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Original Article

Free-radical Scavenging properties and Cytotoxic Activity Evaluation of Latex C-serum from *Hevea brasiliensis* RRIM 600

Leandra Ernst Kerche-Silva[1], Dalita Gomes Silva Morais Cavalcante[1], Caroline Silva Danna[1], Andressa Silva Gomes[1], Iriana Moratto Carrara[2], Alessandra Lourenço Cecchini[2], Eidi Yoshihara[3], Aldo Eloizo Job[1*]

[1]Departamento de Física, Química e Biologia, Faculdade de Ciências e Tecnologia/UNESP, Presidente Prudente, SP, 19060-080, BRAZIL.
[2]Departamento de Patologia Geral, Centro de Ciências Biológicas, Universidade Estadual de Londrina, Londrina, PR, 86.057-970, BRAZIL.
[3]Agência Paulista de Tecnologia dos Agronegócios (APTA), Presidente Prudente, SP, 19015-970, BRAZIL.

ABSTRACT

Background: There are few studies exploring the antioxidant properties of the latex C-serum of the *Hevea brasiliensis* latex and their possible use for therapeutic application. **Objective:** This study investigated the *in vitro* antioxidant activity of the latex C-serum of *H. brasiliensis* and assessed the cytotoxicity of this fraction in two human cell lines derived from normal tissue (MRC-5 and CCD 1059sk. Chemical characterization was also assessed. **Methods:** Latex from *H. brasiliensis* was centrifuged and the C-serum phase was tested for *in vitro* free radical scavenging assays, such as hydroxyl and nitric oxide radicals, hydrogen peroxide and total antioxidant capacity by the phosphomolibdate method. Latex C-serum was also investigated for cytotoxic properties by the MTT test. Latex C-serum was chemical characterized by Infrared Spectroscopy Fourier Transform (FTIR). **Results:** Latex C-serum effectively scavenged hydroxyl and nitric oxide radicals and hydrogen peroxide and presented potent antioxidant activity. The results were compared to ascorbic acid, a standard antioxidant. The cytotoxicity evaluation of latex C-serum demonstrated cell line dependency and for CCD 1059sk cells derived from skin fibroblasts, latex C-serum was not cytotoxic. FTIR showed the presence of proteins in latex C-serum. **Conclusion:** Latex C-serum from *Hevea brasiliensis* presented strong antioxidant potential and these results openthe possibility of further studies aiming the use of latex C-serum as treatment for skin diseases related to reactive oxygenspecies or skin preventive pharmacological formulations.

Key words: Latex C-serum, Antioxidant, *Hevea brasiliensis*, FTIR, Cell viability.

Correspondence :

Aldo E. Job,
Departamento de Física,
Química e Biologia, Faculdade de Ciências e Tecnologia/UNESP,
Presidente Prudente, SP, 19060-080, Brazil.
Mobile no: +55-18-32295750

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INTRODUCTION

It is generally known that oxidative stress can be linked with aging and several diseases. Lately, scientific research has been looking for new compounds with antioxidant effects which can be used as pharmaceuticals or cosmetic ingredients. Exogenous antioxidants can avoid or attenuate the organic damage caused by the overproduction of reactive species.[1] Antioxidants obtained from parts of plants and also from its derivatives such as latex have been increasingly studied, once they can be used as an alternative in preventing or fighting diseases related to oxidative damages.[2,3] Besides being an antioxidant, latex from different specieshave been reported about their pharmacological, anticancer, antimicrobial and analgesic activitiesand the antioxidant properties of *in natura* latex from *Hevea brasiliensis* have been described in scientific literature.[4,5]

The latex from *H. brasiliensis* RRIM 600 is the raw material most commonly used for natural rubber production around the world. Natural rubber is used in a bunch of industrial applications and manufactured products, like tires and gloves, but its presumable biological properties are not explored as it should be.[6] Latex and its constituents have been specifically implicated in multiple biological propertiesand it is known to stimulate angiogenic activity, cell adhesion, extracellular matrix formation, and to improve wound healing in skin tissue.[7,8] Ferreira *et al.* (2009),[9] showed that the non-rubber constituents of *H. brasiliensis* latex are the bioactive fraction responsible for angiogenic properties of this material.Latexconstituents can be separated in three parts when centrifuged at high speed, consisting of the rubber cream, centrifuged serum (C-serum) and the bottom fraction (B-serum).[7,9] The B-serum contains vacuole-like organelles known as lutoids bodies. C-serum is the phase that contains the soluble substances found in plant cells cytoplasm such as proteins, amino acids, carbohydrates, organic acids, inorganic salts and nucleotidic materials.[10]

Only limited research has been performed to elucidate medicinal properties of *H. brasiliensis* latex C-serum. Ong *et al.*(2009)[11] made the first suggestion of therapeutic application when a subfraction of latex B and C-sera showed to exert specific anti-proliferative properties against cell line of malignant origin. The second suggestion was made by Lam *et al.*(2012),[12] when latex C-serum dialysed and pre-heated sub-fractions leaded specifically hepatocellular carcinoma cells (HepG2) to death in a non-apoptotic way, not altering the proliferation of normal cells. Daruliza *et al.*(2011)[13] also discovered that *H. brasiliensis* latex C-serum can be used as fungicide and could contribute with pharmaceutical and health departments.

