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IN VITRO ASSESSMENT OF ANTITUMOR AND ANTIPARASITIC EFFECTS OF EXTRACTS AND FRACTIONS FROM *Persea Americana* AND *Syzygium cumini*

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Trabalho de Conclusão de Curso apresentado à Comissão de estágio e TCC (CETCC) do curso de graduação em Biomedicina como critério para aprovação na disciplina de TCCII.

Orientadora: Profa. Dra. Fernanda Maria Santiago.

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Uberlândia, 19 de novembro de 2024.

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Profa. Dra. Fernanda Maria Santiago

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ABSTRACT

Introduction: *Persea americana* (Avocado) of the Lauraceae family and *Syzygium cumini* (Jambolan) of the Myrtaceae family are food and medicinal plants widely used with antioxidant, anti-inflammatory, antitumor, and antiparasitic activities. **Objective:** The present study investigated *in vitro* the antitumor and antiparasitic effects of extracts and fractions purified from *P. americana* and *S. cumini*. **Materials and Methods:** Fractions were obtained from the crude ethanolic extract of *P. americana* seed (EtOHPaS), *P. americana* fruit peel (EtOHPaP) and *Syzygium cumini* leaves (EtOHScL) using polar solvents: hexane (fractions FHPA and EHSc), dichloromethane (fractions FDMPA and EDMSc), ethyl acetate (fraction FAePA), n-butanol (fractions FnBPA and EnBSc) and water (fractions FaqPA and EAqSc). Immunohemolysis and MTT assays were performed against the HFF, Beas-2B and Pnt-2 non-tumor cells to assess the cytotoxicity of the samples. To evaluate the antitumor activity, MTT assays were performed using PC3 (prostate cancer cell) and A549 (lung cancer cell). Colony assays were also performed to evaluate the inhibitory effect of the samples on colony formation. Regarding the antiparasitic activity, plaque assays were performed to analyze the effect of the samples on the invasion and proliferation process of *Toxoplasma gondii* tachyzoites. **Results:** The results showed that the FAePA fraction of EtOHPaS exhibited high antitumor activity at a concentration of 400 μg/ml, with a A549 tumor cell death percentage of 45%. The EtOHScL and the EHSc and EDMSc fractions had higher antiproliferative effects against PC3 tumor cell. Furthermore, EtOHPaP and its FHPA fraction; the FAePA, FDMPA, FaqPA fractions of EtOHPaS; and EtOHScL and its EnBSc, EAqSc, EDMSc, and EHSc fractions demonstrated antiparasitic properties indicating that these treatments effectively inhibited the proliferation and reinvasion of *T. gondii* tachyzoites. **Conclusions:** In that sense, the fractions obtained from EtOHPaP, EtOHPaS and EtOHScL show promising therapeutic potential. More studies are required to investigate the mechanisms which may be responsible for the biological activities of these plants.

Keywords: *Syzygium cumini*; *Persea americana*; tumor cells; *Toxoplasma gondii*

1. INTRODUCTION

Natural products use is gaining global importance in clinical practices due to the need for new medications that work effectively and reduce the side effects. Consequently, these products have become the subject of numerous studies investigating the biological activities of components derived from natural sources by analyzing their potential anticancer, anti-inflammatory, antimicrobial, antiparasitic, and antioxidant effects, among others (Harvey et al., 2015; Thomford et al., 2018; Ekiert et al., 2020; Jiang et al., 2022; Naeem et al., 2022).

Lung and prostate cancer are a significant health problem. Both are estimated to be responsible for more than 20% (18.7% and 4.1%, respectively) of global cancer-related deaths. Cancer is characterized by specific cells that evade apoptosis, proliferate unlimitedly, and are capable of invading various tissues through a process known as metastasis. If these characteristics are not properly inhibited, they lead to the progression of the disease, which can be fatal to the patient (Subramaniam et al., 2019). The most commonly used types of treatments for this disease are chemotherapy, radiotherapy, and surgery (Muhammad et al., 2022). Although these treatments have shown considerable positive results in combating cancer and increasing survival rates, they also generate a range of side effects. The potential consequences of these therapies include issues with the skin, bones, blood, kidneys, and gastrointestinal organs, and may also affect the heart, brain, and lungs (Wang et al., 2018; Schirrmacher et al., 2019). Recent studies have reported the anticancer effects of different plant species as observed by Cinnamomum (Lauraceae family) and *Syzygium aromaticum* (Myrtaceae family), capable of inducing apoptosis, inhibiting the proliferation and migration of lung, colon, cervical breast and prostate cancer cells (Ekiert et al., 2020; Zari et al., 2021; Caserta et al., 2023).

Another focus of several studies is the antiparasitic effects of various plants. Currently, more than 300 species of parasites can be transmitted to humans. Parasites cause numerous diseases worldwide, each with different levels of severity and etiological agents (Theel and Pritt, 2020). Toxoplasmosis is a zoonosis caused by the intracellular parasite *Toxoplasma gondii* of the phylum Apicomplexa, that manifests differently in immunocompetent individuals, generally asymptomatic, and in immunocompromised individuals, in whom it can lead to as encephalitis, pneumonia, neurological disorders, miscarriages, among others (Aguirre et al., 2019; Attias et al., 2020; Sanchez et al., 2021). Regarding the treatment of toxoplasmosis, it is carried out through a combination of medications that have high rates of ineffectiveness when it comes to chronic infections, in addition to causing serious side effects such as, hepatic necrosis and thrombocytopenia. Despite significant advances in pharmacological and safety research, we still lack the ideal and proper agent for treating chronic toxoplasmosis (Jiang et

al., 2022; Qi et al., 2022). Nowadays, natural products with fewer side effects than synthetic drugs have attracted the attention of researchers. Recent studies demonstrated that extracts and molecules purified from plants of the Lauraceae and Myrtaceae families are highly effective against the infection and proliferation of *T. gondii* tachyzoites, probably with immunomodulation of host cells (Mirzaalizadeh et al. 2018; Mottaghi et al., 2024).

Persea americana (Avocado) is a Central American tree of the Lauraceae family used as a nutrition source and treatment of diseases due to its anti-inflammatory, antioxidant, anti-hemolytic, antiparasitic and promising anticancer properties (Dreher et al., 2013; Jiménez-Arellanes et al., 2013; Nicolella et al., 2017). Ahmed and colleagues (2022) observed that the *P. Americana* fruit and seed extracts have many biological benefits, like chemopreventive effects against hepatocarcinogenesis, inhibiting cell proliferation and promoting apoptosis.

