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Analysis Physical-Chemical, Mutagenic and Antimutagenic of *Morinda citrifolia L.* (Rubiaceae: Rubioideae) Noni, Germinaded in the Region of Brazilian West Amazon

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Abstract

Given the widespread use of the juice of the fruit of *Morinda citrifolia* by population in different regions of Brazil, this study aimed to perform the analysis: physical chemistry, anti-mutagenic and mutagenic action of M citrifolia, germinated in the Western Brazilian Amazon region. The physical-chemical analyzes were performed in triplicate, using the pulp of the fruit fresh and following the Analytical Standards Institute Adolfo Lutz (1988). The mutagenicity and anti-mutagenicity analysis were performed using the micronucleus test in *Allium cepa*. It was evident that the Noni fruit in the western Brazilian Amazon region is aqueous, acidic, has high moisture content, reducing sugars and ash, which suggests a large amount of minerals. Moreover, it presents higher values of soluble solids and proteins, as compared to fruits from others region and high nutritional value of the fruit. However, it contains a low ratio SS/TA. It was found that the pulp of the fruit of *M. citrifolia* showed no mutagenic effect in the concentrations studied and still had a great ant-mutagenic activity in all concentrations from the pulp of the fruit and the extract.

Keywords: *Morinda Citrifolia*; Mutagenicity; Anti-mutagenicity; Physical chemistry

Introduction

The *Morinda citrifolia*, commonly known as noni, belongs to the family Rubiaceae, and its genre *Morinda* covers 80 species, found mainly in tropical regions [1]. The fruit *M. Citrifolia* is consumed in Asia for over two thousand years, whoever their introduction in Brazil was only few years ago, occurring because there are several disclosures about its benefits, especially in combating pain, tumors, inflammation, hypertension, and fatigue [2]. It's common to use all parts of the plant specially the leaf and the fruit pulp crushed, and mixed with water or grape juice, however, is not scientifically proven that herbal medicine has all these pharmacological action, even though according to a Wang and Su [3], approximately 160 phytochemical compounds have been identified in the plant and the major micronutrients are phenolic compounds, organic acids and alkaloids, although, has not been studied whether there may be variations of the compounds of germinated plants in different regions, and demonstrate that the same action at the cell level.

Therefore, against the widespread use of the fruit juice of *M. citrifolia* by population in different regions of Brazil and because contain little information regarding, will possible induce changes in the cell genome at the level of mutation, is important and necessary to evaluate genotoxic product in order to enrich the scientific knowledge of the same.

These changes genotoxic can be observed through the formation of micronuclei (Figure 1) which are small bodies containing Deoxyribonucleic acid (ADN) located in the cytoplasm, manifested by cells division with chromosome breakage results, forming acentric fragments or whole chromosomes with sequences which are not attach to the mitotic spindle and thus do not reach the poles of the cells during mitosis or meiosis [4,5]. The system micronucleus test in roots of species *Allium cepa*, is defined as one of the best studies for environmental monitoring and mutagenicity of medicinal plants for their sensitivity and accuracy, and considering the roots of *A. cepa* strain have a cell division process similar to the humans [5,6]. However to understand the genotoxic action, it is essential to determine the chemical composition of the plant, because through chemical analysis physical-chemical or Physical, can be performed the evaluation of a nutritional assessment of a product, quality control of food and even develop new products

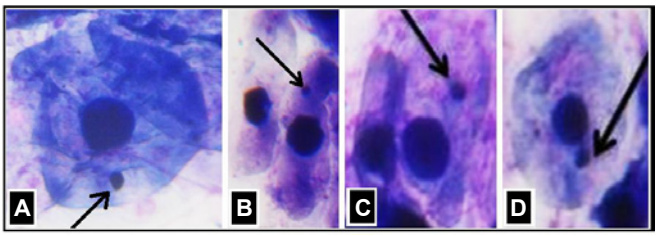

Figure 1: Micronuclei in cell *Allium cepa* exposed (ocular: 10x, objective: 40x).

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[7]. The main purpose of determining the chemical composition is to quantify the moisture, ashes, proteins, carbohydrates, fibers, lipids, vitamins and minerals, as well as establishing other parameters such as water activity, color and texture [8]. This study aimed to perform the analysis: physical chemistry, anti-mutagenic and mutagenic action of *Morinda citrifolia*, germinated in the Western Brazilian Amazon region.