Some few *in vitro* studies reported that *H. brasiliensis* latex C-serumhas antioxidant properties and this factopens new perspectives about possible biomedical applications.[14,15] One of the possibilities could be the use of *H. brasiliensis* latex C-serumas adjuvantin antioxidant therapies. Another possibility could be its use in preventive medicine and esthetics. However, forlatex C-serumto be used as treatment or preventive drug, several pre-clinical and clinical trials are needed in order to elucidate its safety for humans. The very first stage to evaluate a new therapeutic product is to perform an *in vitro* characterization. The results of these *in vitro* trials are used to assess the possibility of this material to be consid-

ered a candidate for *in vivo* characterization, so the systemic toxicity of this new therapeutic product could be evaluated.[16]

Thus, the aim of this work was to assess *in vitro* antioxidant activity of *H. brasiliensis* latex C-serum as reactive oxygenspecies scavenger and to evaluate its cytotoxicity intwo human cell lines derived from **normal-tissue**. Two cell lines derived from normal tissue is justified by the attempt to recreate normal biological conditions, since for biomedical applications it is required that the compound tested does not cause any deleterious effects on healthy cells. The results found in this study is very important as initial screening evaluation for the use of *H. brasiliensis* latex C-serum as an antioxidant product for medical purposes.

MATERIALS AND METHODS

General Experimental Procedures

Compounds used in the assays was purchased from Sigma-Aldrich Chemical (São Paulo, Brazil). Absorbance of scavenging *in vitro* activity assay was measured using a spectrometer Quimis - Q798DP Model. Absorbances of MTT assay was measured using an Enzyme-Linked Immunoabsorbent Assay (ELISA) Reader (Readwell Touch, Robonik, India). Spectra was obtained on a FTIR spectrometer (Bruker FTIR Vector 22 model) in the wavelengths region of 4000-600 cm^{-1}, with 2 cm^{-1} resolution.

Latex collection and preparation of C-serum subfractions

Latex was collected from field-grown RRIM 600 trees at Indiana Farm, in the state of São Paulo, Brazil. To prepare latex C-serum, fresh latex was fractionated by centrifugation at 17968 g for 30 min. At this speed, latex separates into three distinct parts. Using a syringe, the clear aqueous phase C-serum (Figure 1) was removed and separated in a *falcon* tube to be used in the experiments. C-serum was then freezed at -4ºC so the remaining rubber particles could precipitate and be removed later. For the experiments of antioxidant activity and cytotoxicity evaluation, latex C-serum was primarily filtered in a 14 µm pore membrane, after in a 0.22 µm pore membrane, and diluted in Phosphate Buffer Saline (PBS) to a final concentration of 200 µg/mL. From that concentration, ten working concentrations raging from 0.02 to 200 µg/mL (0.02, 0.2, 1, 2, 5, 10, 20, 50, 100 and 200 µg/mL) was prepared. All concentrations had their pH corrected to 7.4.

In vitro antioxidant assays

Hydroxyl ($HO^{\bullet}$) radical scavenging of latex C-serum

$HO^{\bullet}$ scavenging activity was measured according to Liu *et al*. (2010).[17] $HO^{\bullet}$ radicals was generated from $FeSO_4$ and H_2O_2, and detected by their ability to hydroxylate salicylate. The reaction mixture (2 mL) contained 0.5 mL $FeSO_4$ (1.5 mM), 0.35 mL H_2O_2 (6 mM), 0.15 mL sodium salicylate (20 mM), and 1 mL of ten different concentrations of latex C-serum. L-ascorbic acid (1000 µg/mL) was used as the positive control. After incubation for 1 hour at 37ºC, the absorbance of the hydroxylated salicylate complex was measured spectrophotometrically at 562 nm. The percentage scavenging effect was calculated as:

$$\%HO^{\bullet}scavenged = [1 - \frac{(A_1 - A_2)}{A_0}]x100$$

where A_1 was the absorbance of the sample or ascorbic acid, A_0 was the absorbance of the control, and A_2 was the absorbance of the reagent blank without sodium salicylate.

H_2O_2 scavenging of latex C-serum

The H_2O_2 capacity was measured according to Liu *et al.* (2010).[17] The reaction mixture contained 1.0 mL of 0.1 mM H_2O_2 (freshly made), 1.0 mL of ten concentrations of latex C-serum, 0.10 mL of ammonium molybdate (3%, w/w), 10 mL of 2 M H_2SO_4, and 7 mL of 1.8 M KI. The mixture was titrated against 5 mM $Na_2S_2O_3$ until the color disappeared. The scavenging activity was calculated as:

$$H_2O_2scavenged = \frac{(V_0 - V_1)}{V_0}x100,$$

where V_0 was the volume of $Na_2S_2O_3$ solution used to titrate the control mixture and V_1 was the volume titrated of the mixture containing the samples. L-ascorbic acid (1000 µg/mL) was used as the positive control.