Syzygium cumini (Jambolan) of the Myrtaceae family is a medicinal plant widely used with antioxidant, anti-inflammatory, analgesic, antipyretic, antimalarial, anticancer, and antidiabetic activities (Qamar et al., 2022). Li and colleagues (2021) demonstrated that phytochemicals extracted with chloroform from *S. cumini* fruit had antiproliferative effect (above 90%) against PA-1 ovarian cancer cell tumors.

Considering the essential role of natural products as potential therapeutic targets, the aim of the present work was to analyze analyze the *in vitro* effects of isolated fractions from *P. americana* and *S. cumini* against prostate (PC3) and lung (A549) cancer cells and *Toxoplasma gondii* tachyzoites.

2. MATERIAL AND METHODS

2.1. Ethanolic extraction and liquid-liquid partitioning of *Persea Americana* and *Syzygium cumini* plants

Seeds and fruit peel of *P. Americana* and leaves of *S. cumini* cultivated in Brazilian soil, were purchased from pharmaceutical supplier Florien® (Piracicaba, SP, Brazil), (Lot number 058671), and authenticated by Dr. Paula Mariana Pezzatti and Karina Maria da Silva. To obtain the crude ethanolic extract of *P. americana* seed (EtOHPaS*), P. americana* fruit peel (EtOHPaP) and *S. cumini* leaves (EtOHScL), ethanolic extraction was done as previously described (Justino et al., 2018). After the ethanolic extraction an liquid-liquid partitioning was done as previously described (Justino et al., 2018). The *P. americana* and *S. cumini* extracts were analyzed previously by HPLC-ESI-MS/MS and their phenolics, flavonoids and proanthocyanidins contents were quantified (Justino et al., 2018). The fractions were obtained from the EtOHPaP, EtOHPaS and EtOHScL extracts utilizing increasingly polar solvents: hexane (fractions FHPA

and EHSc), dichloromethane (fractions FDMPA and EDMSc), ethyl acetate (fraction FAePA), n-butanol (fractions FnBPA and EnBSc) and water (fractions FaqPA and EAqSc). The yields were 12.5% of the initial weight of seed and fruit peel for EtOHPa; dried leaves for EtOHSc and for fractions the yields were approximately 12, 21, 9, 23 and 15%, respectively. For *in vitro* analyzes, ethanolic extract and fractions were solubilized in DMSO. A stock solution was prepared (10 mg/ml) and work solutions were diluted in RPMI culture medium.

Fraction/Extract	*Persea americana*		*Syzygium cumini*
	Fruit peel	Seed	Leaves
Crude ethanolic extract	EtOHPaP	EtOHPaS	EtOHScL
Hexane	FHPA	-	EHSc
Dichloromethane	FDMPA	FDMPA	EDMSc
Ethyl acetate	FAePA	FAePA	-
n-butanol	FnBPA	FnBPA	EnBSc
Water	FaqPA	FaqPA	EAqSc

Table 1. Extracts and fractions of *P. americana* and *S. cumini*.

2.2. Cell culture and parasite strain

Non-tumor cell lines: human foreskin fibroblasts (HFF); human lung cells (Beas-2B); human prostate cells (Pnt-2) and human cancer cell lines: lung cancer cells (A549) and prostate cancer cells (PC3), were grown in the Gibco Roswell Park Memorial Institute 1640 medium (RPMI). The RH strain of *Toxoplasma gondii* was maintained in human foreskin fibroblasts (HFF) cells. All culture media were supplemented with 10% Fetal Bovine Serum (Gibco), 2 mM glutamine, 100 U/ml penicillin, and 100 µg/ml streptomycin, at 37°C in a 5% CO_2, as previously described (Li, et., 2009; Sohn et.al., 2011).

2.3. Animals

The management of blood collected and euthanasia protocols of the animals were approved by the Animal Research Ethics Committee of the Federal University of Uberlândia, Brazil (CEUA/UFU 109/16).

2.4. Cytotoxicity assessment

2.4.1. Immunohemolysis

Red blood cells (RBCs) from Balb/c mice were collected at the Rodent Bioterium Network (Rebir-UFU) and approved by the Ethics Committee. After collection, the RBCs were washed

in a 0.95% NaCl solution and centrifuged at 400g for 5 minutes. The RBCs were adjusted to a concentration of 0.5%. In a 96-well U-bottom plate, the EtOHPaP, EtOHPaS, EtOHScL extracts and fractions were serially diluted in 0.95% NaCl (starting at a concentration of 1000 µg/ml up to 1.95 µg/ml). Then 50 µl of 0.5% red blood cells were added to all the wells and incubated for 60 minutes at 37°C. The negative control consisted only of red blood cells with NaCl, while the cell death control used 1% Triton X-100 and red blood cells. After incubation, the plate was centrifuged at 500g for 10 minutes, and only the supernatant was removed for reading on the spectrophotometer (540nm). The samples were considered cytotoxic when hemolysis occurred.

2.4.2. Evaluation of cellular viability of HFF; Beas-2B and Pnt-2 treated with EtOH crude extract and fractions of *P. americana* and *S. cumini*

To evaluate the cytotoxicity of the samples of interest were tested in non-tumor cells by MTT (3-(4,5-1094 dimethylthiazol-2yl)-2,5-diphenyl tetrazolium bromide) colorimetric assay. Initially, HFF, Beas-2B and Pnt-2 were cultured in 96-well plates, adjusted to a concentration of $1x10^4$ cells/well in a complete RPMI medium and incubated for 24h at 37°C, 5% CO_2. Subsequently, the cells were treated with different concentrations (400 up to 50 µg/ml) of EtOHPaP, EtOHPaS, EtOHScL and its fractions and incubated for 24 hours. After the incubation interval, an MTT solution (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was added in the proportion of 10 µl/well, along with 90 µl of RPMI medium containing 10% fetal bovine serum. The plates were incubated for 4 hours. Afterward, the plate was centrifuged at 500g for 10 minutes at 37ºC, and 200 µl/well of a solution of 10% SDS (Sodium Dodecyl Sulphate) and 50% DMF (dimethylformamide) was added to solubilize the crystals formed. After 24 hours, the crystals were read on a spectrophotometer at a wavelength of 570 nm (Garn et al., 1994). As a control, the cells were treated only with the medium. The percentage of viable cells was calculated under the experimental conditions in relation to the control (untreated cells), which corresponded to 100% viability.

