# Antioxidant effect of *Physalis angulata* fruit on cells exposed to 2,4-dichlorophenoxyacetic acid

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## Abstract

*Several in vitro studies have described the erythrocyte toxicity mechanism response to the 2,4-dichlorophenoxyacetic acid (2,4-D) and its metabolites, strongly related to oxidative stress. Compounds such as ascorbic acid and flavonoids, present in various fructiferous plants like Physalis angulata L.-Solanaceae, are exogenous sources of antioxidants, which have aroused interest because of its beneficial biological effects to health by capturing oxygen reactive species. Here, was investigated the cytotoxic effects of the 2,4-D in human erythrocytes exposed to this herbicide and treated with the juice of P. angulata fruits. Analyzing the oxidant and antioxidant mechanisms in these cells in vitro, was demonstrated that the damage mechanism by the Physalis juice occurred at the level of cellular proteins and membranes, altering levels of endogenous antioxidants components such as reduced glutathione and uric acid, and exogenous like vitamin C. Our results indicate new insights into the mechanism of human erythrocytes response exposed to the P. angulata juice, providing wayforward to future studies of cytotoxicity.*

**Keywords:** 2,4-dichlorophenoxyacetic acid; Oxygen reactive species; Physalis.

## 1. Introduction

The application of herbicides for weed control has been a common activity in global agriculture, aiming to increase crop productivity. However, when these compounds are employed in an uncontrolled manner, they can impact non-target organisms such as those living in the environment, including humans (Nwani et al., 2010).

Herbicide levels in water, food and soil have increased considerably, which accentuates the risk of environmental and toxicological problems. 2,4-Dichlorophenoxy acetic acid (2,4-D) is commonly used for annual weed control, preemergence of broadleaf in cereals, corn, pasture, rice and non-agricultural (Bongiovanni et al., 2012). According to the National Health Surveillance Agency (ANVISA), 2,4-D is a hormonal herbicide belonging to toxicological class I due to the acute effects it can produce in a single exposure (Anvisa, 2018).

Prolonged inhalation of 2.4-D in humans may cause acute effects such as dizziness, coughing, weakness

and even temporary loss of muscle coordination, as the main and most recurrent effects are due to improper handling of the product, which may lead to skin and eye irritation (Macedo Neto, Froehner and Machado, 2012).

In addition, some studies show that 2,4-D alters cellular energy metabolism and redox balance, favoring the occurrence of Oxidative Stress (OS) in humans (Bongiovanni et al., 2011; Liu et al., 2017). OS can be caused by increased production of Reactive Species (RSs) or decreased antioxidant system action that is activated to prevent the buildup of RSs that damage cells and molecules by attacking the body's lipids, proteins, polysaccharides and nucleic acids (Gelatti et al., 2018).

For this reason, erythrocytes, being anucleated and unable to repair damage through synthesis of news components, become an excellent research model for oxidative damage, since any modification that occurs cannot be masked by the repair mechanism in these cells. Nicotinamide Adenine Dinucleotide Phosphate (NADPH) is used as a cofactor in the reduction of Oxidized Glutathione (GSSG) in Reduced Glutathione (GSH), which is the primary protective mechanism against oxidative stress (Greer et al., 2003; Zhou et al., 2017).

Erythrocytes have a hemoglobin solution essentially surrounded by a lipid bilayer complex containing about 42% lipids, 52% protein and 7% carbohydrates. These membrane components can be highly damaged when oxygen (O2) is reduced and generates Reactive Oxygen Species (ROS), such as the hydroxyl radical (OH-), which acts as an oxidant (Furman, 2011). Oxidative damage can be counteracted by endogenous antioxidants such as GSH (free thiol group tripeptide) and uric acid (metal ion chelator from the capture of ROS by its urate group) or exogenous as Ascorbic Acid (ASA), which despite being at levels lower levels within erythrocytes (0.043 mol/L) is essential for action synergism along α-tocopherol (vitamin E), which acts as a chain reaction terminator, preventing the spread of ROS (Furman, 2011; Vaos and Zavras, 2017).

In this context, certain nutrients and food components have stood out due to their antioxidant activity, ie, with the ability to transform and/or reduce the oxidation action of RSs, preventing their harmful effects on the body (Tureck et al., 2017). Phenolic compounds such as simple and glycosylated flavonoids, present in various fruits, are also exogenous sources of antioxidants, which aroused interest due to their beneficial health biological effects (Camlofski, 2014). Thus, the present research carried out experiments with a fruit widely marketed and cultivated in southern Brazil, called *Physalis angulata* L., an annual branched shrub belonging to the Solanaceae Family, known by various names as Mullaca, camapu, gooseberry cape, wild tomatoes, among others, widely distributed in tropical regions and their extracts or infusions have been used in many countries in folk medicine to treat a variety of diseases such as malaria, asthma, hepatitis, dermatitis and rheumatism (Rathi et al., 2017). Therefore, the present study aimed to evaluate the cytotoxic effects of 2,4-D on human erythrocytes exposed to this herbicide and treated with *Physalis angulata* L. fruit juice.