Nitric Oxide ($NO^{\bullet}$) radical scavenging of latex C-serum

Nitric oxide generated from sodium nitroprusside was measured using Griess reagent by the method of Marcocci *et al*. (1994).[18] Ten concentrations of the latex C-serum extract and sodium nitroprusside (5 mM) in phosphate buffer saline (PBS) in a final volume of 3 mL was incubated at 25ºC for 150 min. After incubation, sample (0.5 mL) was emoved and diluted with 0.5 mL Griess reagent (1% sulfanilamide, 2% *o*-phosphoric acid and 0.1% naphthyl ethylenediamine). The absorbance of the chromophore formed was measured at 546 nm and the inhibition of $NO^{\bullet}$ generation was estimated by comparing the absorbance values to that of the control without the latex C-serum concentrations. The scavenging activity was calculated as:

$$NO^{\bullet}scavenged = \left[1 - \frac{A_1}{A_2}\right]x100,$$

where A_0 was the absorbance of the control, and A_1 was the absorbance of the latex C-serum or ascorbic acid (1000 µg/mL) after the reaction.

Total in vitro antioxidant capacity of latex C-serum

Total antioxidant capacity was determined according to Pietro *et al*. (1999).[19] The concentrations of latex C-serum was added in an Eppendorf tube and mixed with 1 mL of a reagent solution containing sulfuric acid (0.6 M), sodium phosphate (28 mM), and ammonium molybdate (4 mM). Tube was capped and incubated at 95ºC for 90 min. Absorbance was measured at 695 nm after the samples had cooled to room temperature. Ascorbic acid (1000 µg/mL) was used as positive control. The scavenging activity was calculated as:

$$Total\ antioxidant\ capacity = \left[1 - \frac{A_1}{A_0}\right]x100$$

where A_1 was the absorbance of the sample in the presence of scavenger and A_0 was the absorbance of the control.

Cytotoxicity evaluation

Cell culture and Exposure Protocol

Cell lines MRC-5–human fetal lung fibroblasts, and CCD 1059sk–human normal skin fibroblasts, was purchased from the Rio de Janeiro Cell Bank(Rio de Janeiro, Brazil). Cells were cultured in Dulbecco's modified Eagle's medium (DMEM) with Ham's F10 nutrient mixtures (Sigma-Aldrich–Saint Louis, MO, USA), supplemented with foetal calf serum (10% v/v), penicillin (100 units/mL) and streptomycin (100 µg/mL) (Sigma). Culture was maintained at 37ºC in a water-saturated atmosphere containing 5% CO_2. For the exposure, the cells (10.000/well in 100 µL cell

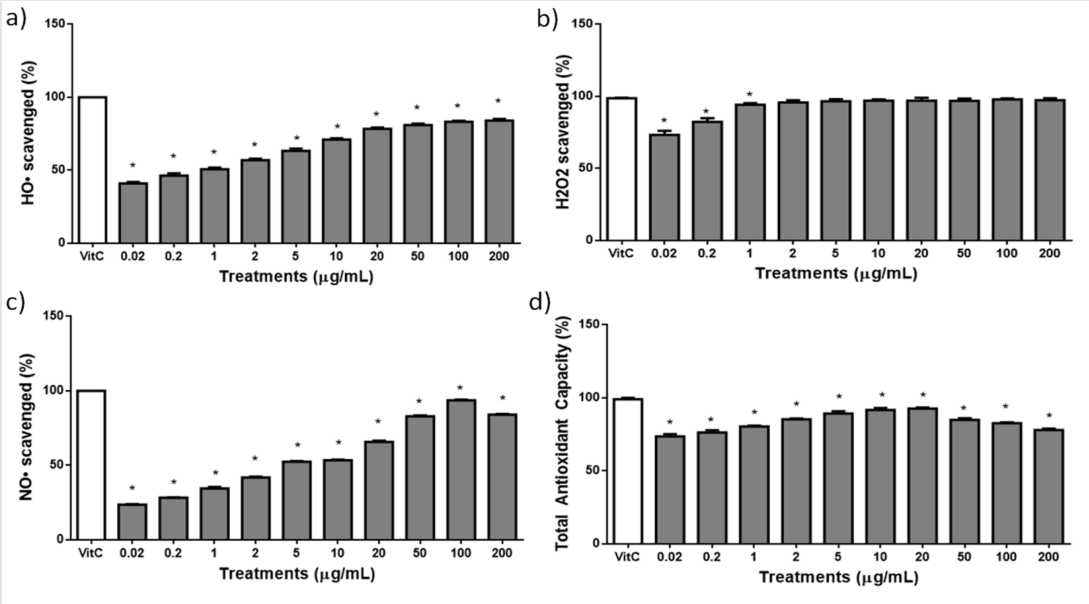

Figure 1: a) Hydroxyl radical scavenging activities of latex C-serum. b) Hydrogen peroxide scavenging activities of latex C-serum. c) Nitric Oxide scavenging activities of latex C-serum. d) Total antioxidant capacity of latex C-serum. The absorbance values were converted to scavenging effects (%) and data plotted as means of triplicate scavenging effects (%) ± SD.

