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Bioprospecting the Cytotoxic Activity of Patchouli Essential Oil (*Pogostemon cablin* Benth) Against Human Blood Cells

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ABSTRACT

Natural products, particularly those of plant origin, are the main promising candidates for new drugs due to availability, low cost, and fewer side effects, and this logic, highlights the Essential Oils (EOs), which are a complex mixture of volatile compounds and have several relevant biological activities, but there are few studies on the potential beneficial and/or adverse effects, making necessary the investigation of these EOs, especially the evaluation of toxicity. Therefore, the objective of this study was to evaluate the cytotoxic activity of Patchouli essential oil (*Pogostemon cablin* Benth) against human blood cells. This was a laboratory-type experimental study, with an inductive approach and quantitative. To carry out the cytotoxic activity studies, human red blood cells of blood types A, B, and O were used and the hemolytic and anti-hemolytic activity was evaluated. The analyses performed revealed that they showed a low percentage of hemolysis for human erythrocytes of the ABO system at a concentration of 50 to 100 µg/mL. In addition, it showed a moderate anti-hemolytic effect at concentrations of 500 to 1000 µg/mL for blood types B and O. Considering the results obtained in the in vitro cytotoxicity study, Patchouli EOs become a promising candidate for the use of its bioactive properties in herbal medicines. However, auxiliary studies such as *in vivo*toxicity are necessary to better elucidate the mechanisms of action of this compound.

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INTRODUCTION

Natural products, particularly those of plant origin, are the main promising candidates and play an important role in drug development.[1,2] Several ethnobotanical studies have focused on identifying possible therapeutic plants for even more effective management of health problems, demonstrating the importance of medicinal plants in the health care delivery system. [3,4,5,6] Ease of availability, low cost, and fewer side effects make herbal preparations the main actor of all available therapies, especially in rural areas. [7,8]

In this context, we highlight essential oils, which are a complex mixture of volatile compounds obtained by distillation of different parts of aromatic plants, or by the cold expression for citrus EOs.[9] They are complex substances, composed of mixtures of several components containing hundreds of chemicals.[10,11] Some of the most commonly reported properties of EOs are antibacterial[12], antifungal[13], antiviral[14,] and antioxidant.[15] The increasing use of EOs demonstrates that many people seem to consider essential oils as safe alternatives to more invasive pharmacological forms of treatment due to the concept that they are more "natural." However, only a modest amount of research has been conducted on essential oils. This leaves the potential beneficial and/or adverse effects unclear, making research on these oils necessary to verify their true effects on human health.[16]

Among OEs worth mentioning is Patchouli ("Guanghuoxiang") or scientifically known as *Pogostemon cablin* Benth. This oil has unique characteristics, including a long-lasting, woody, camphorous, and earthy odor.[17] It is listed as Generally Regarded As Safe (GRAS) and therefore approved as a natural food additive by the U.S. Food and Drug Administration.[18] Given its multicomponent nature, Patchouli has been found to promote numerous pharmacological activities and has been shown to protect against inflammation, microorganisms, tumors, aging, oxidation, analgesic, antiplatelet, antithrombotic, aphrodisiac, antidepressant, antimutagenic, antiemetic, fibrinolytic, and cytotoxic.[19,20]

Faced with the need to develop new drugs with greater efficacy and safety, toxicity evaluation is indispensable to consider safe treatment. There are several cases of nephropathy with the use of specific Chinese medicinal plants.21 For example, *Aristolochia fangchi*, Chinese herbal medicine used for weight loss, caused nephropathy and morbid cases.[22] On the other hand, since

toxicological information and antidotes of some medicinal herbs are not available, the toxicity of plants deserves more attention.[23]

Toxicity evaluation is initially based on *vitro* toxicity tests. According to the International Standard Organization, ISO 10993, *in vitro* cytotoxicity testing is the first test to assess the biocompatibility of any material for use in biomedical devices. Cytotoxicity assessment is a fundamental biological measuring and screening test applied on tissue cells as an *in vitro* sample to distinguish the rate of cell proliferation, reproduction, as well as the morphological effects of substances.[24] It was a crucial preliminary method needed to develop drugs or biomaterial compounds and predict the initial doses that can treat diseases without affecting normal cells.[25]

Based on this information, it the importance of investigating the toxicological activity of essential oils, especially the cytotoxicity of the essential oil *Pogostemon cablin* Benth.