Materials and Methods

Origin and obtaining the fruit and leaf de *M. Citrifolia*

The laboratory fruits were collected from a total of 15 fruits of *M. citrifolia*, obtained on the Sitio Pontal on the line C-70 BR 364 in Ariquemes, Rondonia (RO) Brazil, where the owner reported that the plants were never sprayed with pesticides and foliage fertilizers. For the leaves, was collected 2.300 kg of foliage *M. citrifolia* medium size, firm texture and alive coloration. After harvesting, they were sent to the Laboratory of Pharmacognosy, Faculty of Education and Environment (FAEMA) in Ariquemes, RO.

Obtaining pulp of *M. Citrifolia*

In the laboratory fruits were selected according with some criteria of exclusion: signs of rot; damage by larva or worms, insects or rodents; rigid texture presence, suggesting that fruit comes early (not ripe), cracks in the peel.

After selection of fruits, these were washed in distilled water and placed in sterile glass containers. For obtaining the fruit pulp, was removed all the seeds, which were crushed by hand and sieved with a sterile stainless steel sieve with openings of 2 mm to obtain a pulp. 2.300 kg was collected from *Morinda citrifolia* leaf.

Physical and chemical analysis

The physical and chemical analyzes were performed in triplicate, using the pulp of the fruit fresh and following the Analytical Standards Institute Adolfo Lutz [9], except for the analysis of proteins.

It was determinate the values of pH, water activity, relative equilibrium of humidity, ashes, moisture ,titrated acidity (AT), reducing sugars into glucose, soluble solids (SS), proteins and ratio SS/AT, with mean of results expressed in standard deviation.

Determination of pH values

For the determination of pH, on an analytical balance brand Gehaka, model AG 200, were weighed 10 g of sample diluted in 100 mL of distilled water.

The solution was mixed for about 3 minutes. Then, was allowed to standing for settling. The pH was determined by direct immersion of the electrode in the solution, using a digital pH meter, brand pHTEK; model PHS-3B, calibrated with buffer solutions of pH 4, 7 and 10.

Water activity analyses and relative equilibrium of humidity

At the time of analysis, we used the hygrometer mark Hygropalm-Rotronic, model HP-23A, connected to previously stabilized and calibrated with the distilled water, which has a water activity value equal to 1.0.

Quantification of moisture content

The determination was performed by drying in an oven at 105°C, using oven Medicate brand, and model MD 1.2. First, the equipment was turn on for preheating. Then weighed on an analytical balance Gehaka brand, model AG 200, 5.0 g in dry porcelain crucible and weighed. The transport of the crucibles was done with the aid of tweezers to prevent the passage of moisture of hands. The crucibles were placed in an oven at 105°C for approximately three hours, then removed from the oven with forceps and transferred into a desiccator over silica gel until they reach room temperature. Afterwards the whole sample was weighed crucible more. This procedure was repeated until the sample reached constant mass. Analyzes and data of collection were made every interval of one hour. The mass of the empty crucible was deducted to obtain the mass of dry sample. The calculations for determining the moisture content were made in accordance with the equation shown in (Figure 2A).

Determination of ashes

For determination weighed on an analytical balance, 5.0 g of the sample in a porcelain crucible previously dried, cooled and weighed. Then the flask was brought to the set marks Quimis, model Q-318M25T temperature of 550°C to obtain white or slightly gray ash. After the sample incinerated, withdrew from the crucible furnace, put it in a desiccator containing silica gel to cool and weighed the sample. The ash content was calculated according to the equation shown in (Figure 2B).

Analysis of total Acidity Treatable (AT)

For the determination, weighed on an analytical balance, 1.0 g of the sample then was transferred to a 125 ml Erlenmeyer flask with the help of 50 mL of distilled water. The samples were titrated with NaOH 0.1 mol/L, previously standardized with potassium biphthalate, using 1% phenolphthalein as an indicator. This determination was made by volumetric neutralizing acidity calculated according to the equation shown in (Figure 2C).