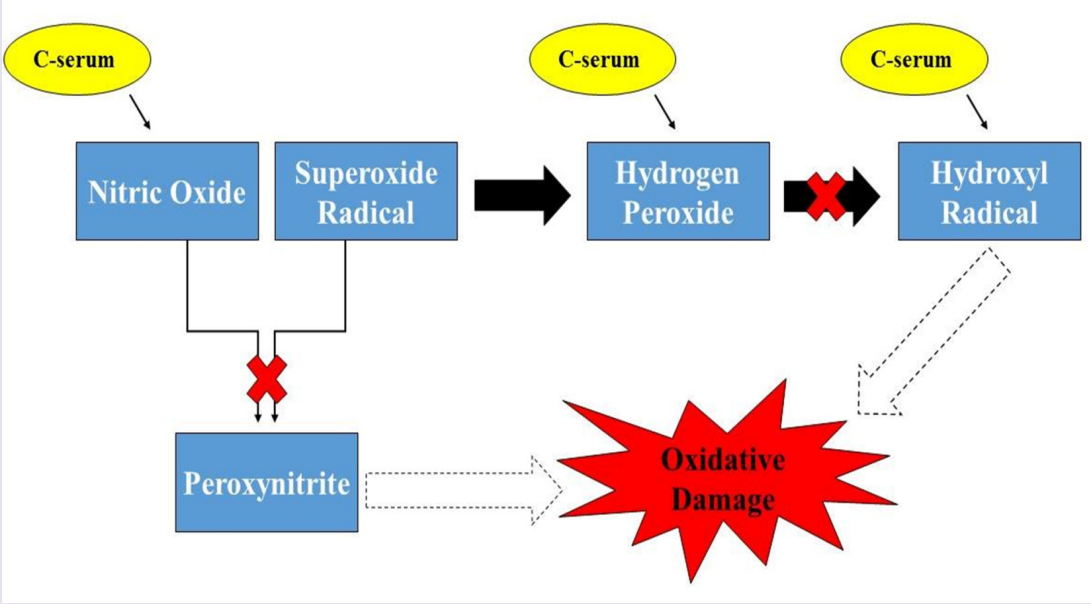

Figure 2: Scheme shows that *H. brasiliensis* latex C-serum possesses ability to scavenge species in a direct or indirect form.