METHODOLOGY

Test Substance

The product submitted to the biological tests was the substance coded as OE- *Pogostemon cablin* Benth from the Quinarí plant located in Ponta Grossa-Paraná. For the in vitro assays, solutions containing *Pogostemon cablin* Benth essential oil in concentrations of 50, 100, 500, and 1000 µg/mL were used.

Human erythrocytes

Human erythrocytes of blood types A, B, and O were obtained from healthy donors. For this assay, the legal and ethical aspects of research involving humans were taken into consideration. The research ethics committee of the Fundação Francisco Mascarenhas/ FaculdadeIntegrada de Patos, with cep: 58704000, approved this research, protocol N 2.373.249.

Analysis of cytotoxicity in human erythrocytes

Hemolytic activity evaluations

Human blood samples A, B, and O were mixed with 0.9 % NaCl in a 1:30 ratio and centrifuged at 2500 rpm for 5 minutes to obtain the erythrocytes. This procedure was repeated twice more,

and the sediment of the last centrifugation was resuspended in 0.9% NaCl to obtain a 0.5% suspension free of white blood cells and platelets. *Pogostemon cablin* Benth essential oil was added to 2 mL of the RBC suspension at different concentrations (50, 100, 500, and 1000 µg/mL). The negative control (RBC suspension without addition of the products - 0 % of hemolysis) and the positive control (RBC suspension plus 1% Triton X-100 - 100 % of hemolysis) was performed. After that, the samples were incubated for 1 hour at 22 ± 2 ºC under slow and constant agitation. After this time, the samples were centrifuged at 2500 rpm for 5 minutes and the hemolysis was quantified by spectrophotometry at a wavelength of 540 nm.[26]All experiments were performed in triplicate. The results are expressed as a percentage representing the arithmetic mean of three measurements.

Evaluation of anti-hemolytic activity

Evaluation of the osmotic fragility of human erythrocytes was performed with a 0.5% suspension of erythrocytes. Solutions containing different concentrations (50, 100, 500, and 1000 µg/mL) of *Pogostemon cablin* Benth essential oil were incubated in tubes containing 2 mL of an erythrocyte suspension for 1h at 22 ± 2 °C. After this time, the preparations were centrifuged at 2500 rpm for 5 min and the supernatant was discarded.

The red cells were resuspended in 0.24% sodium chloride hypotonic solution and shaken at 100 rpm for 1 h at 22 ± 2 °C. After this period, the samples were centrifuged at 2500 rpm for 5 minutes and hemolysis was quantified by spectrophotometry at a wavelength of 540 nm.[27] The negative control (red cell suspension without the addition of *Pogostemon cablin* Benth essential oil - 0% hemolysis) and the positive control (red cell suspension plus hypotonic solution - 100% hemolysis) were performed. The tests were performed in triplicate. The results are expressed as a percentage representing the arithmetic mean of three measurements.

Statistical Analysis

Experiments were performed in triplicate and results were expressed as percentages representing the arithmetic mean ± standard error of the mean. Data were analyzed by One-way Analysis of Variance (ANOVA) and Bonferroni posthoc test using GraphPad Prism 7.0 software. Differences were considered significant when $p < 0.05$.

RESULT AND DISCUSSION

Natural/synthetic substances with high cytotoxic potential can cause the death of cells or tissues present in the mucosa, generate local and systemic absorption, and/or cause harmful effects on the individual's health.[28]

The toxic effect produced by certain drug candidates may be directly related to high hemolytic capacities, since red blood cells are easily sensitized by certain substances, promoting lysis and releasing hemoglobin.[29]

In vitro cytotoxicity tests, using erythrocytes, are often performed to screen and determine the toxicity of various compounds.[30]This cytotoxic profile assesses the direct interaction of various chemical compounds under erythrocytes. Hemolytic assays are widely used to examine the toxicity of a xenobiotic, primarily to investigate direct effects on cell membrane integrity.[31]

In this study, the *in Vitro* cytotoxicity test was used to evaluate the toxic potential of Patchouli essential oil against human blood cells, since through this analysis possible harmful effects of the essential oil on the cell membrane can be evidenced.