Quantification of reducers sugars in glucose

First solutions were prepared Fehling A and Fehling B which are solutions of copper sulphate pentahydrate ($CuSO_4.5H_{20}$) in acid (H_2SO_4) solution and tartrate of sodium and potassium ($C_4H_4KNaO_6.4H_{20}$) in alkaline (NaOH), respectively. Then, weighed 5.0 g of sample was diluted in 500 mL of distilled water and then filtered on paper quality. This sample solution was stored for later analysis. In a 250 mL Erlenmeyer flask were added 10 ml of Fehling solution A, 10 ml

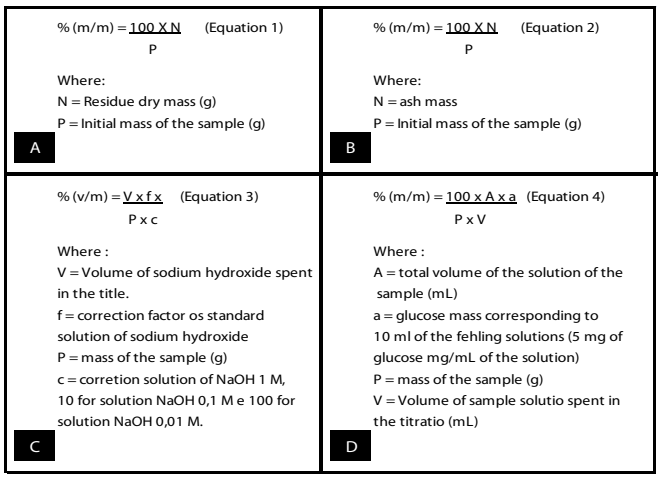

Figure 2: Formulas used for physical and chemical analyzes: A) Calculation of moisture content, B) Ash content, C) acidity content, D) Reducing sugars.

of Fehling solution B and 40 ml of distilled water. Then, this solution was taken to the heating blanket and heater to boil, was titrated with the sample solution. After boiling, were added three drops of blue methylene indicator 1% and then titration was continued until the blue color began to fade, with formation of a brick red precipitate of cuprous oxide (Cu_2O) in the bottom of the flasks. The calculation for the content of reducing sugars was made according to the equation shown in (Figure 2D).

Determination of soluble solids (SS)

The determination was made in a bench refractometer; model Biobrix by reading directly from a small amount of sample. The results were expressed as °Brix.

Determination of protein content

The determination was performed by the biuret method [10]. For this, was initially prepared the biuret reagent by dissolving 0.15 g of copper sulfate and 0.6 g of potassium sodium tartrate in 50 mL of distilled water. Then was added 30 mL of 10% NaOH solution under constant agitation. Subsequently, diluted with distilled water in a volumetric flask of 100 ml and stored in the reagent bottle of polyethylene.

To quantitative proteins in the sample, we constructed a calibration curve of casein (protein standard). To this, a solution prepared casein 5.00 mg/mL, weighing 2.5 g casein which was diluted in 20 mL of distilled water and 5.0 ml of NaOH solution 0.5 mol/L. The solution was heated on hot plate briefly to solubilize the protein. Was transferred to a 250 mL volumetric flask and completed with distilled water. For the preparation of the standard curve of protein casein solutions were prepared in concentrations of 0, 0.25, 0.50, 0.75, 1.00, 1.25, 1.50, 2.50, 3.50 and 4, 50 mg/mL obtained by diluting the 5.0 mg/mL was added in test tubes previously enumerated 1.0 mL of each standard solution of casein at different concentrations and 4.0 ml of biuret reagent. Stirred the tubers allowed to rest for 30 minutes and then read the absorbance at 540 nm in a spectrophotometer visible digital microprocessor model Q798DP, brand Quimis Scientific Apparatus Ltd. With the data of absorbance and concentration of casein was constructed calibration curve.

For the preparation of sample, weighed 2.0 g thereof, transferred to a beaker and added to 20 ml of distilled water and 1.0 ml of NaOH solution 0.5 mol/L. Stir the solution with the aid of a glass rod and heated on hot plate, waiting three minutes from the time of boiling, the protein to be solubilized. After cooled, the sample was transferred to a 50 mL volumetric flask, where it had completed its volume with distilled water. We carried out the filtration of the sample solution and then placed 1.0 ml of the filtered sample in the test tube. Was added 4.0 ml of biuret reagent, stirred and allowed to rest 30 minutes. Subsequently, the absorbance read at 540 nm in a spectrophotometer visible digital microprocessor Q798DP model, brand Quimis Scientific Instruments Llc. The protein content of the sample was calculated by interpolation from the calibration curve.