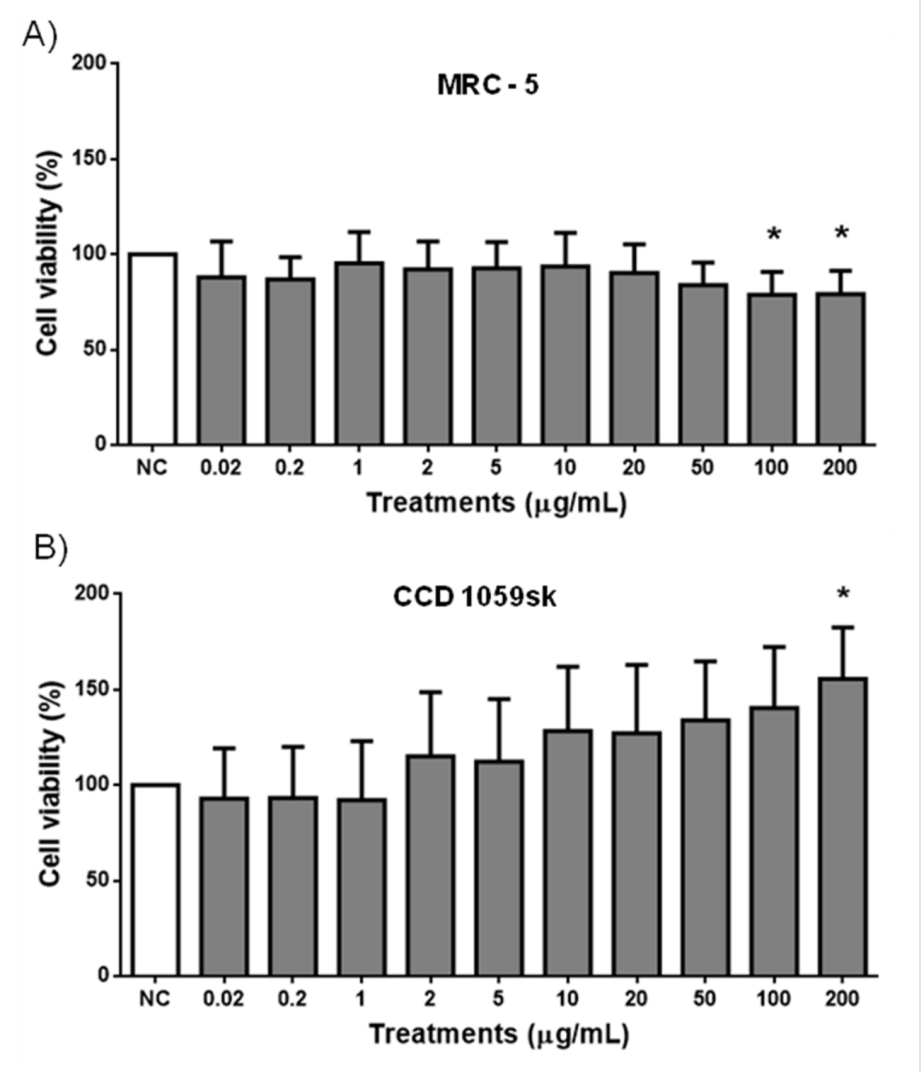

Figure 3: Impact of treatment of latex C-serum on the viability of MRC-5 cells (A) and CCD 1059sk cells (B). The cultures were exposed to the compound for the period of 24 h. Bars represent means ± SD of results. *Indicate statistical difference between control group and treated cell groups ($p \leq 0.05$).

culture medium for each cell line) was seeded in a 96-well micro plate and allowed to adhere to the well walls for 24 h at 37ºC in a water-saturated atmosphere containing 5% CO_2. The cellswasexposed to ten different concentrations of latex C-serum and kept again in the incubator at 37 ºC for 24 h. PBS was used as negative control (NC).

Cell viability assay

The MTT assay measures cell viability by an *in vitro* procedure that indicates changes in metabolic events of living cells. After exposure time (24 h), the medium containing latex C-serum was aspirated and discarded, and to each well was added 100 µL DMEM/Ham-F-10 culture medium containing 0.5 mg/mL MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; Sigma-Aldrich, USA) in phosphate buffered saline (PBS), and the plates re-incubated at 37ºC for a further 4 h. The reaction mixture was then carefully removed, and 200 µL of DMSO added to each well and mixed thoroughly. After 10 min, the absorbance at 492 nm was measured using a microplate reader. Results of the experiments are expressed as percentage of viable cells in relation to the control group (NC).

Qualitative chemical characterization of latex C-serum using FTIR spectroscopy

To investigate the molecular chemical structure of latex C-serum, Fourier Transform Infrared (FTIR) technique was used. For further comparison, *in nature* latexand rubber cream fraction samples was also analyzed.

Statistical analysis

The resultswereshown as mean ± SD. All tests were statistically significant at the 95% confidence level and comparisons between multiple groups were performed using One-Way ANOVA with Dunnet in GraphPad Prima® 6.01.