Rangel et al. (1997) classified through erythrocyte hemolysis different levels of cellular cytotoxicity where: the percentage of hemolysis between (0 to 40%) is considered as low, between (40 to 80%) moderate, and above (80%) considered as high.[32]

The results obtained reveal a similarity between the percentage of hemolysis in the different blood types tested, which showed a low percentage of hemolysis < 40% at the tested concentrations of 50µg/mL and 100 µg/mL. However, the 500µg/mL and 1000 µg/mL concentrations showed moderate hemolytic effects with the percentage ranging from 50% to 75% hemolysis, as can be seen in figures 1, 2, and 3.

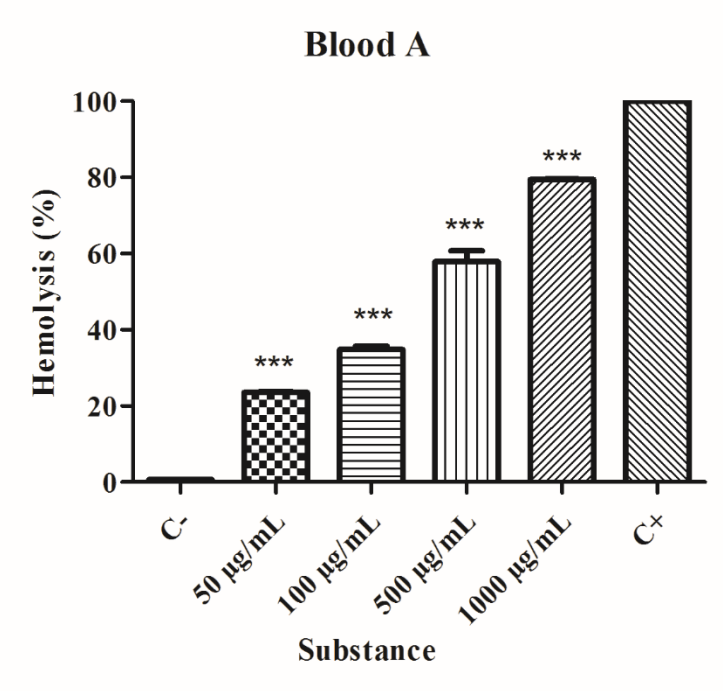

Figure 1. Hemolytic evaluation of Patchouli on type A erythrocytes. (C-) Negative control (0.5% suspension), (C+) Positive control (1% Triton X-100). P < 0.001 (***) versus positive control

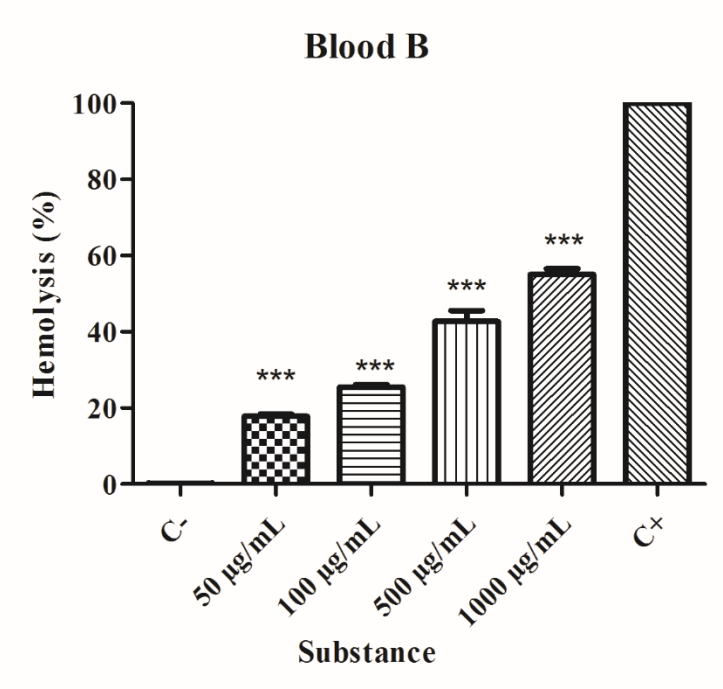

Figure 2. Hemolytic evaluation of Patchouli on type B erythrocytes. (C-) Negative control (0.5% suspension), (C+) Positive control (1% Triton X-100). P < 0.001 (***) versus positive control