Obtaining a pure extract of the leaf *M. citrifolia* for Anti-mutagenicity

The leaves were cut with scissors and distributed in three aluminum trays and placed to dry in an oven at 40°C, remaining seven days. After these days, these leaves were ground in a blender to obtain a final weight of 312,361 g leaf powder of *M. citrifolia*. Then, the crushed divided into two beakers, and 65.649 g in a beaker of 600ml which was added methyl alcohol to 96% to approximately 480 ml, and in another beaker of 1000 ml was placed 245,077 g of crushed and added 800 ml of methanol and is waited for 5 days. After that time, it was made the filtration of solutions on filter paper 2, 5 cm in diameter. Transferred the filtrate to an amber glass 1000 ml, obtaining approximately 700 ml of mixture, and passed on a rotary evaporator at 70°C stirring 3 to obtain the extract, this procedure has occurred for 2 days to obtain the extract with final weight of 12,909 g.

Mutagenicity and ant-mutagenicity analysis of the fruit pulp

Was used 140 copies of A. strain (bulbs) small size, uniform, same origin, ungerminated and healthy, acquired in the municipal market at Ariquemes, Rondônia, Brazil, on July 22. On the same day, the bulbs were placed to germinate for a period of 72 hours at 25°C, in appropriate containers, with the lower part immersed in a solution containing different concentrations. For mutagenicity analysis of the fruit of *M. citrifolia*, it was used 10 bulbs put to germinated placed in bottles of 50 ml for each of four concentrations (2.5%, 5%, 10% and 20% of the pulp). We also used bulbs 10 at a concentration of 100% mineral water to the negative control.

For the ant-mutagenic analysis, it used the same procedure as mutagenic analysis, being added 0.04 g of copper sulphate ($CuSO_4.5H_2O$-mutagen) [11] to each container and a positive control containing only H_2O and mutagenicity agent.

Analysis of the ant-mutagenicity pure leaf extract

The extract was divided into three treatments with it: 0,1 ml, 0,3 ml and 0,5 ml diluted in 50 ml of mineral water, following the same procedure of analysis of the pulp.

Preparations and reading of blades

The meristems were collected when they reached length of 0, 5 to 3, 0 cm, being carried out hydrolysis of the same in a solution of 1NHCl for 10 minutes in a water bath at a temperature of 60°C, and subsequent washing in distilled water. Then the smears were performed on two slides for each repetition, waiting drying at room temperature.

After drying, the blades were stained according Meneguetti et al. [5] with a Quick Kit Panoptic LB which is comprised of three containers: one containing triarilmetano 0.1%, the second with 0.1% xatenos and the third thiazine 0.1%. Thus, the blades were immersed 10 times in each container with a second submersion, and then washed with distilled water and left to dry at room temperature. The blades were examined under an optical microscope with a 40x objective and a 10x eyepiece having a 400x magnification. Micronuclei were observed every 1000 cells per blade. This procedure has been used successfully in other studies [12,13]. For statistical analysis, we used the analysis of variance (ANOVA), TUKEY tests, made by Software Graphed PRISM 5.0.

Results and Discussion

Physical and chemical analysis

The results obtained after the physical-chemical analyzes can be seen in (Table 1). The pH-value obtained is similar to others reported in the literature and indicates the character of the fruit acid, as described by Canuto et al. [14], wherein the pH value was 4.1, being close to the guava-Ox (4.0) and cajarana (4.4) [15]. The result of the water activity was very close to the value of the water activity of Biriba determined by Marques [16], which corresponds to 0.984. According Gava,

Parameters	Values obtained s*
pH	4,31 ± 0,02
Water Activity (28°C)	0,986 ± 0,003
Relative equilibrium of humidity (%)	98,6 ± 0,3
Moisture (%)	86,35 ± 0,19
Ashes (%)	0,79 ± 0,03
Analysis of total acidity treatable (AT)	6,91 ± 0,17
Quantification of reducers sugars in glucose (%)	12,30 ± 0,29
Soluble Solids (SS) (°Brix)	15,2 ± 0,0
Proteins (%)	2,72 ± 0,28
SS/AT	2,20

*average ± standard deviation (n=3)

Table 1: Physical-chemical characterization of the fresh pulp of noni fruit.