2.5. Antitumor Activity

2.5.1. MTT assay

To evaluate the antitumor activity of EtOHPaP, EtOHPaS, EtOHScL and its fractions, cell lines PC3 and A549 were used and tested by MTT (3-(4,5 dimethylthiazol-2yl)-2, bromide method 5-diphenyl tetrazolium), as described previously. However, the treatment was carried out at concentrations that did not show cytotoxicity for non-tumor cells.

2.5.2. Clonogenic assay

The colony assay was carried out to analyze the survival of PC3 and A549 tumor cells, which provides information on the proliferation and colony-forming potential of cells when exposed to treatment with EtOHPaP, EtOHPaS, EtOHScL and its fractions. The cells were plated in a 24-well plate at a ratio of 200 cells/well and were subsequently treated with the samples at concentrations of 25; 50; 100; 200 and 400 μg/ml. The experiment was carried out in duplicate. As a control, the cells were treated with a complete RPMI medium and were kept at 37ºC, 5% CO_2, for 7 days. After this period, the reaction was stopped by adding ice-cold methanol for 5 minutes. Subsequently, the wells were washed 3 times with PBS and stained with 1% crystal violet to visualize an optical microscope.

2.6. Antiparasite Activity

2.6.1. Plaque assay

To assess the invasion capacity of the RH strain of *Toxoplasma gondii*, and consequently cause cell lysis, HFF cells were distributed in 24-well plates, at a density of $1x10^5$ cells per well, and incubated for 24 hours. Subsequently, HFF cells monolayer were infected with 500 parasites/well for 2 hours and then treated with EtOHPaP, EtOHPaS, EtOHScL and its fractions at concentrations of 100 up to 25 μg/ml. As a control, the infected cells were treated with 10% DMEM medium and incubated for 7 days at 37°C, 5% CO_2. At the end of this period, the wells were washed with PBS, fixed with ice-cold 100% methanol for 10 minutes and stained with 1% crystal violet for visualization under an optical microscope. Number and area of plaques were calculated using ImageJ.

2.7. Statistic analysis

Cell viability and MTT assays analysis used Two-way ANOVA and Dunnett´s multiple comparisons test. The antitumor and antiparasitic activity used Two-way ANOVA. All analyses and graphics were performed using GraphPad Prism Software 9.0 (GraphPad Software, Inc., San Diego, CA, USA). Values of $p<0.05$ were considered statistically significant in the Cell viability and antitumor assays and values of $p<0.0001$ were considered statistically significant in the antiparasitic assay.

3. RESULTS AND DISCUSSION

3.1. Cytotoxicity Evaluation

The hemolysis assay was performed to evaluate the cytotoxicity of EtOHPaP, EtOHPaS, EtOHScL, and fractions on erythrocytes at different concentrations, starting at 1000 μg/ml.

Firstly, the cytotoxic effect was examined with EtOHPaP and its fractions. The FDMPA fraction showed a statistically significant effect with cell mortality of 100% at concentrations of 1000 up to 125 ug/ml, $p < 0.05$. FAePA had significant hemolytic activity at 1000 and 500 µg/ml (100% and 62% respectively), while the fractions FHPA, FnBPA and EtOHPaP showed cytotoxic effect only at the highest concentration (1000 µg/ml) with 100%, 100% and 62% cell death, respectively. In contrast, the FaqPA fraction not demonstrated cytotoxic activity at all tested concentrations (Figure 1A).

When evaluating the hemolytic activity, the fractions obtained from EtOHPaS, FDMPA showed the highest cytotoxic effect at concentrations of 1000 up to 250 µg/ml (100%, 43% and 33% cell death, respectively). Regarding the EtOHPaS and FnBPA fractions, was observed that both presented significant cell death at concentrations of 1000 and 500 µg/ml (above 40%), while the FAePA fraction was cytotoxic only at the highest concentration, with 81% cell death. The FaqPA fraction did not induce hemolysis at the evaluated concentrations (Figure 1B).

Concerning the cytotoxic effect of the fractions of *S. cumini* leaves, the EnBSc, EAqSc and EHSc fractions showed a statistically significant effect, $p < 0.05$, when used at concentrations of 1000 and 500 µg/ml (cytotoxicity above 57% and 24% respectively), while the EtOHScL fraction demonstrated a high effect only at the highest concentration (1000 µg/ml) with 72% cell death. The EDMSc fraction did not show any change of the cell viability (Figure 1C).