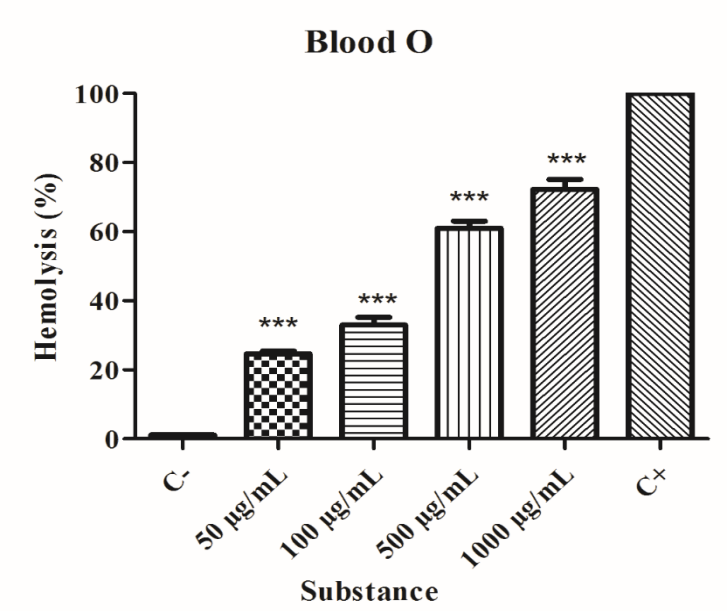

Figure 3. Hemolytic evaluation of Patchouli on type O erythrocytes. (C-) Negative control (0.5% suspension), (C+) Positive control (1% Triton X-100). P < 0.001 (***) versus positive control

Essential oils are aromatic products of secondary metabolism in plants. Their composition possesses several pharmacological properties, mostly due to their volatility.[33]

The essential oil of *Pogostemon cablin* Benth. (Patchouli) demonstrates biological activities such as: antimicrobial, anti-inflammatory, antifungal, cicatrizing, antiseptic, neuroprotective, anti-influenza, and anthelmintic.[34] chemically, this essential oil is characterized by presenting sesquiterpene compounds, where its main component is Patchouli.[35]

In vitro and *Vivo,* studies have shown effective results on different types of essential oils, from a wide variety of plant species, against mouth, lung, breast, prostate, liver, colon, brain, and leukemia cancers. According to the results of Maeda (2016), the vapor of *Pogostemon cablin* essential oil inhibited the growth of cancer cells.[36]

In a second step, the anti-hemolytic test was performed to check the effect of Patchouli essential oil on the osmotic fragility of erythrocytes. This way, divergence in the form of action of the substance against the different blood groups could be observed.

Type A did not show anti-hemolytic potential in any concentration evaluated. Type B and O erythrocytes showed moderate hemolysis ranging from 59% to 75% in the evaluated concentrations of 500µg/mL and 1000 µg/mL, as can be seen in figures 4, 5, and 6.

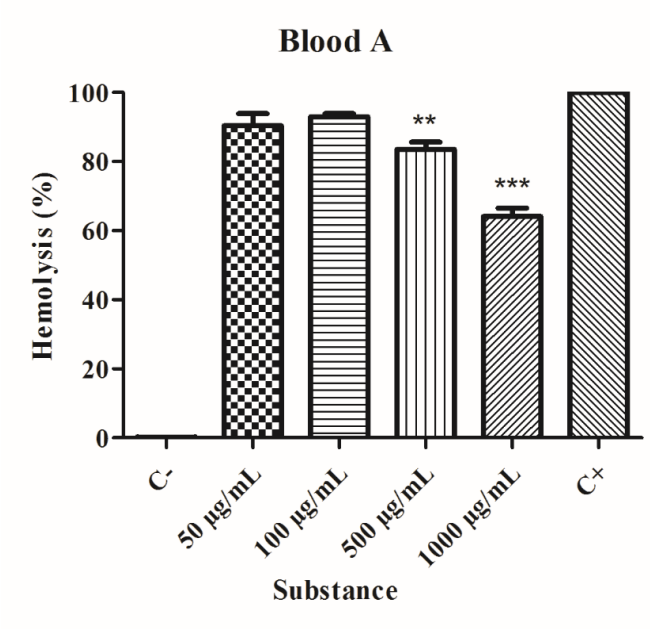

Figure 4. Anti-hemolytic evaluation of Patchouli on type A erythrocytes. (C-) Negative control (0.5% suspension), (C+) Positive control (1% Triton X-100). P < 0.01 (**) and P < 0.001 (***) versus positive control