## 2. Method

### *2.1 Ethical Aspects*

This project was submitted to the Ethics Committee of the University of Cruz Alta and was approved under protocol number: 15510413.3.0000.5322. Included in these subsections the information essential to

comprehend and replicate the study. Insufficient detail leaves the reader with questions; too much detail burdens the reader with irrelevant information. Consider using appendices and/or a supplemental website for more detailed information.

*2.2 Physalis angulata cultivation and 2,4-D preparation*

The *Physalis* were planted in the experimental area of the Vegetal Multiplication Laboratory, University Campus of Unicruz - Technological Pole Alto Jacuí in the town of Cruz Alta, located in the Northwest of Rio Grande do Sul state. The soil is characterized as Typical Dystrophic Red Latosol, type A moderate and medium texture (Embrapa, 2006). The climate, according to the Koeppen classification is subtropical, type cfa 2a, with rains uniformly distributed throughout the year. Samplings of fruits were obtained after 60 days from lab seedlings transplantation to the field (approximately February 20, 2014). For the production of the juice, the fruit were picked in the end of maturation and weighed (250g), followed by grinding of the fresh fruit and dilution with water to obtain the concentration of 250g/L, while the other concentrations (50 and 100g/L) were made from the initial solution. For the 2,4-D preparation, 1.1 grams of 2,4-D were measured, followed by dilution to one liter, to achieve the concentration of 1.1g/L.

*2.3 Physalis Fruit characterization*

Measurements of total polyphenols and condensates tannins were performed using 50mL *Physalis angulata* juice at a concentration of 250g/L lyophilized.

2.3.1 Determination of Total Phenolics

The determination of the total phenolics was made according to the method described by Chandra and Mejia (2004). Infusion of 250g/L lyophilized *P. angulata* was diluted with distilled water to a concentration of 0.15mg/mL. To this solution was added sodium carbonate to 20% and after 5 minutes was added 2N Folin-Ciocalteu reagent. This reaction mixture was incubated for 10 minutes and the spectrophotometer readings taken in visible 730nm. The tests were performed in triplicate and for calculating the dosage of total phenolics used was a standard curve of gallic acid. The results were expressed as mg gallic acid/g dry weight.

2.3.2 Determination of Condensed Tannins

The determination of condensed tannins was carried out using the method described by Burns (1971). Infusion of 250g/L lyophilized *P. angulata* was diluted with methanol to a concentration of 25mg/mL. To this solution was added methanol, vanillin solution 0.01g/mL and 0.08M hydrochloric acid. The mixture was heated at 60°C for 10 minutes. Readings were taken in visible spectrophotometer at 420nm. The tests were carried out in triplicate to calculate the assay was condensed tannins using a catechin standard curve. The results were expressed as catechin mg/g dry mass.

2.3.3 Blood collection, isolation of erythrocytes, exposition and treatment

Blood samples were obtained from 20 healthy volunteers, the blood was colleted after fasting for eight hours by intravenous puncture into Vacutainer® tubes with ethylenediaminetetra acetic acid (EDTA). The study protocol was executed in conformity with the guidelines of the Institutional Ethical Committee. After the measurement of hematocrit and hemoglobin into total blood, the samples were centrifuged at 3000rpm for 10 minutes and the plasma removed. Erythrocytes were washed three times with isotonic saline (0.9%) at 37ºC, centrifuged and suspended with some adjustments (Catalgol et al., 2007). The hematocrit was

diluted at 5% and the suspensions of erythrocytes were divided into five different groups with the same patients' samples:

1) Control, not exposed to 2,4-D nor treated with *P. angulata* juice

2) Exposed to 1.1g/L 2,4-D for one hour;

3, 4 and 5) Exposed to 2,4-D for one hour and treated with *P. angulata* juice at 50g/L, 100g/L and 250g/L for one more hour, respectively.

After exposure and processing the suspension of erythrocytes at 5% hematocrit were hemolyzed by vortexing, followed by centrifugation and separation of the supernatant, where a new hemoglobin measurement was performed and the remaining stored in the freezer for subsequent biochemical analysis.