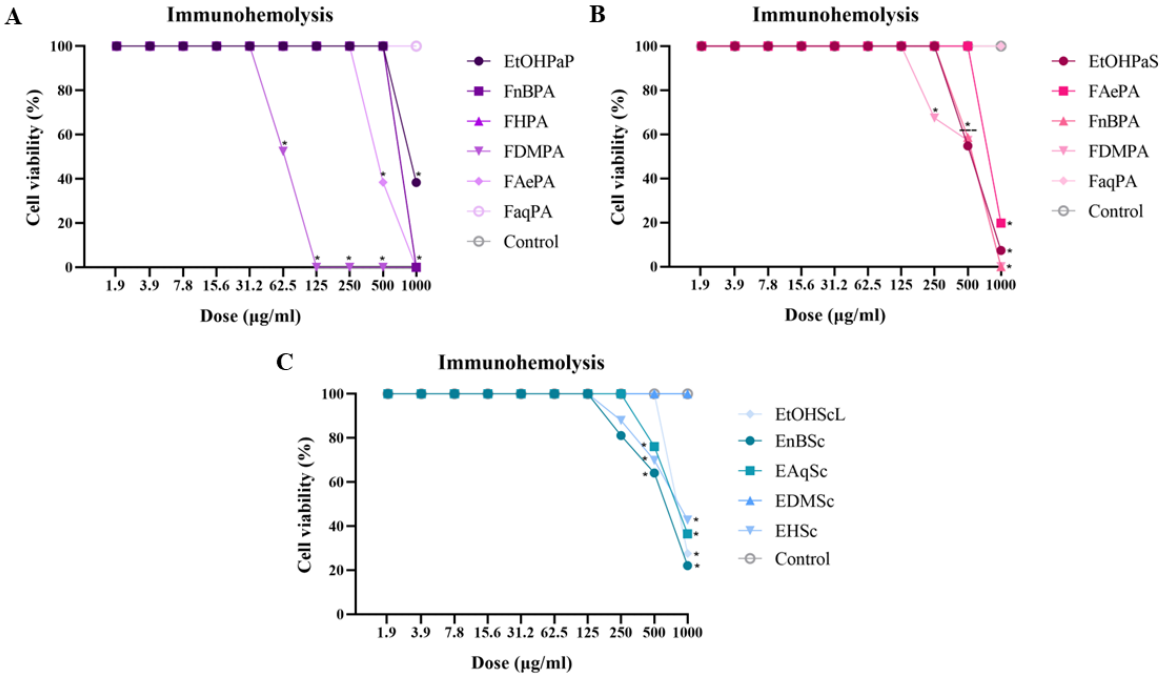

Figure 1. Immunohemolysis. RBCs were incubated with different concentrations (1000 µg/ml up to 1.95 µg/ml) of EtOHPaP, EtOHPaS, EtOHScL and its fractions. **(A)** RBCs incubated with EtOHPaP and fractions. **(B)** RBCs incubated with EtOHPaS and fractions. **(C)** RBCs incubated with EtOHScL and fractions. The value of * $p<0.05$.

The hemolytic activity is one of the indexes to evaluate the safety in the use of compounds. According to Costa et al. (2023), when molecules obtained of natural products shows high cytotoxicity, its application should be limited. Rodrigues and colleagues (2015) demonstrated by hemolysis assay the cytotoxic effect of the essential oil from *S. cumini* (Myrtaceae) and its compound α-pinene in human erythrocytes with half-maximal hemolytic concentration (HC_{50}) of 874.3 µg/mL and 233.3 µg/mL, respectively. In another study, the ethanolic extract of bark (EEB) and leaves (EEL) of *Ocotea minarum* (Lauraceae) induced hemolysis of human erythrocytes at concentration of 125 µg/ml in EEB (above 90%) and in EEL (above 70%) (Rodrigues et al., 2019).

The MTT test is also used to evaluate the cytotoxicity of samples and establish safe concentrations for subsequent biological analysis experiments. In this study, in addition to evaluating cytotoxicity in red blood cells, its toxicity was also investigated in HFF fibroblasts; Beas-2B and Pnt-2 cells, using concentrations lower than 1000 ug/ml due to the high toxicity of most samples when tested by hemolysis assay.

Regarding the MTT assays with HFF cells, it was observed that the FDMPA fraction, derived from the ethanol extract of the *P. americana* fruit peel, was the only sample with statistically significant cytotoxic effect, $p<0.05$, at concentrations of 400 up to 100 µg/ml (94%, 52% and 28%, respectively) (Figure 2A). All other fractions did not show any change in cell viability in the presence of different concentrations tested.

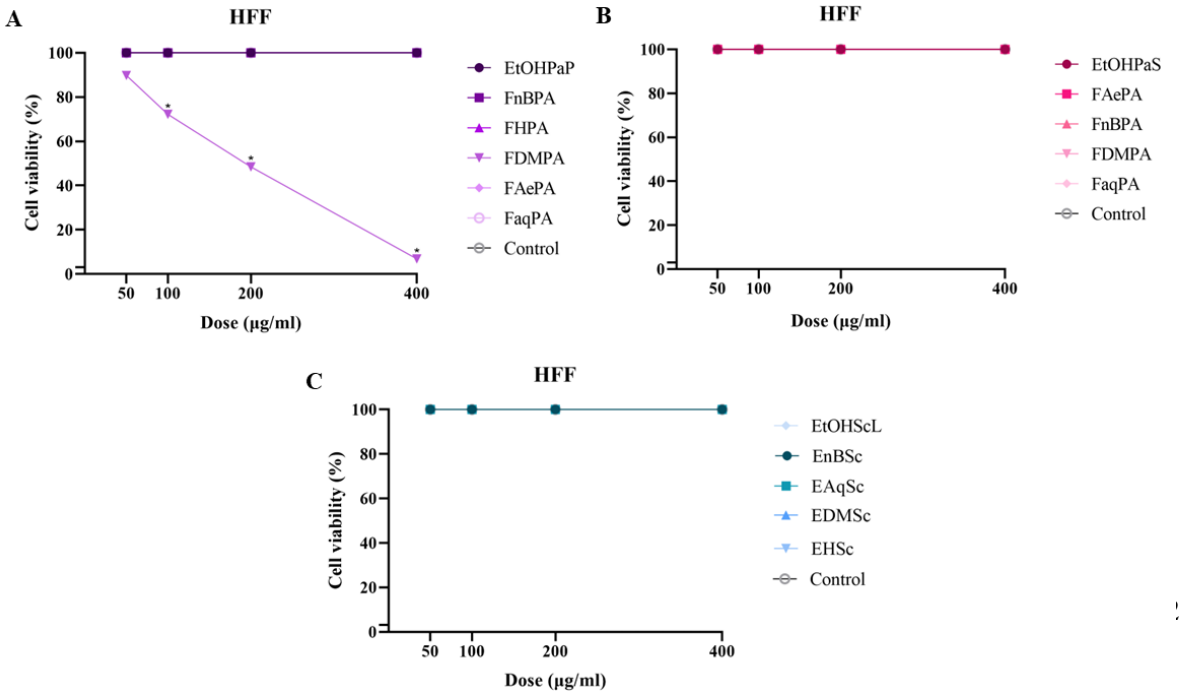

Figure 2. Evaluation of cellular viability of HFF. HFF cells were cultured with different concentrations (400 up to 50 µg/ml) of EtOHPaP, EtOHPaS, EtOHScL and its fractions during 24h. The results were expressed as the percentage of viable cells in the control. **(A)** Treatment with EtOHPaP and fractions. **(B)** Treatment with EtOHPaS and fractions. **(C)** Treatment with EtOHScL and fractions. The value of * $p<0.05$.