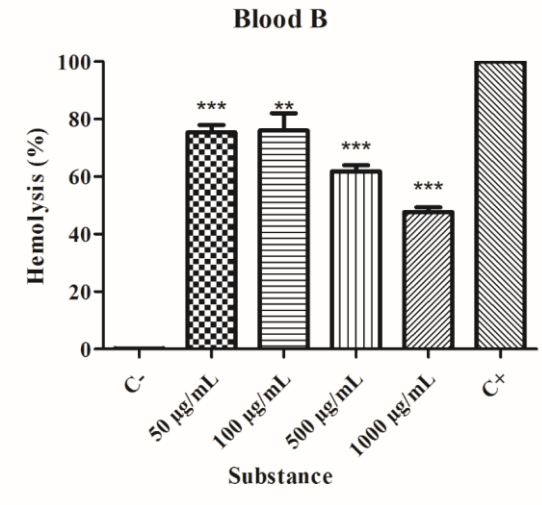

Figure 5. Anti-hemolytic evaluation of Patchouli on type B erythrocytes. (C-) Negative control (0.5% suspension), (C+) Positive control (1% Triton X-100). P < 0.01 (**) and P < 0.001 (***) versus positive control

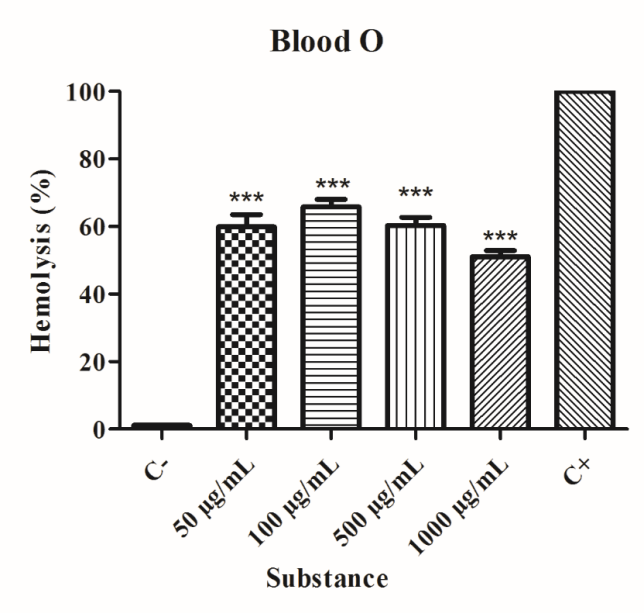

Figure 6. Anti-hemolytic evaluation of Patchouli on type O erythrocytes. (C-) Negative control (0.5% suspension), (C+) Positive control (1% Triton X-100). $P < 0.001$ (***) versus positive control

The anti-hemolytic test is considered an important screening test in determining the biocompatibility of the compound. The osmotic stability characteristic of a substance attributes value to its potential for pharmacological application. Since hemolysis releases the products contained within the erythrocytes, adjacent cells are more susceptible to the impact of cytotoxic effects generated by reactive oxygen species, the level of which increases considerably due to the release of iron and hemoglobin. [37]

FINAL CONSIDERATIONS

Considering the results obtained in the in vitro cytotoxicity study on Patchouli essential oil, it is concluded that the natural origin product tested showed a low percentage of hemolysis for human erythrocytes of the ABO system at the concentration of 50 to 100 µg/mL. In addition, it shows a moderate anti-hemolytic effect at concentrations of 500 to 1000 µg/mL for blood types B and O.

Thus, Patchouli essential oil becomes a promising candidate for the use of its bioactive properties in herbal medicines. However, auxiliary studies such as in vivo toxicity are necessary

to better elucidate the mechanisms of action of this compound. Contributing to the enrichment of the natural heritage of Brazil, through the use of plants with medicinal potential.

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