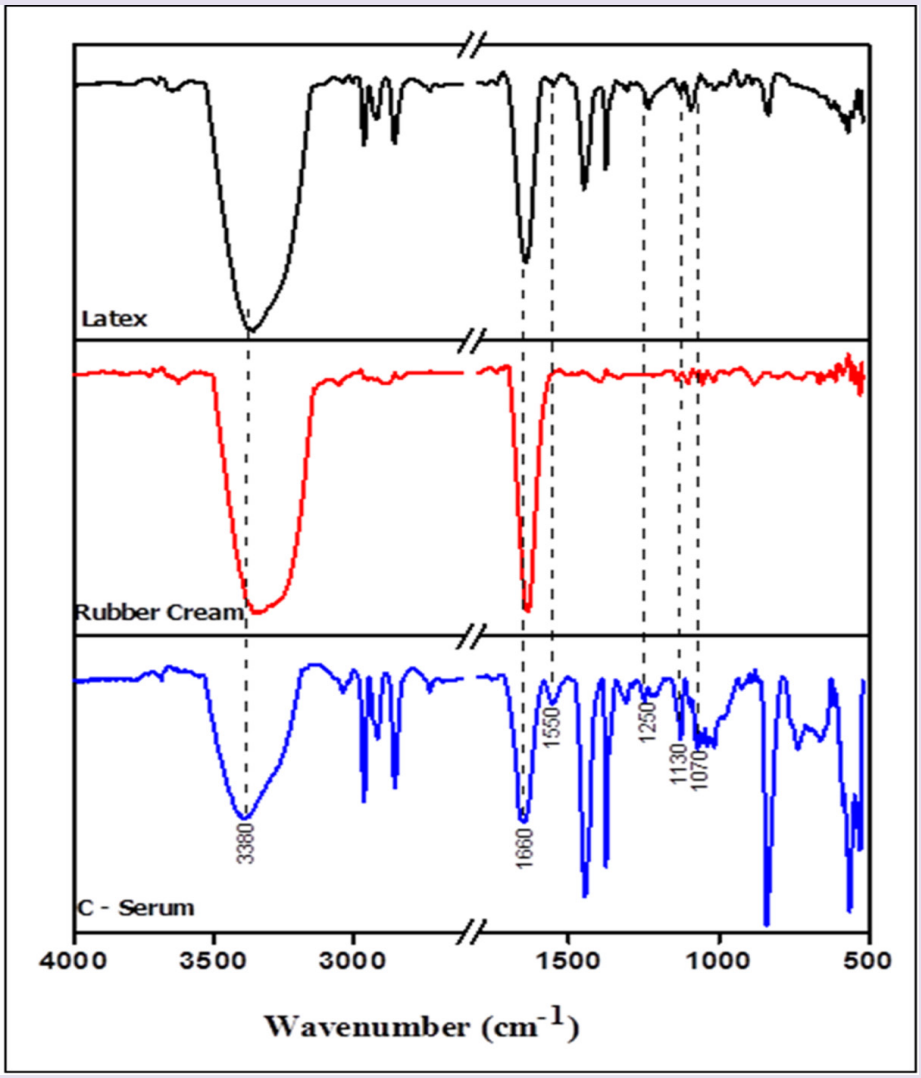

Figure 4: Absorbance spectra of latex, rubber cream and C-serum phase determined by Fourier Transform Infrared (FTIR).

RESULTS

In vitro antioxidant assays

Hydroxyl ($HO^{\cdot}$) radical scavenging of latex C-serum

The scavenging of hydroxyl radicals by latex C-serum from *H. brasiliensis* was increased in a dose-dependent manner as shown in Figure 1a. At concentration of 200 µg/mL latex C-serum reached the maximum scavenging effect, corresponding to 84.17% of the ascorbic acid scavenging ability.

H_2O_2 scavenging of latex C-serum

Latex C-serum from *H. brasiliensis* capacity to scavenge H_2O_2 peroxide is shown in Figure 1b. Concentrations ranging from 1 to 200 µg/mL showed ability to scavenge hydrogen peroxide similar to ascorbic acid (95% to 97%).

Nitric Oxide ($NO^{\cdot}$) radical scavenging of latex C-serum

The scavenging of nitric oxide radicals by latex C-serum from *H. brasiliensis* was also increased in a dose-dependent manner, reaching its peak in 100 µg/mL, as shown in Figure 1c. At the concentration of 100 µg/mL, the capacity of latex C-serum to scavenge nitric oxide radicals corresponded to 93.71% of the ascorbic acid scavenging ability.

Total in vitro antioxidant capacity of latex C-serum

Total antioxidant capacity of latex C-serum from *H. brasiliensis* was assessed by measuring the ability of latex C-serum to reduce molibdate ions, and the results are shown in Figure 1d. All concentrations tested of latex C-serum, ranging from 0.02 to 200 µg/mL, was able to reduce molibdate ions, and its maximum reduction capacity was reached at the concentration of 20 µg/mL (92.67% of the ascorbic acid capacity).

Cytotoxicity evaluation

Cell viability assay

Cytotoxicity evaluation was assessed by the MTT test and the results are shown in Figure 3. MRC-5 cell line that were exposed to latex C-serum up to the concentration of 50 µg/mL showed no reduction of the cell viability, when compared to the control group. MRC-5 cells that were exposed to the concentration of 100 µg/mL and 200 µg/mL presented

reduction in cell viability ~20%, when compared to control group (Figure 3a).

CCD 1059sk cell line showed no cell viability reduction in any of the concentrations tested. To this cell line, an increase in the cell viability ~50% was noticed in the concentration of 200 µg/mL, when compared to the control group (Figure 3b).

Qualitative chemical characterization of latex C-serum using FTIR spectroscopy

Figure 4 shows absorption bands and peaks that are related to proteins in the latex C-serum spectra. Secondary amide can be associated to 1550 cm^{-1} region, tertiary amide can be associated to 1250 cm^{-1} band and primary amide can be associated to 1070 cm^{-1} band. *In natura* latex shows bands and peaks related to proteins that are not found in the rubber cream phase.

DISCUSSION

Moses Gomberg was the first to describe free radicals more than a century ago.[20] In a great amount of time it was believed that free radicals were not present in biological systems[21] and only in the 1950th they were discovered in the cells[22] and it was supposed that they were involved in pathological process[23] and aging.[24] After proving the existence of reactive oxygen species (ROS) in living organisms, the focus of researchers became the identification of the mechanisms of generation.[21] 10% of the oxygen consumed is not reduced to water by cytochrome oxidase in mitochondria[22] and results in superoxide anion radical ($O_2^{\cdot}$) followed by one-electron reduction with concomitant acceptance of two protons generating hydrogen peroxide (H_2O_2).[21] H_2O_2 molecule results in the formation of hydroxyl radical ($HO^{\cdot}$) that are able to provoke lesions in diverse parts of the cells.

Since ROS generation was related to different types of diseases, including cancer, antioxidants substances became the focus of diverse researches worldwide. Different types of plants and its derivatives have been studied for antioxidant properties and have indicated free radical scavenging activity.[26,27] But only few investigations have been done to elucidate the antioxidant capacity of *H. brasiliensis* latex, and even less studies tried to accomplish the antioxidant activity of the C-serum phase.[4,28] Amnuaikit and Boonme (2014)[14] also evaluated C-serum latex from *H. brasiliensis* through the reduction method of DPPH and found antioxidant activity. Siriwong *et al*. (2015)[15] detected phenolic compounds in the non-rubber fraction of *H. brasiliensis* latex and observed that it exerts antioxidant activity through FRAP and DPPH reduction assays.