*2.3.4 Analytical determinations*

2.5.1 Determination of hemoglobin and hematocrit

The measurement of hemoglobin levels was performed using a commercial kit Labtest®, according to the manufacturer's instructions. The hematocrit was determined by the microhematocrit method for the sealing of the capillary tubes followed by centrifugation at 11.500rpm for 5 minutes. With the aid of the hematocrit reading card, each microtube was read to obtain the percentage of cells relative to the total volume of solution.

2.5.2 *Determination of total protein carbonylated proteins and uric acid*

The dosage of total proteins and uric acid were done by use of a commercial kit Labtes$^{t®,}$ according to the manufacturer's instructions. The carbonylated proteins were analyzed according to the methodology described by Levine (1990) with some adjustments. 250µL of 10 mM 2,4-dinitrophenylhydrazine (DNPH) to 250µL of 2M hydrochloric acid were added, followed by incubation in the dark, and washing with the solution containing ethanol and ethyl acetate. 1500µL of the denaturing sodium dodecyl sulphate buffer (3% SDS, pH 8.0) were added and the samples were incubated for 10 minutes in a water bath at 37°C. After formation of colored product, the mixture was measured spectrophotometrically at 370nm and expressed as nmolcarbonyl/mg total protein.

2.5.3 *Determination of Reactive Substances Thiobarbituric Acid (TBARS)*

For the quantitative assessment of lipid peroxidation, the determination of TBARS was performed by the formation of malondialdehyde (MDA) following the procedure described by Stocks and Dormandy, (1971) with some adjustments. 200uL of supernatant were added to 550µL of water and 1mL 28% trichloroacetic acid (TCA). After centrifugation at 2000rpm for 15 minutes, the supernatant was collected and added to 500µL of 1% thiobarbituric acid (TBA). The solution was subjected to heating in a boiling bath at 95ºC for 15 minutes. After cooling, the samples were read at 532nm through the pink color formation and the results expressed as nmol MDA/Hb.

2.5.4 *Determination of Reduced Glutathione (GSH)*

850uL of potassium phosphate buffer (TFK) 1M pH 7.4 and 50uL of 5,5-dithio-bis-2-nitrobenzoic acid (DTNB) 10 mM were added to 100uL of the supernatant. The mixture was read by a spectrophotometer at 421nm and the results were expressed in µmol GSH/Hb (Ellman, 1959).

2.5.5 *Determination of Ascorbic Acid (ASA)*

Quantification of Ascorbic Acid (ASA) was performed with the addition of 50uL of supernatant into water

at 150uL, 300uL of 13.3% TCA and 75Ul of DNPH. Incubation was performed at 37°C for 1 hour and 30 minutes with the formation of colored product. The reaction was stopped by the addition of 500uL of sulfuric acid ($H_2SO_4$ 65%), followed by centrifugation for 10 minutes at 3500rpm reading on a spectrophotometer at 520nm and the results expressed as μmol ASA/Hb (Roe, 1954).

2.6 *Statistical analyses*

Data normality was assessed by the Shapiro-Wilk test. Nonparametric results of GSH, TBARS, carbonylated proteins and ASA were analyzed statistically by the Kruskal-Wallis test followed by the Dunns post-test. Parametric data of total protein, uric acid and hemoglobin were analyzed by 1-way ANOVA repeated measures followed by the Tukey post-test, considering significant differences at $p<0.05$, through analyses using the statistical program GraphPad Prism 5 and results showing the mean ± standard error of the mean (SEM).

## 3. Results

3.1 *Phytochemical characterization of the fruit of P. angulata*

There was a concentration of 5.5±0.24mg/g of total phenolics and 1.5±0.48mg/g of condensed tannins in *P. angulata* juice at a concentration of 250g/L, which indicates that the plant has a low concentration of compounds with antioxidant potential compared with other juices (Vargas et al., 2008; Rocha et al., 2011).

3.2 *Haematologic assessments by hematocrit and hemoglobin*

The mean (± SD %) hematocrit (Ht) of the subjects' total blood was 39±2.0, while the average (g/dL ± SD) hemoglobin (Hb) was 14.8±2.1 demonstrating the absence of pathologies associated with these parameters.

3.3 *Induction of formation of ROS*

The data demonstrate that the cells that have been exposed to 1.1g/L of 2,4-D did not alter the total protein (TP) levels and the carbonyls levels. However, there was a decrease of TP in cells exposed to *P. angulata* juice (Figure 1 A) and increased protein carbonylation in the group treated with 50g/L of juice (Figure 1 B). These data suggest that the lowest concentration tested appears to be toxic. We believe that TP reduction occurred by the increase of protein carbonylation generated by oxygen reactive species.