Similar results were also observed when the FDMPA fraction of the EtOHPaP, was tested with non-tumor prostate cells (Pnt-2), showing high cytotoxicity at concentrations at concentrations of 400 up to 100 µg/ml (86%, 72%, and 46%, respectively) (Figure 3A). The other samples, including the fractions of EtOHPaS and EtOHScL, exhibited cell viability above 80% at the evaluated concentrations.

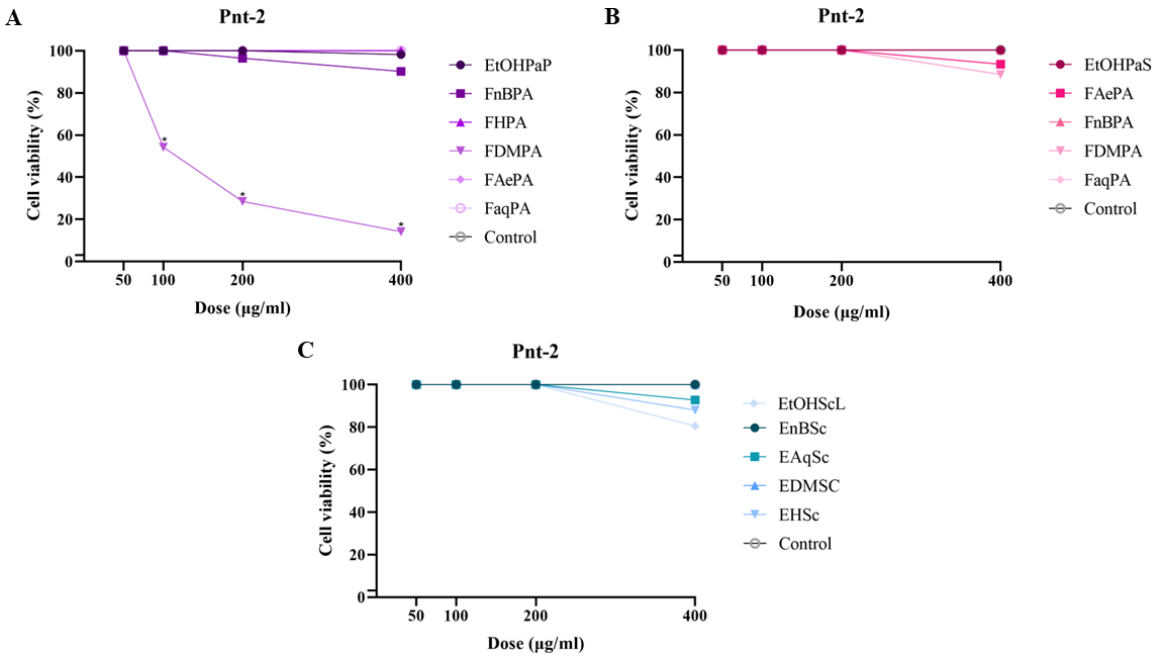

Figure 3. Evaluation of cellular viability of Pnt-2. Pnt-2 cells were cultured with different concentrations (400 up to 50 µg/ml) of EtOHPaP, EtOHPaS, EtOHScL and its fractions during 24h. The results were expressed as the percentage of viable cells in the control. **(A)** Treatment with EtOHPaP and fractions. **(B)** Treatment with EtOHPaS and fractions. **(C)** Treatment with EtOHScL and fractions. The value of * $p<0.05$.

When analyzing the cytotoxic effect of the samples in normal lung cells (Beas-2B), it was observed that the FDMPA fraction of EtOHPaP demonstrated high cell mortality when tested at concentrations of 400 up to 100 µg/ml (91%, 89% and 45%, respectively) (Figure 4A). The cells treated with EtOHPaS and its fractions demonstrated cell viability above 94% at all tested

concentrations (Figure 4B). Regarding the crude ethanolic extract of *S. cumini* leaves (EtOHScL) and EnBSc and EHSc fractions, a statistically significant cytotoxic effect, $p<0.05$, was observed only at the highest concentration of 400 µg/ml (21%, 39% and 29%, respectively) (Figure 4C).

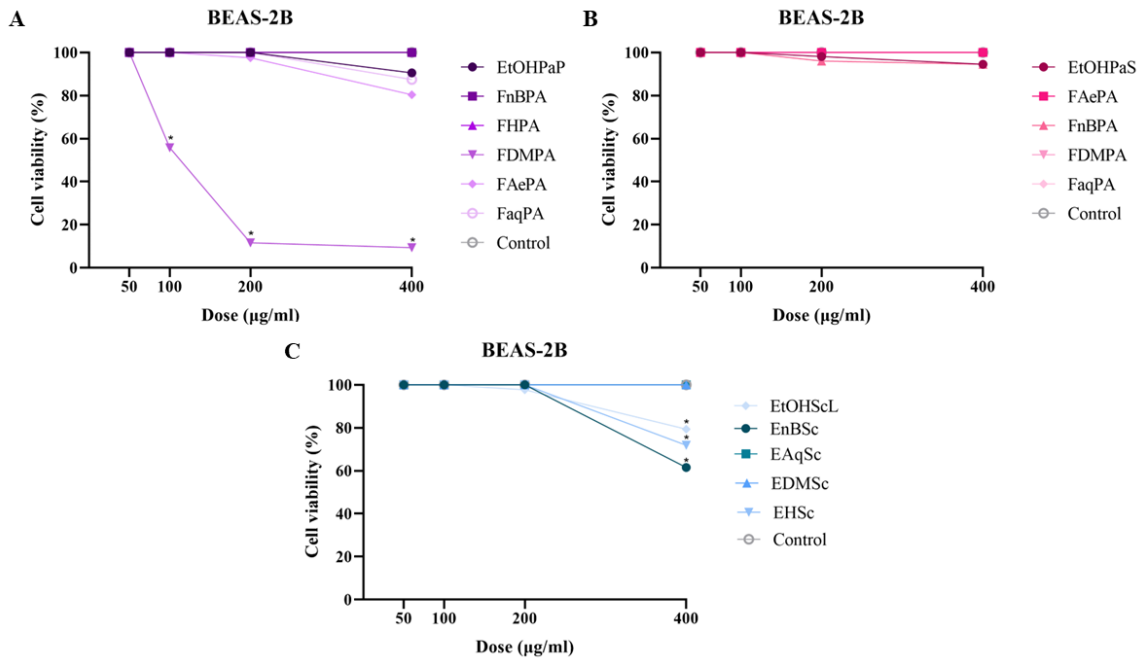

Figure 4. Evaluation of cellular viability of Beas-2B. Beas-2B cells were cultured with different concentrations (400 up to 50 µg/ml) of EtOHPaP, EtOHPaS and EtOHScL and its fractions during 24h. The results were expressed as the percentage of viable cells in comparation of the control. **(A)** Treatment with EtOHPaP and fractions. **(B)** Treatment with EtOHPaS and fractions. **(C)** Treatment with EtOHScL and fractions. The value of * $p<0.05$.

