# Evaluation of *in vitro* Cytotoxic Effects of Especifico Pessoa Phytotherapic Tincture on Ehrlich Tumor Cells and Mice Spleen Cells

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**ABSTRACT**

Especifico Pessoa (EP) is traditionally used for the treatment of snakebite envenoming. The traditional use of EP and its properties have been reported. In this study, we evaluated the *in vitro* cytotoxic effects of EP on Ehrlich tumor and mice spleen cells. Cytotoxicity assay was carried out by using Trypan blue exclusion method. Spleen cell suspension was prepared (n=2) with RPMI medium and tumor cell suspension was prepared from ascitic fluid of Ehrlich tumor-bearing mice (n=1); both the suspensions contained 4 x $10^6$ cells $mL^{-1}$. Pure EP or EP diluted in RPMI (1:2; 1:4) was used. The results were expressed as percentage of cell viability and demonstrate that EP is toxic to Ehrlich cells at all concentrations (Control: 96.42 ± 3.40; Pure: 1.55 ± 2.91; 1:2: 4.85 ± 5.04; 1:4: 13.39 ± 5.08), but nontoxic to spleen cells in at the lowest dilution (Control: 72.86 ± 13.79; Pure: 13.52 ± 6,36; 1:2: 41.36 ± 13.51; 1:4: 56.59 ± 8.62). Therefore, the results demonstrate that EP has cytotoxic effects, depending on the dose and the cell line evaluated.

**Keywords:** Especifico Pessoa; Phytotherapic; Ehrlich Tumor; Cytotoxicity.

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The use of medicinal plants in simple or complex formulations is an integral part of popular cultures worldwide and has been accompanied by development of civilizations (Akran et al. 2014). Several studies have reported the biological effects of compounds extracted from plants, fungi, marine animals, amphibians, and microorganisms (Kaneno et al. 2004; Martins et al. 2008; Ferreira et al. 2013; Sultana et al. 2014; Albiero et al. 2016).

Several pharmaceutical products that are currently used, such as reserpine, deserpidine, vinblastine, and paclitaxel are medicinal plant-based drugs (Sultana et al. 2014). The National Cancer Institute in the United States has evaluated approximately 114,000 natural extracts with anticancer activity (Sultana et al. 2014). In Brazil, unconventional therapeutic agents are regulated by law, which legitimizes their use in therapies such as acupuncture, phytotherapy, and homeopathy (Brasil 2013).

The scientific community recommends that products of unknown action be initially evaluated for their biocompatibility through *in vitro* cell culture studies (Martins et al. 2009). *In vitro* cytotoxicity tests involve the culturing of mammalian cells via direct or indirect contact with a compound or material and the observation of cellular alterations (Rogero et al. 2003).

One of the most commonly used parameters to evaluate cellular changes is cell viability, which can be visualized using vital dyes such as neutral red or Trypan blue (Rogero et al. 2003; Oliveira et al. 2010). In this study, we used Trypan blue. Another alternative for the measurement of cell viability is the reduction of MTT (3-[4,5-dimethyl-2-thiazolyl]-2,5-diphenyl-2H-tetrazolium) to formazan crystals by mitochondrial NADPH of viable cells (Martins et al. 2009; Oliveira et al. 2010).

The main objective of *in vitro* cytotoxicity assays is to facilitate the study of cell behavior in a controlled environment free of complexity of the living organism and in an easy, fast, and inexpensive way (Martins et al. 2009). Following *in vitro* studies, the material or compound may subsequently enter animal studies and human clinical trials (Rogero et al. 2003; Martins et al. 2009).

Especifico Pessoa is a phytotherapic tincture prepared from Brazilian plants and has been traditionally used in the treatment of snakebite envenoming, particularly in the North and Northeast of Brazil (Reichert et al. 2014; Moura and Mourão 2012; Pierini et al. 1996). It is a hydroalcoholic extract of the root of a plant popularly known as "cabeça-de-negro," which includes anise and orange, manufactured in Ceará (Brazil). Especifico Pessoa is normally administered orally (Reichert et al. 2014; Pierini et al. 1996).

Several studies have reported the biological effects of EP tincture in different types of animals, restoring and preserving of the physiological conditions of envenomed animals, such mice and beagle dogs (Nakagawa et al. 1982).