Silva and Frias [15], water is probally the individual factor that most influences the change of food and the higher the water content, the more perishable foods are, presenting a higher risk of multiplication of pathogenic bacteria. The moisture content was close to the values found in the literature, according to Correia [17], the largest component of the fruit of *M. citrifolia* is water, ranging between 90 and 92%, the moisture content found by the authors was 89,44%, as of Canuto et al. [14] was 90.2%, both getting close guava 90.1% and 91.7% cajá. The ash content was not significantly different when compared to the result of another region, similar to the level obtained by Correia [17], in the state of Ceará (0.80%). The ashes constitute the mineral fraction of foods, are formed by macro and micro nutrients can vary according to the composition of the soil in which the plant was cultivated [16]. The acidity identified by Silva et al. [18] equal to 0.39%, which differs significantly from the value determined in this study (6.31%). In Canuto et al. [14], the acidity of noni is 3.2%, similar to the level of acidity found in cupuaçu 3.5% [18] also point out that noni has low acidity compared to other fruits such as pineapple, umbu-caja and jabuticaba. The acidity of acerola pulps was also evaluated by Oliveira et al. [19], which had a range between 0.47 and 1.56%. The content of reducing sugars into glucose determined in fresh pulp of noni was higher when compared with the caja 2,70% and 5,74% cashew determined by Oliveira et al. [19] in the states of Paraíba and Pernambuco, and the sugar content of Tootles (1,13%) occurred in Marques [16]. The main soluble sugars in the fruits are glucose, fructose and sucrose, and the proportions vary depending on each species. And the reducing sugar content increases as the fruit ripening [17].

The amount of soluble solids found in the pulp of noni was superior to that found by Canuto et al. [14] (9.0 °Brix) and Silva et al. [18] (10.33 °Brix). According Oliveira et al. [19], it is noteworthy that the variation of soluble solids can occur due to rainfall during the harvest, climatic factors, variety, and soil, among others. The protein content was determined by interpolation from the standard curve of casein, which had the same straight line equation Y=0.0496. 0.05928+X (r^2=0.997). The amount of protein found in noni fruit was higher than the value determined by Correia [17] (0.68%).

Rubio-Pino et al. [20] claimed that the SS/TA ratio is critical in assessing flavor, being more efficient than the quantification of sugars and acidity. There is a big difference in the ratio SS/TA noni obtained in this work with the value found by Silva et al. [18] (26.49). This difference can be explained by the fact that there are variations in climate and soil of each region and due to the extent of maturation and storage of fruits [17].

Analysis of mutagenicity and ant-mutagenicity

Mutagenicity: The results obtained are represented in the analysis (Table 2). Observing the data in the above table, note the presence of 5,7 micronuclei per 1000 cells at a concentration of distilled water to 100%, and it was considered as negative control, showing that it is within normal values.

For concentrations containing 2.5%, 5%, 10% and 20% pulp M. citrifolia were obtained 2.8 respectively (P<0.001) 3.7 (P<0.01); 2.1 (P<0.001) and 3.9 (P<0.05) micronuclei per 1000 cells, showing statistical significance when compared and the negative control, showing that the tested concentrations did not show a mutagenic effect on cells analyzed (Figure 3). Concentrations containing 2.5% and 10% pulp of the fruit of *M. citrifolia* showed greater statistical significance when compared to the control group.

	C-	2,5 %	5 %	10%	20%
***A. cepa* 01**	3	2	2	3	6
	3	2	2	3	6
***A. cepa* 02**	5	2	6	3	3
	4	2	3	3	4
***A. cepa* 03**	4	2	6	2	3
	13	3	3	2	4
***A. cepa* 04**	8	3	4	2	4
	7	2	3	2	3
***A. cepa* 05**	6	3	3	2	3
	6	2	5	1	4
***A. cepa* 06**	4	3	6	1	2
	3	3	2	2	3
***A. cepa* 07**	6	3	3	1	4
	2	2	4	3	6
***A. cepa* 08**	4	4	2	1	4
	7	3	5	2	4
***A. cepa* 09**	1	4	7	4	3
	8	6	3	2	4
***A. cepa* 10**	8	3	3	1	3
	12	2	2	2	5
Count	**114**	**56**	**74**	**42**	**78**
Average	**5,7**	**2,8**	**3,7**	**2,1**	**3,9**

Table 2: Number and average of micronuclei in *A. cepa* on each 1000 cells to blades and concentration.