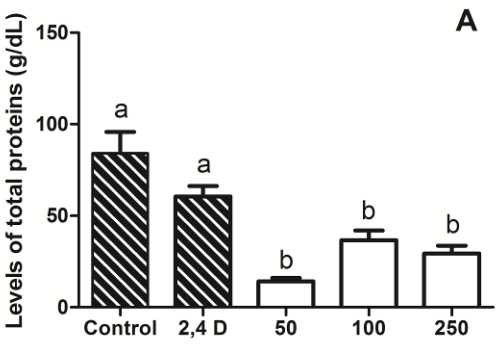


Figure 1 A. Levels of total proteins (g/dL)

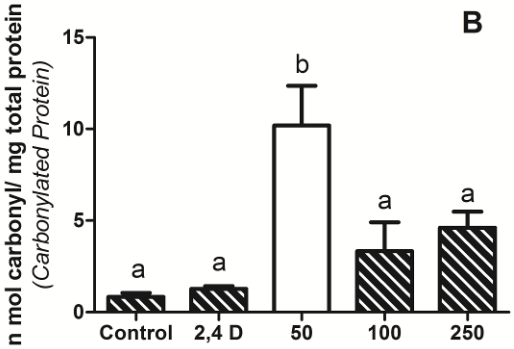


Figure 1 B. Carbonylated protein (nmol carbonyl/mg)

3.4 *Activation of the antioxidant system against damage in the cell membrane*

The data suggests that *P. angulata* juice has a dose-dependent effect, therefore, the uric acid levels increased as the juice concentrations increased (Figure 2 B). Declining levels of TBARS (Figure 2 A) in concentrations may be occurring in proportion to the amount of uric acid, in other words, the higher the antioxidant levels the better to reduce the damage in cell membranes. The results demonstrate a greater cytotoxicity level of cell membranes exposed to concentration of 50g/L of *P. angulata* juice, suggesting an increase in the ROS generation, confirming the carbonylation protein levels presented in this study.

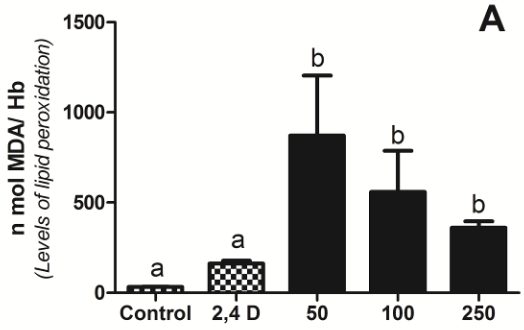


Figure 2 A. Levels of lipid peroxidation (nmol MDA/Hb)

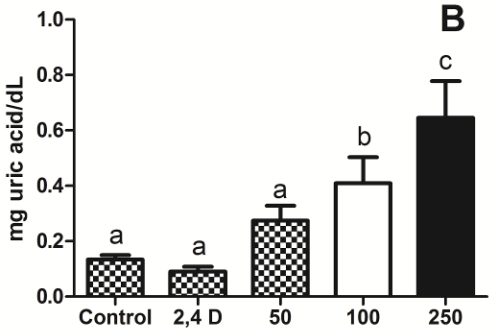


Figure 2 B. Uric acid (mg/dl)

3.5 *Increased GSH activity concomitant with consumption of ASA*

The results show that the erythrocytes exposed to concentration of 50g/L *P. angulata* juice had higher consumption of ASA and GSH activity (Figure 3 A; B) which possibly happened due to increased oxidative damage already described in this study.

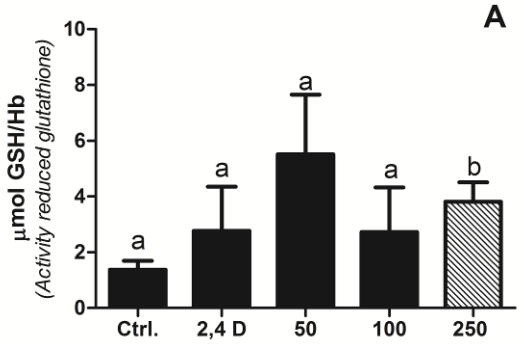


Figure 3 A. Activity Reduced Glutathione

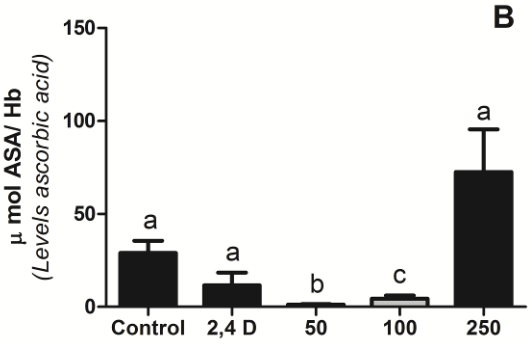


Figure 3 B. Levels Ascorbic Acid

## 4. Discussion

The aim of the present study was to evaluate the antioxidant or oxidative action of *Physalis angulata* L-Solanaceae in human erythrocytes exposed to the herbicide 2,4-D. The choice of this plant was due to phytochemical studies according to Lashin and Elhaw (2016) that revealed constituents with a wide range of biological activities presented by the genus *Physalis,* possibly due to the metabolic and structural diversity of its compounds with pharmacological properties such as alkaloids, flavonoids, glycosides, saponins, tannins, steroids, among others, attributed to these many of the documented anti-cancer, anti-