Four Brazilian plant species are designated as "cabeça-de-negro": *Cayaponia tayuya* (Kell.) Cogn., *Cayaponia espelina* Cogn., *Annona coriacea* (Mart.), and *Wilbrandia* sp. (Silva et al. 2015; Moura, Mourão, and Dos-Santos 2015). EP's antiophidic property was evaluated by Nakagawa and Nakanishi (1982), who first identified and isolated two new pterocarpans, cabenegrins A-I and A-II.

In the studies of Nakagawa et al. (1982), mice were intraperitoneally injected with 2.5-fold the lethal dose of *Bothrops atrox* snake venom, and then immediately injected with cabenegrins A-I (44 mg) and A-II (1 mg). After 24 h, the physiological conditions of the animals were restored (Nakagawa et al. 1982). The cardiovascular toxic effects of *B. atrox* lethal dose (2.5 mg/kg) venom was also evaluated in male beagle dogs (Nakagawa et al. 1982). Pretreatment with cabenegrin A-I (1.0 mg/kg) restored respiration, blood pressure, and electrocardiogram to normal after 60–90 min (Nakagawa et al. 1982). Conversely, when cabenegrin A-I was injected in an isolated heart preparation of beagle dogs several minutes after envenoming, the toxic effects were reversed (Nakagawa et al. 1982).

Cabenegrin A-I and cabenegrin A-II were the first pterocarpans isolated and synthesized from Especifico Pessoa tincture (Nakagawa et al. 1982). This designation came from the belief that this tincture is prepared from a plant called "cabeça-de-negro" (Silva, Matos, and Silveira 1997). However, Nakagawa & Nakaniski could not identify the plant, and the factory in Sobral, Ceará, Brazil does not present the scientific name of the species used (Moura, Mourão, and Dos-Santos 2015).

*H. brasiliana* Benth (Leguminosae–Papilionoideae) is a Northeastern Brazilian shrub called "raiz-de-cobra" and is used by people for treating snake bites (Silva et al. 1999). The pterocarpans harpalyce (Silva et al. 1999) and edunol (Silva et al. 2004; Silva, Matos, and Silveira 1997) were isolated from *H. brasiliana*.

Silva, Matos, and Silveira (1997) performed structural determination of edunol and cabenegrin A-I through absolute stereochemistry and spectral analysis, particularly CD and 2D NMR techniques, and observed the same absolute stereochemistry for both the substances. Owing to the vicinity of *H. brasiliana* collection site and Especifico Pessoa preparation factory, the authors believe that cabenegrin A-I and edunol are the same substance, therefore "raiz-de-cobra" and not "cabeça-de-cobra" is one of the materials used for EP (Silva, Matos, and Silveira 1997).

Other studies with Especifico Pessoa did not show beneficial effects on *B. atrox* venom, being suggested its prohibition (Moura, Mourão, and Dos-Santos 2015; Borges, Sadahiro, and Santos 1999).

The main effect of snake venom is its myotoxicity, which induces tissue hemorrhage and myonecrosis, due to the presence of proteolytic and phospholipase A2 enzyme activities (Silva et al. 2004).

Silva et al. (2004) observed that *H. brasiliana* pterocarpan inhibited myotoxic activity *in vitro* against mice muscle damage induced by *Bothrops jararacussu* (0.1–3 μM, > 40% of the inhibition).

Ximenes et al. (2012)demonstrated that harpalycin 2, an isoflavone isolated from *H. brasilia*na, inhibited secretory phospholipase $A_2$ activity by around 58.7% for *Bothrops pirajai*, 78.8% for *Crotalus durissis terrificus*, 87.7% for *Apis mellifera*, and 88.1% for *Naja naja* when pre-incubated at equal mass for 30 min before evaluation. This study also evaluated the neutralization of secretory phospholipase $A_2$-induced paw edema by harpalycin 2, and the results showed that the significant inhibition of initial edema phase may be correlated with the inhibition of phospholipid catalysis by isoflavone (Ximenes, Alves, et al. 2012) . Similar to the study by Silva et al. (2004), Ximenes et al. (2012) also showed that harpalycin 2 can inhibit the myotoxic activity of secretory phospholipase $A_2$ of snake venom. Thus, harpalycin 2 seems to induce a chemical modification of crucial amino acid residues and a partial unfolding of secretory phospholipase $A_2$. Such modifications lead to an irreversible loss of enzymatic activity and/or its ability to bind to the cell membrane (Ximenes, Rabello, et al. 2012).