Singh et al. (2009) investigated the cytotoxicity of an aqueous extract of *Cinnamomum zeylanicum* bark (Lauraceae family), in normal cells (mouse hepatocytes and primary fibroblasts). The results showed a dose-dependent effect, highlighting the importance of selecting a significantly non-toxic dose to test biological effects. In another study, the MTT assay was used to determine a non-toxic concentration of *Persea americana* Mill oil and extracts against Vero cells, demonstrating that none of the concentrations tested reduced cell viability (Queiroz Junior et al., 2021). Therefore, cytotoxicity assays are important to understand the degree of toxicity of extracts of the plants and ensure their safe use (Dwivedi et al., 2021).

Based on the data described, the FDMPA fraction of EtOHPaP exhibited high cytotoxicity against all non-tumor cells tested, so it was excluded from further experiments, ensuring that the results were not compromised by the cytotoxic effect.

3.2. Antitumor Activity

To evaluate the inhibition of tumor cell proliferation and colony formation, the tumor cells were exposed to the different treatments of *P. americana* and *S. cumini* fractions.

Regarding to A549 tumor cell line, the FAePA fraction of EtOHPaS exhibited high antitumor activity at a concentration of 400 µg/ml, with a cell death percentage of 45% (Figure 5B). The remaining samples did not show significant results (Figures 5).

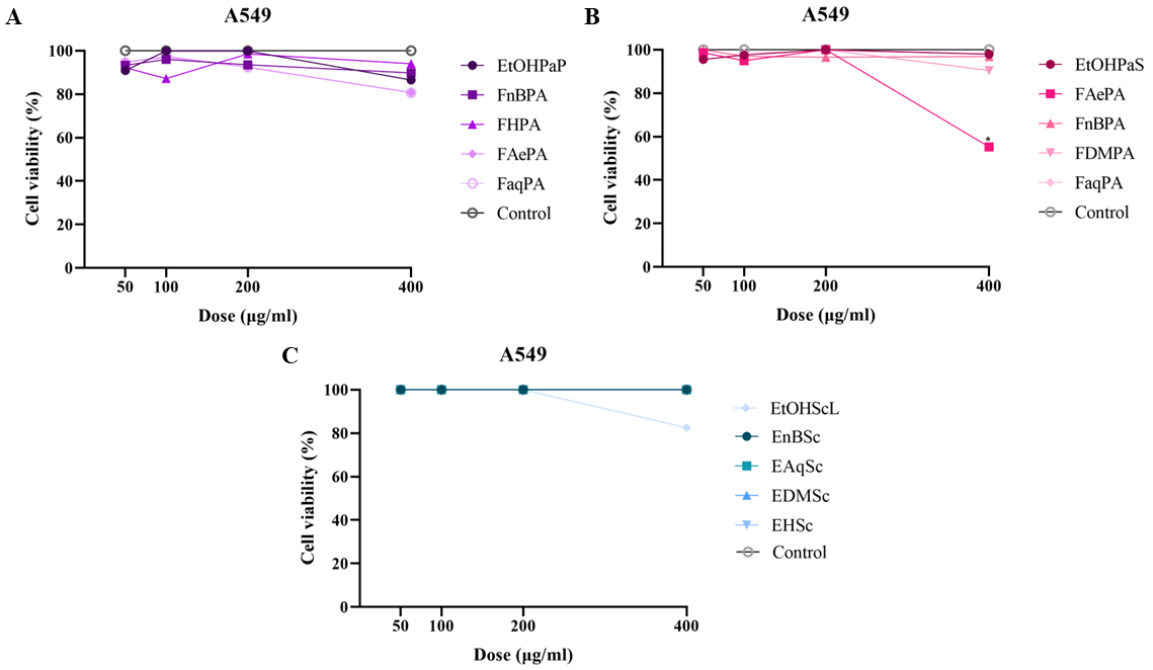

Figure 5. Antitumor effects on A549. A549 cells were treated with different concentrations (400 up to 50 µg/ml) of EtOHPaP, EtOHPaS, EtOHScL and its fractions during 24h. **(A)** Treatment with EtOHPaP and fractions. **(B)** Treatment with EtOHPaS and fractions. **(C)** Treatment with EtOHScL and fractions. The value of * $p<0.05$.

Concerning the antiproliferative activity of EtOHScL and its fractions on PC3 tumor cell line, EtOHScL, EHSc and EDMSc showed a statistically significant effect, $p<0.05$, however, the crude ethanolic extract *S. cumini* leaves (EtOHScL) had higher antitumor properties in 400 µg/ml (75% cell death) when compared with the other fractions. The EHSc fraction presented 51% cell death at 400 µg/ml while the EDMSc fraction demonstrated antitumor activity of 47% and 33% at concentrations of 400 and 200 µg/ml, respectively (Figure 6C). The samples obtained from *P. americana* fruit peel (Figure 6A) and seeds (Figure 6B) did not show significant effects.