The results found in this study show that different concentrations of latex C-serum from *H. brasiliensis* present scavenging properties against hydroxyl and nitric oxide radicals and hydrogen peroxide (Figure 1) and the scavenging ability of latex C-serum was compared to a standard non-enzymatic antioxidant compound, ascorbic acid. Hydroxyl and nitric oxide radicals were scavenged in a dose-dependent manner (Figure 1a and 1c, respectively) and the scavenging capacity was very similar to ascorbic acid. Latex C-serum was also very effective in scavenging hydrogen peroxide presenting 97% of the scavenger capacity of ascorbic acid (Figure 1b). Total antioxidant capacity evaluation of latex C-serum was performed by phosphomolybdate method, and the results show that latex C-serum was able to reduce Mo^{6+} to Mo^{5+} at all concentrations tested (Figure 1d).

Among reactive species, hydroxyl radical is the most deleterious to all organisms inducing DNA, RNA, proteins, lipids, nuclear and mitochondrial cell membranes damages. Hydrogen peroxide is less reactive to the organic molecules in the absence of transition metals, however when in oxidative stress it can easily overpass the cell membranes and generate hydroxyl radicals.[29] In the other hand, nitric oxide radical can react to anion superoxide radical creating peroxynitrite, and this one can interact to lipids, DNA and proteins causing oxidative injuries.[30] Therefore, the results presented in this work show that *H. brasiliensis* latex C-serum has the ability to scavenge species highly reactive and potentially deleterious to the biological system in a direct (hydroxyl radical and nitric oxide) or indirect (hydroxyl radical through hydrogen peroxide) form (Figure 2).

Since different concentrations of latex C-serum presented antioxidant properties, it is very important for therapeutic purposes to establish the potential toxicity of *H. brasiliensis* latex C-serum. Only a few studies in the literature evaluated the *in vitro* cytotoxicity of latex C-serum of *H. brasiliensis*.[11,12] Nogueira *et al*. (2011)[31] recommend that different cell lines from different origins must be used to screen the toxicological effects of new products with potential biomedical applications. For initial screening of latex C-serum potential toxicity, two human cell lines were chosen: MRC-5, derived from lung fibroblasts, and CCD 1059sk, derived from skin fibroblasts.MRC-5 cell line that were exposed to latex C-serum up to the concentration of 50 µg/mL showed no reduction of the cell viability, when compared to the control group. However, the cells exposed to the concentration of 100 µg/mL and 200 µg/mL presented a significant reduction in cell viability around 20%, when compared to non-exposed to C-serum cells (Figure 3A). CCD 1059sk cell line showed no cell viability reduction in any of the tested concentrations. To this cell line, it was noticed an increase in the cell viability around 50% in the concentration of 200 µg/mL, when compared to the control group (Figure 3B). It is very important to highlight that this increase in the cell viability may not be related to an increase in the cell proliferation, since the measure of the cell viability by the MTT test is an indirect quantification of the cell metabolic activity.[32] Therefore, the compounds existing on latex C-serum could change these cells metabolism anyhow, forcing them to reduce the tetrazolium salt into formazan compounds in a better way and so leading to a raise in the intensity of the bluish color, resulting in an increase in the absorbance reading during the experiments. MTT test results analysis permit us to conclude that MRC-5 cells are more sensitive to latex C-serum than CCD 1059sk cells, and perhaps its cytotoxicity depends on the cell line tested. Specific responses related to the cell line tested are described in the literature with different materials.[31] It is possible to infer that for CCD 1059sk cells derived from skin fibroblasts, latex C-serum was not cytotoxic at the concentrations tested. Although more studies of pre-clinical and clinical toxicity are needed, this results can be an indicative of possible therapeutic application of latex C-serum as a treatment for skin diseases related to reactive species or even preventive skin pharmacological formulations.[33]

Since little is known about the constitution of latex C-serum from *H. brasiliensis*, chemical characterization is important to understand the biological properties of this compound.FTIR spectroscopy is one of the oldest and useful tools for the determination of secondary structure of proteins.[34] A great number of studies inferring the vibrational spectra of *in natura* latex and the rubber fraction can be found in the literature and a various numbers of vibrational groups were found in this material by the use of FTIR spectroscopy.[35] In the other hand, for latex C-serum only a few studies were performed in order to analyze the vibrational spectra of this material by FTIR spectroscopy. Ferreira *et al*. (2009)[9] used FTIR spectroscopy to analyze *H. brasiliensis* latex C-serum and they identified absorption bands that can be associated to secondary structure of proteins, such as primary and secondary amide. In the present study, absorption bands and peaks related to proteins were found in the latex C-serum spectra (Figure 4). For example, 1550 cm^{-1} region shows a band that can be associated to secondary amide, 1250 cm^{-1} shows a band that

can be associated to tertiary amide, and 1070 cm^{-1} shows a band associated to primary amide.[36,37] As expected, absorption bands and peaks related to proteins found in *in natura* latex are absent in the rubber cream phase, since during the ultracentrifugation process proteins tend to migrate to C and B-sera fractions. However, remnant proteins can be found in the rubber cream phase since 3380 cm^{-1} region shows a band related to the overlap of OH and N-H groups descendant from amine group.[36,37] Remnant rubber can also be found in latex C-serum since 1660 cm^{-1} region shows a band related to C=C stretching which is a vibrational mode characteristic from rubber main chain.[36] With these results it is possible to ensure that *H. brasiliensis* latex C-serum obtained through ultracentrifugation process is composed by protein parts with minimum amount of rubber. And although this study only identified proteins in C-serum phase, others constituents such as carbohydrates, organic acids, and nucleic acids can also be found in the composition of C serum phase of *H. brasiliensis* latex.[10]