Since then, several studies have been conducted and the biological properties of Especifico Pessoa compounds including antibacterial, anti-inflammatory, antiproliferative, and cytotoxic effects have been reported. Natural and synthetic molecules of EP have been isolated and its molecular mechanism of action has been studied (Reichert et al. 2014; Ximenes, Rabello, et al. 2012; Vieira et al. 2008; Militão et al. 2007; Silva et al. 2004; Silva et al. 1999; Engler et al. 1993).

Therefore, the objective of this study was to evaluate the *in vitro* cytotoxic effects of Especifico Pessoa tincture on co-culture with Ehrlich tumor cells and mice spleen cells. This is the first study on the effects of EP tincture in these types of cells. The findings of this study are important to corroborate the biological action of Especifico Pessoa and its ethnobotanical use.

## MATERIALS AND METHODS

### ANIMALS AND ETHICAL ASPECTS

Male Swiss mice (n=2), aged 40 to 50 days, were obtained from the Central Animal Facility of Federal University of Mato Grosso–UFMT, Cuiabá (Brazil). The animals were kept in polypropylene boxes with xylan substrate (Suprimart Mercantil, Itaquaquecetuba, SP, Brazil) at 22°C with 12/12h light/dark cycles. The mice received filtered water and pelleted feed (Purina, St. Louis, Missouri, USA) *ad libitum*. All procedures were conducted in accordance with the recommendations of the Brazilian College of Animal Experimentation and were approved by the Ethics Committee on Animal Use (Comitê de Ética no Uso de Animais - CEUA) of Federal University of Mato Grosso-UFMT (CEUA Protocol nº 23108.702149/13-0).

### ESPECIFICO PESSOA PHYTOTHERAPIC TINCTURE

Especifico Pessoa tincture is a hydroalcoholic plant extract that is manufactured in Ceará, Brazil; Register number 262 in the Department of Public Health of Rio de Janeiro, Brazil. The tincture used in this present work was purchased from a local farm store (Sinop, Brazil). The usual adult human being dosage is 1.0 mL diluted in 14.0 mL of water, one to three times daily (Reichert et al. 2014). For *in vitro* analysis, the tincture was used in pure form or diluted in complete medium (RPMI 1640 supplemented with 20% of heat-inactivated fetal bovine serum-Cultilab, Campinas, SP, Brazil). Doses were chosen according to *in vivo* studies performed by Reichert et al. (2014).

### TOTAL SPLEEN CELL SUSPENSION

Mice (n=2) were sacrificed by cervical dislocation and the spleens were removed after laparotomy. Spleen cell suspension was obtained by teasing the spleens on a sterile fine nylon screen in RPMI 1640 medium (Cultilab, Campinas - SP, Brazil), according to Castoldi et al. (2007). The cell suspension was centrifuged at 1,500 rpm for 10 min and suspended in 1 mL complete medium. The concentration of splenic mice cells was adjusted to $4 \times 10^6$ viable cells $mL^{-1}$ (Albiero et al. 2016) using Trypan blue exclusion test, and a minimum viability of 70% was considered.

### EHRLICH TUMOR CELLS

Ehrlich tumor cells were kindly provided by Rondon Tosta Ramalho, Ph.D., from Federal University of Mato Grosso do Sul, Campo Grande, Brazil. The cells were maintained by intraperitoneal inoculation (ascitic form) in Swiss mice, every 7 days, during 14 days. The ascitic fluid of animals with the ascitic form of Ehrlich tumor was aspirated from the peritoneum, and tumor cell suspensions were

prepared in sterile phosphate-buffered saline to obtain a final concentration of 4 $x10^6$ viable cells $mL^{-1}$. Viability, assessed by Trypan Blue dye exclusion method, was always found to be at least 70%.

### CYTOTOXICITY ASSAY BY TRYPAN BLUE EXCLUSION METHOD

The toxic effect of Especifico Pessoa tincture was determined by measuring the viability of both spleen cells and Ehrlich tumor cells. Two animals were used (male Swiss mice) to prepare spleen cell suspension, according to the protocol described previously (Section: total spleen cell suspensions). One animal bearing ascitic form of Ehrlich tumor was the source of tumor cells. The cells (mice spleen cell or Ehrlich tumor cell, $4x10^6$ cells $mL^{-1}$) were distributed (50 μL/well) on a 96-well flat-bottomed microculture plate and the effect of tincture was tested using the pure form and dilutions (1:2 and 1:4). Mice spleen cells suspensions were distributed in triplicate on microplate for each group (basal control, EP pure, EP 1:2, and EP 1:4). Ehrlich tumor cell suspension was distributed in six repetitions for each group. The basal control group was formed by complete medium (50 μL/well) and cells (50 μL/well, mice spleen or Ehrlich tumor), without EP. The plates were cultured for 24 h at 37°C under 5% $CO_2$. Then, cell viability was assessed by Trypan blue exclusion method in a Neubauer chamber (Militão et al. 2006).