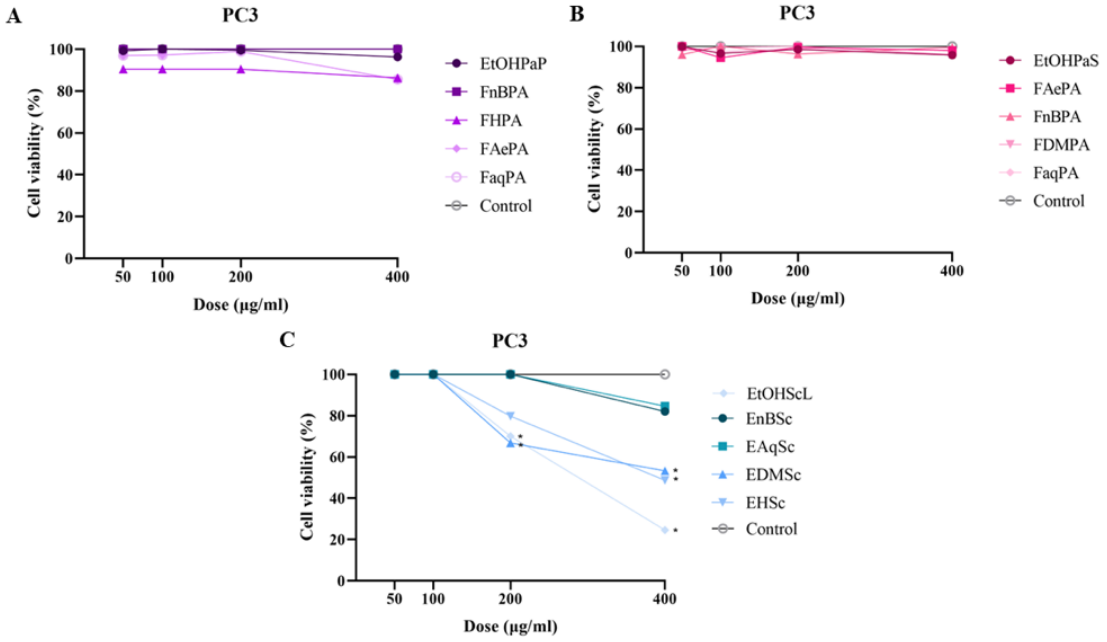

Figure 6. Antitumor effects on PC3. PC3 cells were treated with different concentrations (400 up to 50 µg/ml) of EtOHPaP, EtOHPaS, EtOHScL and its fractions during 24h. **(A)** Treatment with EtOHPaP and fractions. **(B)** Treatment with EtOHPaS and fractions. **(C)** Treatment with EtOHScL and fractions. The value of * $p<0.05$.

Thus, based on the results obtained, significant antitumor activity was demonstrated by the EtOHScL, EHSc and EDMSc fractions of the *S. cumini* leaves against the PC3 tumor cell and of the FAePA fraction of the *P. americana* seeds for A549 tumor cell. On the other hand, when analyzed in normal cells, all these fractions did not show any change in cell viability. In this regard, the ethanolic extracts derived from the *S. cumini* leaves and *P. americana* seeds might be deliberated as a valuable source of metabolites with potential uses as antitumor drug precursors.

Lung and prostate cancer are a significant health problem, therefore, novel strategies are needed to understand and overcome cellular mechanisms of therapeutic resistance (Bray et al., 2024). Tonga and colleagues (2024) evaluated the cytotoxic effect of *Hypodaphnis zenkeri* (Lauraceae) leaf extract on human prostate cancer cell lines. The results showed that the leaf extract had activity against PC3, with CC_{50} 184 µg/ml, and GC-mass analysis revealed that the extract had a high content of methyl esters with antioxidant, anti-inflammatory and anticancer activities. Regarding the Myrtaceae family, recent studies have demonstrated the anticancer activity of dichloromethane extract (IC_{50} of 24.31 µg/mL) and ethyl acetate extract (IC_{50} of 12.62 µg/mL) of *M. glazioviana* against human cervical cancer (HeLa). The results showed that the antiproliferative activity is due to the existence of phenolic compounds with anticancer

activity which occurs through several mechanisms that include an antiangiogenic effect, antimetastatic effect and an inhibitory effect on the NF-κB and AP-1 protein signaling cascade (Toledo et al., 2024).

To confirm the antitumor effect, the colony formation assay was performed only with samples that demonstrated statistically significant results in MTT assays. The results demonstrated that the EtOHScL and EDMSc, EHSc fractions significantly inhibited growth of PC3 tumor cell at the highest concentrations. EtOHScL demonstrated complete absence of tumor colony formation at concentrations of 400 up to 100 µg/ml and 65% inhibition when tested at 50 µg/ml. As for the EDMSc and EHSc fraction showed no colony formation at concentrations of 400 and 200 µg/ml. High inhibition was showed when tested for the EDMSc fraction at concentrations of 100 µg/ml and 50 µg/ml (95% and 73%, respectively) (Figure 7A). For the A549 tumor cell, the FAePA fraction from *P. americana* seeds inhibited 100% colony formation at concentrations of 400 and 200 µg/ml (Figure 7B).

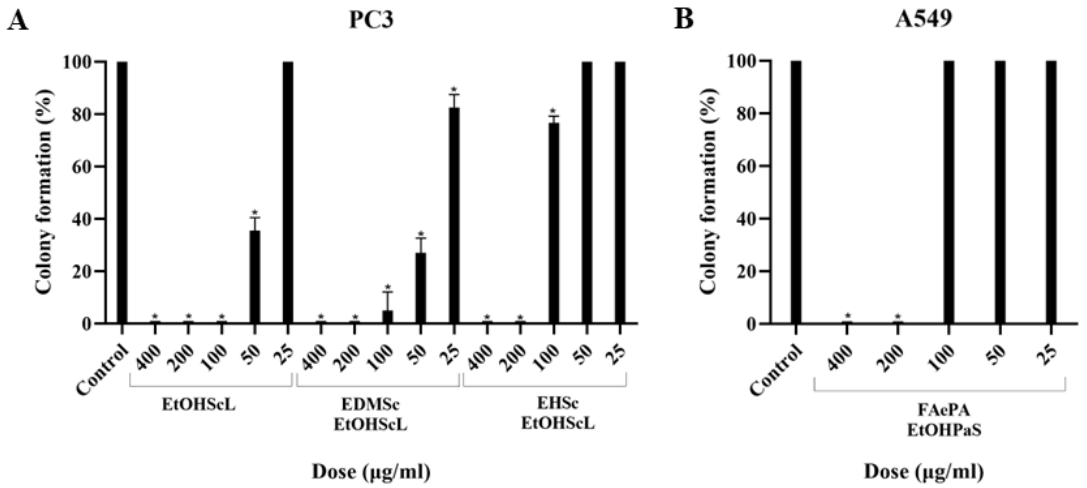

Figure 7. Effects of treatment on the clonogenic assay. Tumor cells were treated with different concentrations (400 up to 25 µg/ml) of EtOHPaP, EtOHPaS, EtOHScL and fractions for 7 days. **(A)** PC3 tumor cells. **(B)** A549 tumor cells. The value of * $p<0.05$.