The proteins constituents found in FTIR investigation could be responsible for the antioxidant properties of latex C-serum found in this work. Havanapan *et al*. (2015)[38] investigated the protein contents of latex serum from *H. brasiliensis* by proteomic analysis and beta-1,3-glucanase, chitinase and lectin were isolated. These proteins are associated with plant defenses against microbes such as fungus. Sunderasan *et al*. (2015)[39] analyzed proteins from latex C-serum supernatant that could elicit anti-proliferative activity on human cancer cells and a number of protease inhibitors were found. Protease inhibitors present beneficial effects that have been attributed to their multiple regulatory activities that can suppress carcinogenesis. Malate dehydrogenase was also found in considerable amounts, and this protein is an essential enzyme in the tricarboxylic acid cycle (TCA) that also plays a role as antioxidant due to their actions of scavenging H_2O_2 and reducing injury and toxicity in the cells.[40]

CONCLUSION

Based on the results presented in this investigation, it can be concluded that *H. brasiliensis* latex C-serum is a solution rich in proteins with high reactive species scavenger ability and total antioxidant capacity similar to ascorbic acid in almost all the concentrations tested. Furthermore, the cytotoxicity of latex C-serum appears to be cell line dependent and concentrations up to 50 µg/mL and up to 200 µg/mL can be used without interference in the cell viability of MRC-5 cells e CCD 1059 sk cells, respectively. These results also show that *H. brasiliensis* latex C-serum is a candidate for *in vivo* investigations, which might suggest its therapeutic applications in diseases related to oxidative stress.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

ABBREVIATION USED

MTT test: (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) test; **FTIR:** Fourier Transform Infrared; **PBS:** Phosphate Buffer Saline; **H_2O_2:** Hydrogen peroxide; **HO•:** Hydroxyl radical; **NO•:** Nitric Oxide radical; **MRC-5:** Human fetal lung fibroblasts; **CCD 1059sk:** Human normal skin fibroblasts; **ROS:** Reactive oxygen species.

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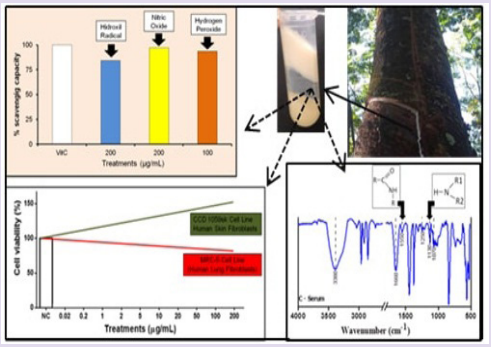
PICTORIAL ABSTRACT



SUMMARY

- Latex C-serum from *Hevea brasiliensis* extensively scavenged free radicals in different assays such as hydroxyl and nitric oxide radical scavenger, and hydrogen peroxide scavenger. Latex C-serum also presented high total antioxidant capacity in different concentrations.
- Latex C-serum also didn't show cytotoxic effects in two different human cell lines.
- FTIR spectroscopy showed high number of proteins in the composition of Latex C-serum of *H. brasiliensis*.

ABOUT AUTHORS

Aldo Eloizo Job: Has completed his graduation in Physics in Universidade Federal do Mato Grosso, Cuiabà, Mato Grosso, Brazil and obtained his Phd degree in Material Science and Engineering in Universidade de Sào Paulo, Sào Carlos, Sào Paulo, Brazil. Currently he is working Associated Professor in the Department of Physics, Chemistry and Biology in Universidade Estadual Paulista "Júlio de Mesquita Filho" in Presidente Prudente, Sào Paulo, Brazil, publishing extensively in the field of Natural Rubber and Biopolymers.

Leandra Ernst Kerche-Silva: Has Completed her graduation in Biological Sciences in Universidade Estadual de Londrina, in Londrina, Paranà, Brazil. Obtained her Master Degree in Biotechnology in Universidade Estadual de Londrina. She is finalizing her Phd degree in Materials Science with focus on Biomaterials in Universidade Estadual Paulista "Júlio de Mesquita Filho" in Presidente Prudente, Sào Paulo, Brazil. Currently she is working as a Professor in the Medical School of Universidade do Oeste Paulista, in Presidente Prudente, Sào Paulo, Brazil. Her experience relies on Biochemistry and Mutagenesis Research.