### STATISTICAL ANALYSIS

Statistical analysis was performed using the Graphpad InStat software, San Diego, California-USA. Analysis of variance (ANOVA) and Tukey-Kramer tests were employed. Differences were considered significant when the probability of error was less than 5% ($p \leq 0.05$).

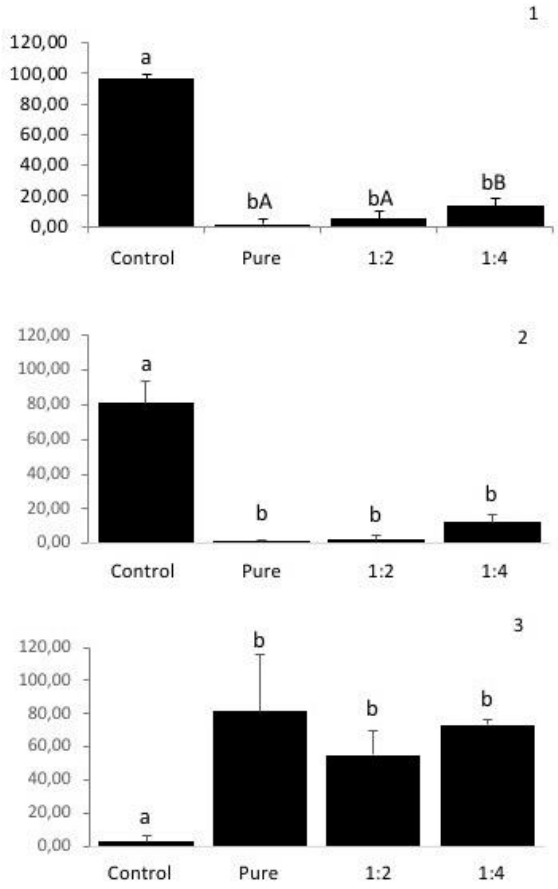
The percentage of cell viability (% CV) was calculated using the following equation: %CV = [(viable cell nº x 100)/(viable cell nº + die cell nº)].

## RESULTS

Figures 01 and 02 show the effects of Especifico Pessoa on co-culture with Ehrlich tumor cells and mice spleen cells, respectively.

Especifico Pessoa tincture reduced the % CV of Ehrlich tumor cells at all dilutions tested (Figure 01, Graphic 01. Control: 96.42 ± 3.40; Pure: 1.55 ± 2.91; 1:2 dilution: 4.85 ± 5.04; 1:4 dilution: 13.39 ± 5.08). For spleen cells, % CV reduced when the cells were treated with pure and 1:2 dilution (Figure 02, Graphic 01. Control: 72.86 ± 13.79; Pure: 13.52 ± 6,36; 1:2 dilution: 41.36 ± 13.51; 1:4 dilution: 56.59 ± 8.62).

**Figure 01.** Percentage cell viability (% CV) of Ehrlic tumor cells in co-culture (37ºC, 5% $CO_2$) with different dilutions of phytotherapic tincture Especifico Pessoa for 24 h.