Chemoprevention involving the use of plant-derived molecules has received considerable attention over decades. A recent study using the essential oil from leaves of *Myrcia splendens* (Myrtaceae) at a concentration of 40 µg/mL observed the potential antitumor effect in the complete inhibition of colony formation in A549 tumor cells. Furthermore, the essential oil was able to induce apoptosis of these tumor cells, demonstrated by the reduction in cytoplasmic volume, and DNA fragmentation (Montalvão et al. 2023).

3.3. Antiparasite Activity

The antiparasitic effect of the *P. americana* and *S. cumini* on *Toxoplasma gondii* infection in HFF cells was analyzed by plaque assays and shown in Figure 8.

The treatment of infected HFF host cells with EtOHPaP and its FHPA fraction showed significant results in lysis formation at the highest concentration (100 µg/ml), reducing the number of plaques by more than 40% (Figure 8A). In an analysis of the fractions obtained from the ethanol extract of *P. americana* seeds, the FDMPA fraction had a higher antiparasitic effect showed significant reduction in the lysis plaque formation when tested at a 100 and 50 µg/ml concentrations (56% and 37%, respectively), while the FAePA and FaqPA fractions demonstrated inhibition only at the highest concentration of 100 µg/ml (57% and 49%, respectively) (Figure 8B). Regarding to *S. cumini* leaves, all fractions demonstrated dose-response inhibition in the lysis plaques formation. However, EAqSc fraction allowed the highest antiparasitic effect at concentrations of the 100 and 50 µg/ml (72% and 51%, respectively) when compared to other fractions (Figure 8C). In contrast, the areas of plaques were statistically significant reduced in treatments with the FnBPA, FHPA fractions obtained from the ethanolic extract of *P. americana* fruit peel and with all fractions obtained from the *P. americana* seed and *S. cumini* leaf (Figure 8D, E, F), indicating that these treatment groups effectively inhibited the proliferation and reinvasion of tachyzoites in HFFs at different concentrations.

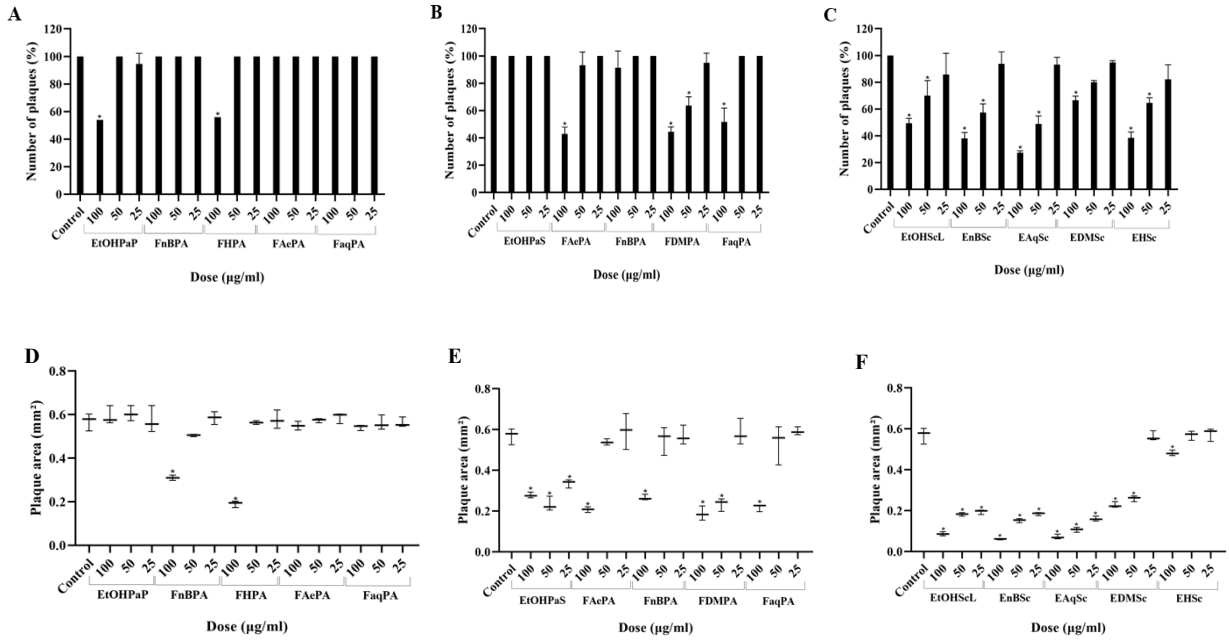

Figure 8. Effects of treatment on lysis formation by *T. gondii* strain RH WT. Plaque assay was carried out for 5 days with increasing concentrations of the EtOHPaP, EtOHPaS EtOHScL and fractions 100, 50 and 25µg/ml.

(A, D) Treatment with EtOHPaP and fractions. **(B, E)** Treatment with EtOHPaS and fractions. **(C, F)** Treatment with EtOHScL and fractions. The value of * $p<0.0001$.

Currently, molecules derived from natural products have been widely considered as an alternative against infectious diseases. A recent study reported that the methanolic extract of *Cinnamomum zeylanicum* (Lauraceae) reduced the replication of *T. gondii* tachyzoites in Vero cells by more than 80% and suggested that molecules with antiparasitic activity are able of modulating the immune response of host cells (Alanazi and Almohammed, 2022).

4. CONCLUSION

In conclusion, the results of this experimental study confirmed the antitumor activity of the FAePA fraction of EtOHPaS against A549 tumor cells and the inhibition of the growth of PC3 tumor cells by the EtOHScL, EDMSc and EHSc fractions. Furthermore, EtOHPaP and its fraction FHPA; the FAePA, FDMPA, FaqPA fractions of EtOHPaS; and EtOHScL and its fractions EnBSc, EAqSc, EDMSc, EHSc demonstrated antiparasitic properties by effectively inhibiting the proliferation and reinvasion of *T. gondii* tachyzoites. This study may open interesting new structure–activity relationship perspectives for molecules purified of the *P. americana* and *S. cumini* with pharmacological interest for future studies related to cancer and infectious diseases.

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