tumor, and anti-leukemic actions, also considered as an effective immune stimulant with antimicrobial properties (Rathi et al., 2017). However, in the present study a low concentration of compounds with antioxidant action was demonstrated.

Phenolic compounds (including flavonoids) can react with RSs in the cell, conferring antioxidant properties that have the potential to inhibit pathological and degenerative processes. Consequently, these antioxidant properties confirm the therapeutic potential of this plant (Ferreira et al., 2019).

In this context, it was possible to verify that the toxicity of *Physalis* juice is dose dependent since, according to the concentration tested, there was an increase in lipid or protein damage, and in the case of treatment with 50 and 250 g infusions/L there was an increase in both lipid peroxidation and protein oxidation. These findings corroborate the results found by Conceição et al. (2017), which shows in their research that the toxicological effect caused by herbal extracts may be dose dependent.

In addition, it is noteworthy that in this study the damage was greater in lipids, probably because the membrane is one of the most affected structures due to oxidative stress due to lipid oxidation, a process in which ROS attack phospholipid polyunsaturated fatty acids cell membranes, disintegrating them and thus allowing these species to enter into intracellular structures. Phospholipase, activated by toxic species disintegrates phospholipids, releasing unsaturated fatty acids, resulting in deleterious actions of lipid peroxides (Kleniewska and Pawliczak, 2017; Thapa and Carroll, 2017).

The entry of toxic substances in the biological system generates RSs, which when not removed by the antioxidant defense system end up generating cellular damage such as oxidation of biomolecules through lipoperoxidation and protein carbonylation, enzymatic inactivation, exaggerated consumption of endogenous antioxidants, excessive activation of proinflammatory genes (Petiz et al., 2017).

From the degradation of aldehydes lipid peroxide products are toxic to cells. Malondialdehyde is a highly reactive molecule, being a dialdehyde capable of reacting with primary amines in proteins or DNA to form crosslinks. The covalent modifications made by this secondary liporexidation messenger can alter the structure and function of proteins and nucleic acids and are responsible for the cytotoxicity of these molecules (Gaschler and Stockwell, 2017).

Regarding proteins, they are biomolecules that are present in abundance in the biological system, being the main oxidative targets in oxidative stress, due to the high affinity with ROS. Protein carbonylation derivatives (aldehydes and ketones) are formed by residues of methionine, cysteine, proline, histidine, arginine, lysine, tryptophan, tyrosine, phenylalanine and valine. As a consequence, carbonyl compounds, nitrated products, disulfide bridges, crosslinking, peroxides, among others are generated (Barreiro, 2016).

Direct reactions of proteins with ROS may also lead to the formation of lipid peroxidation fragments containing highly reactive protein carbonylation. In addition, secondary reactions of primary amino groups of lysine residues with reducing sugars or their oxidation products (glycation and/or glyoxidation reactions) may also generate reactive carbonylation in proteins. Changes in protein oxidation may produce carbonyl groups (ketones and aldehydes) in their side chains leading to impairment of protein functions (Dalle-Donne et al., 2017; Zhou et al., 2017).

Protein carbonylation are typically dysfunctional or nonfunctional, having a major influence on cell viability, tissue and even complete organ dysfunction. It is likely that protein dysfunction after

carbonylation may depend on the quantitative level and/or location of the protein-bound carbonyl group (s) (Dalle-Donne et al., 2017).

GSH levels increased at the concentration tested with *Physalis* juice (50 g/L), which in turn may be related to the increase in TBARS and protein carbonyl in this same treatment. Suggesting that the plant led to excessive production of RSs that have a widespread action on cellular homeostasis, causing biochemical changes on lipids, proteins and DNA (Murussi et al., 2014).

However, on the other hand, the plant also increased the production of the body's main antioxidant agent to probably reduce the damage generated on lipids and erythrocyte proteins exposed to 250 g/L concentration of *Physalis* juice, but without success, taking into account the damage continued to increase at this concentration.