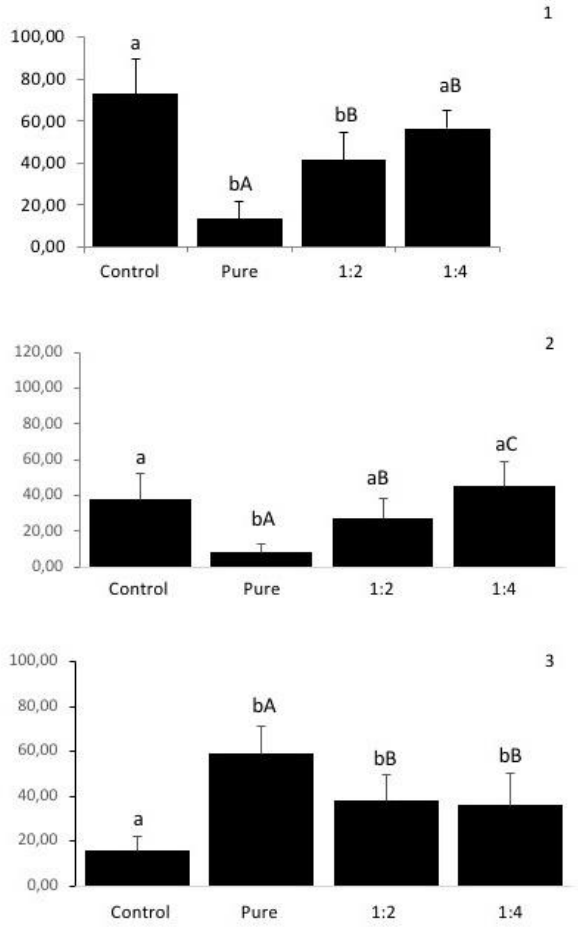

Source: The Authors.

Values are expressed as means ± standard deviation of %CV (1); absolute number of viable cells (2); and absolute number of dead cell (3); The cytotoxicity assay was performed using Trypan blue exclusion method. The %CV was calculated according to the following formula: [(viable cell nº x 100)/(viable cell nº + die cell nº)]. The lowercase letter denotes comparison of control and treated groups. The uppercase letter denotes comparison among treated groups.

The figures also show the absolute number of viable (Graphic 02, Figure 01 and Figure 02) and dead cells (Graphic 03, Figure 01 and Figure 02). A reduction in number of viable Ehrlich tumor cells (Figure 01, Graphic 02. Control: 80.67 ± 12.75; Pure: 0.67 ± 0.82; 1:2 dilution: 2.33 ± 2.25; 1:4 dilution: 11.67 ± 5,24) and an increase in number of dead cells (Figure 01, Graphic 03. Control: 3.00 ± 3.16; Pure: 81.50 ± 34.48; 1:2 dilution: 55.33 ± 14.56; 1:4 dilution: 73.33 ± 3.01) were observed. For mice spleen cells, a reduction in viable cells (Figure 02, Graphic 02. Control: 41.00 ± 13.29; Pure: 7.67 ± 5.47; 1:2 dilution: 26.67 ± 5.47; 1:4 dilution: 45.17 ± 13.44) and increase in dead cells (Figure 02,

Graphic 03. Control: 14.40 ± 6.35; Pure: 58.83 ± 12.62; 1:2 dilution: 38.00 ± 11.66; 1:4 dilution: 35.67 ± 14.47) were observed after treatment with both pure and 1:4 dilution, as % CV.

**Figure 02.** Percentage cell viability (% CV) of Ehrlic tumor cells in co-culture (37ºC, 5% $CO_2$) with different dilutions of phytotherapic tincture Especifico Pessoa for 24 h.




Source: The Authors.

Values are expressed as means ± standard deviation of %CV (1); absolute number of viable cells (2); and absolute number of dead cell (3); The cytotoxicity assay was performed using Trypan blue exclusion method. The %CV was calculated according to the following formula: [(viable cell nº x 100)/(viable cell nº + die cell nº)]. The lowercase letter denotes comparison of control and treated groups. The uppercase letter denotes comparison among treated groups.

## DISCUSSION

The results of this study demonstrate that Especifico Pessoa tincture is toxic to Ehrlich cells at all concentrations, but is nontoxic to spleen cells at the lowest concentration. These effects are corroborated by previous studies, as follows.

Especifico Pessoa has been used for more than 30 years as a supportive therapy for snake and spider bites, particularly in the North and Northeast of Brazil (Silva et al. 2015; Pierini et al. 1996). Its

use is currently disseminated to other regions, such as Midwest and Southern Brazil (Moura, Mourão, and Dos-Santos 2015; Reichert et al. 2014).

It is well known that snakebites are an important cause of morbidity and mortality in tropical and subtropical countries, particularly where agricultural activity (Reichert et al. 2014) and extractivism (Pierini et al. 1996) are intense. The only treatment recommended by the Brazilian Ministry of Health in cases of snakebite envenomation is the application of specific antivenom serotherapy (Moura, Mourão, and Dos-Santos 2015). Nevertheless, in areas without easy access to such therapy in hospitals, many plant extracts described in folk medicine are used to block the biological activities induced by snake venom (Moura, Mourão, and Dos-Santos 2015; Moura and Mourão 2012; Borges, Sadahiro, and Santos 1999; Pierini et al. 1996).