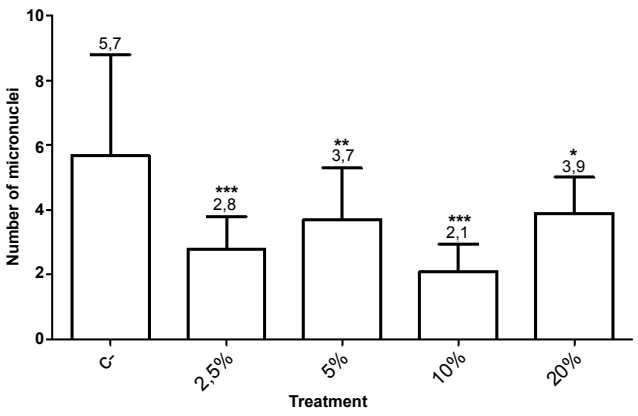

Figure 3: Average numbers of micronuclei per 1000 cells found in *A. cepa* strain concentrations on the pulp of the fruit of *M. citrifolia*. *Significant to (P<0.05), **(P<0.01) and ***(P<0.001).

Consistent with Mathivanan et al. [21], high consumption of *M. citrifolia* is due to the fact that people use it as an alternative for the prevention and treatment of various diseases including cancer [22-24].

It is noted also that according to some studies the *M. citrifolia* fruit of and the components of the root thereof, is unable to cause hepatotoxicity [25,26], but other studies contradict this information describing the consumption of *M. citrifolia* is directly related hepatotoxicity [27,28], where consistent with the work of Chearskul et al. [29], it is believed that the use of components of *M. citrifolia* be used for therapeutic moderately.

Ant-mutagenicity: The results obtained by analyzing mutagenic extract of roots and leaves of *M. citrifolia* are shown in (Table 3). Through Table 3 it can be seen that at all concentrations of the pulp (2.5%, 5.0%, 10% and 20%) and extract (0.1 ml, 0.3 ml and 0.5 ml) observed if anti-mutagenic activity with ($P<0.001$) compared to the positive control, being also less than the mean negative control at all concentrations as can be seen in (Figures 4 and 5).

Some studies have highlighted the beneficial effects of *M. citrifolia* [30,31], and others show that the plant has no genotoxic effects [32,33], but there are few studies that demonstrate the ant-mutagenic action. These effects were tested against Sarcoma 180, which was investigated using a polysaccharide-rich substance called Noni-ppt, the results showed that the antitumor activity of Noni-ppt produced a cure in 25% to 45% of allogeneic rats and that this activity was completely abolished by concomitant administration of specific inhibitors of

	C+	C-	2,5%	5,0%	10%	20%	0,1 mL	0,3 mL	0,5 mL
***A. cepa* 1**	9	2	1	1	1	0	3	1	0
	8	3	1	2	1	1	1	1	0
***A. cepa* 2**	8	2	1	1	1	1	1	1	2
	11	4	2	2	2	1	0	1	1
***A. cepa* 3**	14	4	1	2	1	1	1	1	1
	12	1	1	1	1	1	1	0	1
***A. cepa* 4**	11	4	2	1	1	1	1	0	1
	14	5	1	1	1	1	0	0	1
***A. cepa* 5**	13	3	1	3	3	1	0	2	1
	14	3	6	1	1	0	1	1	1
***A. cepa* 6**	13	2	2	4	1	1	1	1	2
	12	3	1	1	1	0	1	0	1
***A. cepa* 7**	5	1	1	1	3	0	2	0	1
	9	2	1	1	1	1	1	0	0
***A. cepa* 8**	11	4	1	2	1	0	2	1	1
	14	2	2	3	1	0	0	1	1
***A. cepa* 9**	8	1	1	1	1	0	0	1	0
	12	2	1	1	1	0	1	1	0
***A. cepa* 10**	11	2	2	2	1	0	1	1	0
	8	1	1	1	1	0	1	2	1
Count	**217**	**51**	**30**	**32**	**25**	**10**	**19**	**18**	**16**
Average	**10,85**	**2,55**	**1,5**	**1,6**	**1,25**	**0,5**	**0,95**	**0,9**	**0,8**

Table 3: Number and average in *A. cepa* on 1000 cells per blades realized with treatment of leaf and pulp extract.