At the moment was exposed the plasma of the samples with the herbicide an imbalance of this system was caused. Therefore, it was found in this study that although the plant increased GSH production, damage to lipids and proteins were predominant in treatments with *Physalis* juice, greater damage than exposure to 2,4-D, which shows a high toxicity of *Physalis* juice at concentrations 50 and 250 g/L on human erythrocytes.

The major non-enzymatic antioxidant is GSH, a tripeptide formed from glutamic acid, cysteine and glycine, and is present in millimolar concentration (2-10 mmol/L) in all eukaryotic cells. To prevent radical-mediated toxicity, specific enzymes and low molecular weight substances, GSH has the function of eliminating reactive species and helping to maintain cellular redox balance, being involved in various biological functions, playing a central role in biotransformation and elimination of xenobiotics, which are present in large quantities in agricultural pesticides, insecticides, plastics, cleaning products and pharmaceuticals (Kleniewska and Pawliczak, 2017; Oga, Camargo and Batistuzzo, 2014; Vaos and Zavras, 2017).

ASA has the characteristic of enhancing the activity of GSH and also vitamin E within the cells, through an electron donation of vitamin E molecule for the ASA molecule, generating a radical, which in turn oxidizes GSH. In contrast, when the ASA is in fluids it catches and neutralizes ROS (Picchi, 2010).

The increase in ASA levels in erythrocytes exposed to a concentration of 250g/L juice may be due to the higher concentration of P. angulata used in this group, as quantified by Camlofski (2014), the ASA content in fruit of P. angulata (~ 26 mg/100g fruit) confirming the presence of this antioxidant in fruits. Thus, the possible mechanism of action in reducing the levels of lipid peroxidation in this group may be occurring by the concomitant action of uric acid and ASA.

Only hemolysis and antioxidants, in particular the end-product of purine metabolism, uric acid, could bias the measurement of ROS generation. Uric acid is recognized as a potent antioxidant by removing ROS, particularly in combat lipid peroxidation protecting the lysis of erythrocytes. In addition, there is an inverse relationship between uric acid and lipid peroxidation (Peluso et al., 2016).

## 5. Conclusion

Our study has shown that P. angulata juice in concentrations of 50 and 250g/L caused cytotoxicity in human erythrocytes damaging cell membranes and altering proteins. The results presented here provide new

knowledge about the erythrocytic response machinery against the damages caused by the P. angulata juice. Such mechanisms clarified here provide subsidies for future studies of cytotoxicity.

## 6. Acknowledgement

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**Abbreviations:** 2,4D,2,4-dichlorophenoxyacetic acid; ASA, ascorbic acid; ROS, oxygen reactive species; GSH, reduced glutathione; UA, uric acid; GSSG, oxidized glutathione; EDTA, ethylenediaminetetra acetic acid; DNPH, 2,4-dinitrophenylhydrazine; SDS, sodium dodecyl sulphate buffer; TBARS, reactive substances thiobarbituric acid; MDA, malondialdehyde; TCA, trichloroacetic acid; TBA, thiobarbituric acid; TFK, potassium phosphate buffer; DTNB, 5,5-dithio-bis-2-nitrobenzoic acid; Ht, hematocrit, Hb, hemoglobin; TP, protein levels; GO, glutathione oxidase; GSH-Px, glutathione peroxidase; GR, glutathione reductase.

## 7. References

Anvisa – Agência Nacional de Vigilância Sanitária. 2018. Nota técnica nº 24. Disponível em: <http://portal.anvisa.gov.br/documents/219201/4340788/Nota+T%C3%A9cnica+Final+2-4D.pdf/a0ef4bb6-db71-42ae-9174-2fa7e2c9b95f>. (accessed 09/12/18).

Barreiro, E. 2016. Role of protein carbonylation in skeletal muscle mass loss associated with chronic conditions. Proteomes, *4*(2), 18. https://doi.org/10.3390/proteomes4020018

Bongiovanni, B., Ferri, A., Brusco, A., Rassetto, M., Lopez, L. M., de Duffard, A. E., and Duffard, R. 2011. Adverse effects of 2, 4-dichlorophenoxyacetic acid on rat cerebellar granule cell cultures were attenuated by amphetamine. Neurotoxicity research, *19*(4), 544-555. https://doi.org/10.1007/s12640-010-9188-9

Burns, R. E. 1971. Method for estimation of tannin in grain sorghum 1. Agronomy Journal, 63(3), 511-512. doi:10.2134/agronj1971.00021962006300030050x

Camlofski, A. M. D. O. 2014. Avaliação dos compostos bioativos e caracterização das pectinas do fruto de *Physalis angulata* L. (Unpublished doctoral dissertation). Universidade Federal do Paraná, Brasil.