Especifico Pessoa is a hydroalcoholic extract of the root of a plant commonly known "cabeça-de-negro"; however, many plants in Brazil are designated as "cabeça-de-negro" (Silva et al. 2015; Moura, Mourão, and Dos-Santos 2015). Nakagawa et al (1982) reported that the antiophidic property of Especifico Pessoa is related to the presence of two pterocarpans, cabenegrin A-I and cabenegrin A-II.

Pterocarpans are the second largest class of naturally occurring isoflavonoids (Araújo et al. 2008). Characterized by the 6a, 11a-dihydro-*6H*- benzofurol[3,2-*c*][1]benzopyrene skeleton, these compounds are known as phytoalexins, which are produced by plants to contribute to their chemical defense system during infections by fungi, viruses, or bacteria (Araújo et al. 2008). The broad spectrum of their biological activities have attracted attention because some of them are employed as cytotoxics, antimitotics, antitoxins, antifungals, antivirals, and antibacterials (Araújo et al. 2008).

There are pterocarpans isolated from different plants such as *Harpalyce brasiliana* Benth (Ximenes, Alves, et al. 2012; Silva et al. 2004; Silva, Matos, and Silveira 1997), *Platymiscium floribundum* (Falcão et al. 2005; Militão et al. 2005), and *Ulex parviflorus* (Máximo and Lourenço 1998).

*H. brasiliana* Benth (Leguminosae–Papilionoideae) is a Northeastern Brazilian shrub called "raiz-de-cobra" and is used by people for treating snake bites (Silva et al. 1999). Silva, Matos, and Silveira (1997) strongly believe that this plant is one of the main materials used for Especifico Pessoa preparation.

Six bioactive pterocarpan derivatives (4'-dehydroxycabenegrin A-I, leiocarpin, medicarpin, cabenegrins A-I and A-II, and maackiain) were isolated from the chloroform fraction of *H. brasiliana*

extract (Militão et al. 2007). Leiocarpin was found to be the most active derivative in sea urchin eggs, with $IC_{50}$ values ranging from 0.1 to 1.2 mg/mL, demonstrating potent antiproliferative effects. 4'-dehydroxycabenegrin A-I exhibited the most potent cytotoxic activity against leukemia, melanoma, and colon human tumor cell lines, presenting $IC_{50}$ in the range of 3.1–8.5 mg/mL (Militão et al. 2007).

Antiproliferative effects of Especifico Pessoa were also observed in the present study. In both cell types, the absolute number of viable cells decreased (Graphic 02, Figure 01 and Figure 02) and the number of dead cells increased (Graphic 03, Figure 01 and Figure 02). Especifico Pessoa may block cell division and cause membrane damage, allowing the entry of Trypan blue dye into the cytoplasm.

The cytotoxic properties of pterocarpans isolated from *Platymiscium floribundum* were also evaluated. 2,3,9-trimethoxypterocarpan was the most effective compound with a strong cytotoxic activity against several human tumor cell lines (Militão et al. 2006; Falcão et al. 2005), demonstrating maximum activity after 48 h of incubation, with $IC_{50}$ values ranging from 0.1 to 0.8 mg/mL (Militão et al. 2007). 2,3,9-trimethoxypterocarpan did not reduce the number of human peripheral blood mononuclear cells after 24 and 48 h, but 19% inhibition was observed after 72 h (Militão et al. 2007). The inhibitory effect after 72 h indicates that pterocarpans may cause some toxic effects on non-tumor cells, as observed with spleen cells in the present study (Figure 02).

Militão et al. (2005, 2006) suggested that the cytotoxic and antiproliferative effects of *P. floribundum* pterocarpans are due to the inhibition of DNA synthesis and induction of apoptosis with caspase-3 activation. Notably, the increase in hydroxy groups seems to induce cell death by necrosis, whereas methoxy groups induce cell cycle arrest, followed by apoptosis (Militão et al. 2005, 2006).

Thus, the effects observed in the present study on tumor and spleen cells, especially at lower dilution, may be due to the pterocarpans of *H. brasiliana* present in Especifico Pessoa tincture.

However, in the present study, Especifico Pessoa had no toxicity at 1:4 dilution to mice spleen cells, preserving cell integrity (Figure 02). This protective effect was also observed in the study by Vieira et al. (2008).