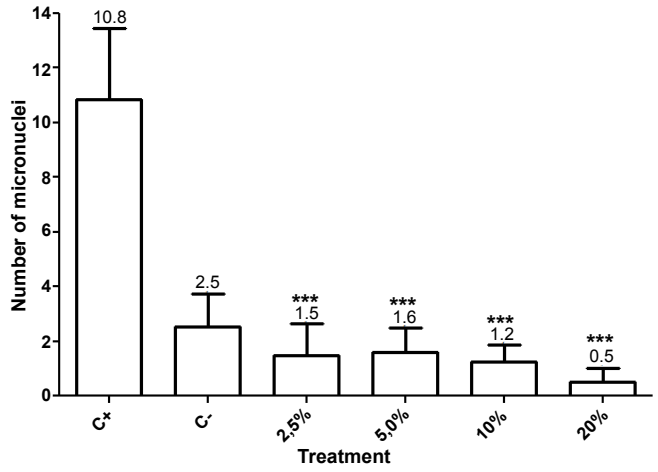

Figure 4: Average of numbers of micronuclei found in 1000 cells of *A. cepa*, per concentration of fruit pulp of *M. citrifolia*. *Significant to ($P<0.05$), **($P<0.01$) and ***($P<0.001$).

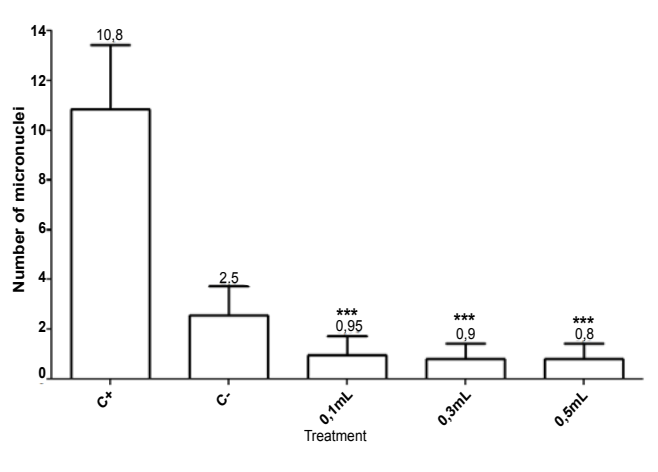

Figure 5: Average of numbers of micronuclei found in 1000 cells of *A. cepa*, per concentration of leaf extracts of *M. citrifolia*. *Significant to ($P<0.05$), **($P<0.01$) and ***($P<0.001$).

macrophages (2-cloroadenosine), T cells (cyclosporine) or natural killer cells [34]. Another study demonstrated that the juice of the fruit of *M. citrifolia* has nutritional and medicinal properties, being able to act as ant-mutagenic, protecting cells against spontaneous and induced mutations [35].

Conclusion

Physical and chemical

Considering the results, it can be stated that the *M. citrifolia* fruit is watery and acidic, has high moisture content, reducing sugars and ash, which suggests a large amount of minerals. Moreover, it presents higher values of soluble solids and proteins when compared with other regions of *M. citrifolia*, showing high nutritional value of the fruit. However, it contains a low SS/TA ratio.

Action mutagenic and ant-mutagenic

It was found that the pulp of the fruit of *M. citrifolia* showed no mutagenic effect in the concentrations studied and still had a great ant-mutagenic activity in all concentrations from the pulp of the fruit and the extract.

Importantly to bounce that these effects ant-mutagenic are encouraging for the use of the plant in combating genotoxics damage stimulated mainly by environmental factors, however, future studies are indicated in animal cells to a better understanding of the results and understanding of the action of the same organism in a satisfaction a physiological system closer to the human being, because in spite of the beneficial effects on plant cells described in the present study the consumption of juice and sweets derived from *M. citrifolia* is not authorized by ANVISA in Brazil, and will not be allowed prior to performing all tests mutagenic and toxicology.

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