Catalgol, B. K., Ozden, S., and Alpertunga, B. 2007. Effects of trichlorfon on malondialdehyde and antioxidant system in human erythrocytes. Toxicology in vitro, 21(8), 1538-1544. DOI: 10.1016/j.tiv.2007.06.002

Chandra, S., and Gonzalez de Mejia, E. 2004. Polyphenolic compounds, antioxidant capacity, and quinone reductase activity of an aqueous extract of Ardisia compressa in comparison to mate (Ilex paraguariensis) and green (Camellia sinensis) teas. Journal of agricultural and food chemistry, 52(11), 3583-3589. DOI: 10.1021/jf0352632

Conceição, K. N., Sampaio, F. A. S. A., da Silva, V. F., and da Silva, A. O. 2017. Poder antioxidante de carotenoides, flavonoides e vitamina e na prevenção da arteriosclerose. Revista Ciência & Saberes-Facema, *2*(4), 320-324.

Dalle-Donne, I., Colombo, G., Gornati, R., Garavaglia, M. L., Portinaro, N., Giustarini, D., ... and Milzani, A. 2017. Protein carbonylation in human smokers and mammalian models of exposure to cigarette smoke: focus on redox proteomic studies. Antioxidants & redox signaling, *26*(8), 406-426. https://doi.org/10.1089/ars.2016.6772

Ellman, G. L. 1959. Tissue sulfhydryl groups. Archives of biochemistry and biophysics, *82*(1), 70-77. https://doi.org/10.1016/0003-9861(59)90090-6

Embrapa - Empresa Brasileira de Pesquisa Agropecuária. 2006. Ministério da Agricultura, Pecuária e Abastecimento. https://www.embrapa.br. (accessed 09/12/18).

Ferreira, L. M. D. S. L., do Vale, A. E., de Souza, A. J., Leite, K. B., Sacramento, C., Moreno, M. L. V., ... and Grassi, M. F. R. 2019. Anatomical and phytochemical characterization of *Physalis angulata* L.: A plant with therapeutic potential. Pharmacognosy Research, 11(2), 171. DOI: 10.4103/pr.pr_97_18

Macedo Neto, D., Froehner, S., and Machado, K. S. 2012. Assessment of transport of 2, 4-dichlorophenoxyacetic acid using a lysimeter. Química Nova, 35(9), 1809-1813. http://dx.doi.org/10.1590/S0100-40422012000900020

Furman, A.E.F. 2011. Ação antioxidante de extrato padronizado de Ginkgo biloba em eritrócitos de indivíduos normais e de portadores de anemia falciforme, submetido à sobrecarga oxidativa, in vitro. (Unpublished doctoral dissertation). Universidade Federal do Paraná, Brasil.

Gaschler, M. M., and Stockwell, B. R. 2017. Lipid peroxidation in cell death. Biochemical and biophysical research communications, 482(3), 419-425. https://doi.org/10.1016/j.bbrc.2016.10.086

Gelatti, G. T., Tissiani, A. C., Mayer, M. S., Felippin, T., Gewehr, D. M., Koefender, J., ... and Horn, R. C. 2018. In Vitro Antioxidant Potential of Baccharis trimera and Baccharis articulata Infusions in Postmenopausal Women. International Journal of Innovation Education and Research, 6(7), 99-112. https://doi.org/10.31686/ijier.Vol6.Iss7.1060

Greer, J.P., Foerster, J., and Lukens, J.N. 2003. Wintrobe's Clinical Hematology. Philadelphia: Lippincott Williams & Wilkins Publishers.

Kleniewska, P., and Pawliczak, R. 2017. The participation of oxidative stress in the pathogenesis of bronchial asthma. Biomedicine & Pharmacotherapy, *94*, 100-108. https://doi.org/10.1016/j.biopha.2017.07.066

Lashin, I. I., and Elhaw, M. H. 2016. Evaluation of secondary metabolites in callus and tissues of *Physalis peruviana*. International Journal of Modern Botany, 1, 10-17. DOI: 10.5923/j.ijmb.20160601.03

Levine, R. L., Garland, D., Oliver, C. N., Amici, A., Climent, I., Lenz, A. G., ... and Stadtman, E. R. 1990. [49] Determination of carbonyl content in oxidatively modified proteins. In Methods in enzymology (Vol. 186, pp. 464-478). Academic Press. https://doi.org/10.1016/0076-6879(90)86141-H

Liu, Z., Zhou, T., Ziegler, A. C., Dimitrion, P., and Zuo, L. 2017. Oxidative stress in neurodegenerative diseases: from molecular mechanisms to clinical applications. Oxidative medicine and cellular longevity, *2017*. https://doi.org/10.1155/2017/2525967