Vieira et al. (2008) showed a concentration-dependent inhibitory effect on the *in vitro* growth of *Trypanosoma cruzi* epimastigotes in co-culture with (-)-2-geranyl-3-hydroxy-8-9-methylenedioxypterocarpan or 4'-dehydroxy-cabenegrin A-I, which are both pterocarpans of *H. brasiliana*, with $IC_{50}$ of 12.2 and 13.3 mg/mL, respectively. Although these compounds exhibit

important trypanocidal activity, they do not demonstrate a cytotoxic effect on human peripheral blood cells and present an $IC_{50}$ greater than 50 mg/mL (Vieira et al. 2008).

It is important to consider that some of these effects may be due to the alcohol present in the formulation (Reichert et al. 2014). The amount of alcohol present in the phytotherapic tincture is 2.77 mg $kg^{-1}$ (Silva et al. 2015).

Reichert et al. (2014) evaluated the safety of a single dose of Especifico Pessoa tincture in normal male Wistar rats and reported that it affects the physiological system, contributing to increases in body weight, lung weight ratio (13.2%), protein synthesis in the brain and liver, total cholesterol levels (29%), low-density lipoprotein (LDL)-cholesterol levels (148.7%), and acetylcholinesterase activity in brain homogenates. In addition, this treatment decreased the brain (15.3%) and heart (12.95 until 20.6%) weight ratio, and serum and liver butyrylcholinesterase activities (Reichert et al. 2014).

Silva et al. (2015) demonstrated that treatment with Especifico Pessoa or 2.77 mg/kg of alcohol for 10, 15, or 30 days increased plasma levels of cholesterol and creatinine. Although the markers of liver function were not altered, microscopic tissue examination revealed hydropic multifocal degeneration. Taken together, the findings of Silva et al. (2015) and Reichert et al. (2014) indicate that although pterocarpans may have beneficial effects, regular exposure to alcohol present in this type of herbal tincture may lead to serious biochemical alterations.

The cytotoxic effect observed in the present study may have been due to the alcohol present in the tincture, especially at higher concentrations of the tincture.

In addition the popular use of Especifico Pessoa, it is important to investigate the pterocarpan types and other types of polyphenolic compounds, such as tannins (Pithayanukul et al. 2005), that can be present in Especifico Pessoa tincture and their biological effects in different types of tumor and non-tumor cells in order to development new strategies for cancer treatment.

## CONCLUSION

The results of this study demonstrate that the phytotherapic tincture Especifico Pessoa has cytotoxic effects, depending on the dose and the cell line evaluated. These findings are important and in agreement with the results of previous studies on Especifico Pessoa, proving the biological action of its compounds and confirming the observations from folk medicine. Further studies are needed to elucidate the actual plant used in tincture preparation, its compounds, and mechanism of action.

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# Avaliação da Citotoxicidade *in vitro* da Tintura Fitoterápica Especifico Pessoa sobre Células do Tumor de Ehrlich e sobre Células Esplênicas de Camundongo

**RESUMO**

Especifico Pessoa (EP) é utilizado no envenenamento por serpentes. Estudos comprovam seu uso tradicional e descrevem outras propriedades, como antitumoral. O objetivo deste estudo foi avaliar seu efeito citotóxico em células do Tumor de Ehrlich e esplênicas de camundongos. O ensaio foi realizado pelo método de exclusão do Azul Tripan. A suspensão de células esplênicas foi obtida (n = 2) em RPMI e a tumoral a partir do fluido ascitico de camundongos portadores do tumor (n = 1), ambos a 4 x $10^6$ células $mL^{-1}$. O EP foi usado puro ou diluído em RPMI (1:2; 1:4). Os resultados são expressos como porcentagem de viabilidade celular e demonstraram que o EP é tóxico para células tumorais, em todas as concentrações (Controle: 96,42 ± 3,40; Puro: 1,55 ± 2,91; 1:2: 4,85 ± 5,04; 1:4: 13,39 ± 5,08), mas atóxico para os esplenócitos na menor diluição (Controle: 72,86 ± 13,79; Puro: 13,52 ± 6,36; 1:2: 41,36 ± 13,51; 1:4: 56,59 ± 8,62). Portanto, os resultados demonstram que o EP tem efeito citotóxico, dependendo da dose e linhagem celular utilizada.

**Palavras-Chave:** Especifico Pessoa; Fitoterápicos; Tumor de Ehrlich; Citotoxicidade.

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