Murussi, C., Horn, R. C., Santi, A., Clasen, B. E., Reis, G., Souza, D., ... and Loro, V. L. 2014. Changes in oxidative markers, endogenous antioxidants and activity of the enzyme acetylcholinesterase in farmers exposed to agricultural pesticides-a pilot study. Ciência Rural, 44(7), 1186-1193. http://dx.doi.org/10.1590/0103-8478cr20130516

Nwani, C. D., Lakra, W. S., Nagpure, N. S., Kumar, R., Kushwaha, B., and Srivastava, S. K. 2010. Toxicity of the herbicide atrazine: effects on lipid peroxidation and activities of antioxidant enzymes in the freshwater fish Channa punctatus (Bloch). International journal of environmental research and public health, 7(8), 3298-3312. https://doi.org/10.3390/ijerph7083298

Oga, S., Camargo, M. M. A., and Batistuzzo, J.A. 2014. Fundamentos de Toxicologia. 4.ed. São Paulo: Atheneu Editora, 704p.

Peluso, I., Manafikhi, H., Reggi, R., Longhitano, Y., Zanza, C., and Palmery M. 2016. Relationship between the peroxidation of leukocytes index ratio and the improvement of postprandial metabolic stress by a functional food. Oxidative Medicine and Cellular Longevity. 2016:10. doi: 10.1155/2016/5630985.5630985

Petiz, L. L., Kunzler, A., Bortolin, R. C., Gasparotto, J., Matté, C., Moreira, J. C. F., and Gelain, D. P. 2017. Role of vitamin A oral supplementation on oxidative stress and inflammatory response in the liver of trained

rats. Applied Physiology, Nutrition, and Metabolism, *42*(11), 1192-1200. https://doi.org/10.1139/apnm-2017-0193

Picchi, M.G. 2010. Efeitos do ácido ascórbico em biomarcadores de estresse oxidativo em nadadores de alto rendimento de Ribeirão Preto/SP. (Unpublished doctoral dissertation). Universidade de São Paulo, Brasil.

Rathi, V., Rathi, J. C., Patel, K., Kanojia, S. S. S., and Tamizharasi, S. 2017. A comprehensive review of *physalis angulata*. World Journal of Pharmaceutical Research SJIF. Impact Factor 7.523 Volume 6, Issue 4, 1503-1512. Review Article ISSN 2277–7105.

Rocha, W. S., Lopes, R. M., Silva, D. D., Vieira, R. F., Silva, J. D., and Agostini-Costa, T. D. S. 2011. Compostos fenólicos totais e taninos condensados em frutas nativas do cerrado. *Revista Brasileira de Fruticultura*, *33*(4), 1215-1221. DOI: http://dx.doi.org/10.1590/S0100-29452011000400021.

Roe, J. H. 1954. Chemical determination of ascorbic, dehydroascorbic, and diketogulonic acids. Methods of biochemical analysis, 1, 118-126. https://doi.org/10.1093/jnci/11.2.313

Stocks, J., and Dormandy, T. L. 1971. The autoxidation of human red cell lipids induced by hydrogen peroxide. British journal of haematology, *20*(1), 95-111. DOI: https://doi.org/10.1111/j.1365-2141.1971.tb00790.x

Thapa, A., and Carroll, N. 2017. Dietary modulation of oxidative stress in Alzheimer's disease. International journal of molecular sciences, 18(7), 1583. https://doi.org/10.3390/ijms18071583

Tureck, C., Locateli, G., Corrêa, V. G., and Koehnlein, E. A. 2017. Avaliação da ingestão de nutrientes antioxidantes pela população brasileira e sua relação com o estado nutricional. Revista Brasileira de Epidemiologia, 20, 30-42. https://doi.org/10.1590/1980-5497201700010003

Vaos, G., and Zavras, N. 2017. Antioxidants in experimental ischemia-reperfusion injury of the testis: Where are we heading towards? World journal of methodology, 7(2), 37. doi: 10.5662/wjm. v7.i2.37

Vargas, P. N., Hoelzel, S. C., and Rosa, C. D. 2008. Determinação do teor de polifenóis totais e atividade antioxidante em sucos de uva comerciais. Alimentos e Nutrição Araraquara, 19(1), 11-15. DOI: https://www.researchgate.net/publication/49599779

Zhou, L., Wen, J., Huang, Z., Nice, E. C., Huang, C., Zhang, H., and Li, Q. 2017. Redox proteomics screening cellular factors associated with oxidative stress in hepatocarcinogenesis. PROTEOMICS–Clinical Applications, 11(3-4), 1600089. https://doi.org/10.1002/prca.201600089