Brazilian Journal of Biology®

ISSN 1519-6984 (Print)
ISSN 1678-4375 (Online)

Original Article

Cytotoxicity of isolated compounds from *Picrasma crenata* (Vell.) Engl. in animal tumor cell (HTC)

Citotoxicidade de compostos isolados de *Picrasma crenata* (Vell.) Engl. em células tumorais animais (HTC)

B. L. Viega[a], A. M. Rocha[a], A. P. Berti[b], C. R. Novello[a], D. J. Nicolin[a], I. V. Almeida[c], V. E. P. Vicentini[d] and E. Düsman[a*]

[a]Universidade Tecnológica Federal do Paraná – UTFPR, Francisco Beltrão, PR, Brasil
[b]Universidade Estadual de Mato Grosso do Sul – UEMS, Dourados, MS, Brasil
[c]Universidade Federal Rural da Amazônia – UFRA, Capitão Poço, PA, Brasil
[d]Universidade Estadual de Maringá – UEM, Maringá, PR, Brasil

Abstract

The study aim was to evaluate the cytotoxic activity, using the MTT test [3-(4,5-Dimethilthiazol-2-yl)-2,5-diphenil tetrazolium bromide], from the crude extract of *Picrasma crenata* (Pau Tenente) and its isolated compounds, quassin and parain, in culture of rat liver tumor cells (HTC). The test was carried out exposing the cells for 24, 48 and 72 hours to concentrations of 5, 10, 50, 100, 200, 300, 400, 500 and 1000 µg of crude extract of Pau Tenente/mL of culture medium and 1, 5, 10, 15, 20, 40, 60, 80 and 100 µg of quassin or parain compounds/mL of culture medium. The absorbances averages results obtained showed that the crude extract did not present cytotoxicity for the HTC cells in all the concentrations and evaluated times. For quassin, the concentrations of 80 and 100 µg/mL were cytotoxic, after 72 hours of treatment. For parain, the concentrations of 1, 5, 20, 40, 60, 80 and 100 µg/mL, in 72 hours, were cytotoxic, revealing a new activity for this compound. Thus, the results demonstrate a first indication of the cytotoxic activity of compounds quassin and parain, adding an important social and economic value to them, and may have application in future research and in pharmaceutical industry.

Keywords: Quassin, Parain, HTC, MTT, antiproliferative, cytotoxicity.

Resumo

O objetivo do estudo foi avaliar a atividade citotóxica, por meio do teste MTT [brometo de 3-(4,5-dimetiltiazol-2-il)-2,5-difenil tetrazólio], do extrato bruto de *Picrasma crenata* (Pau Tenente) e seus compostos isolados, quassina e paraína, em cultura de células tumorais de fígado de rato (HTC). O teste foi realizado expondo as células por 24, 48 e 72 horas às concentrações de 5, 10, 50, 100, 200, 300, 400, 500 e 1000 µg de extrato bruto de Pau Tenente/mL de meio de cultura e 1, 5, 10, 15, 20, 40, 60, 80 e 100 µg de compostos de quassina ou paraína/mL de meio de cultura. Os resultados das médias de absorbâncias obtidos mostraram que o extrato bruto não apresentou citotoxicidade para as células HTC em todas as concentrações e tempos avaliados. Para quassina, as concentrações de 80 e 100 µg/mL foram citotóxicas, após 72 horas de tratamento. Para a paraína, as concentrações de 1, 5, 20, 40, 60, 80 e 100 µg/mL, em 72 horas, foram citotóxicas, revelando uma nova atividade para este composto. Assim, os resultados demonstram uma primeira indicação da atividade citotóxica dos compostos quassina e paraína, agregando-lhes importante valor social e econômico, podendo ter aplicação em pesquisas futuras e na indústria farmacêutica.

Palavras-chave: Quassina, Paraína, HTC, MTT, antiproliferativo, citotoxicidade.

1. Introduction

Medicinal plants are made up of various substances that can be used in therapeutic and alternative medicine, and the recognition of their efficiency and cultural influence has increased their use (Lapa et al., 2004; Bach et al., 2014; Gomes et al., 2014; Vendruscolo et al., 2022). Mainly due to the growing increase in the incidence of cancer and mortality, stimulating the search for new less invasive treatments, since the results are of great interest to the general population and to the pharmaceutical industry (Gomes et al., 2013).

Pau Tenente (*Picrasma crenata* (Vell.) Engl.) is a medicinal plant belonging to the Simaroubaceae family that can be found in the Brazilian states of Rio Grande do Sul, Santa Catarina, Paraná, São Paulo, Rio de Janeiro, Minas Gerais and Bahia, as well, also grows in the wild part of the Atlantic Forest (Pirani, 1997; Alves et al., 2014; Han et al., 2023).

*e-mail: edusman@utfpr.edu.br
Received: April 13, 2023 – Accepted: May 23, 2023

It is also present in the humid subtropical region of Misiones, Argentina, and is confused with other species of bitter wood, such as *Quassia amara* (Alves et al., 2014; Oliveira et al., 2005).

P. crenata is used in folk medicine as a febrifuge (Debenedetti et al., 2002; Oliveira et al., 2015; Xu et al., 2021); antimalarial (Alves et al., 2014; Debenedetti et al., 2002; Oliveira et al., 2015); anti-syphilitic, tonic, insecticide (Alves et al., 2014; Debenedetti et al., 2002); hypoglycemic (Gomes et al., 2014; Rajasekaran et al., 2005); slimming (Teixeira et al., 2014); antispasmodic (Oliveira et al., 2015); is used against Diabetes mellitus with empirical therapeutic indication (Alvarenga et al., 2017; Xu et al., 2021) and in the treatment of vitiligo (Monteiro et al., 2002). The low toxicity, hypoglycemic and anti-ulcer potential of its hydro alcoholic extract also was demonstrated (Novello et al., 2008; Kujawska and Schmeda-Hirschmann, 2022).

Studies show that *P. crenata* has alkaloids (Alves et al., 2014; Martínez et al., 2013) and quassinoids in their composition, these being the main constituents (Alves et al., 2014; Almeida et al., 2007; Xu et al., 2021). Quassinoids have shown a wide range of biological activity, such as antitumor, anti-leukemic, antimalarial, phytotoxic, insecticide, anti-inflammatory and anti-ulcer (Almeida et al., 2007; Boeno et al., 2021). Among the quassinoids present in *P. crenata*, quassin and parain can be mentioned, active ingredients that provide a characteristic and bitter flavor, being used in the production of bitter drinks (Gilbert, 2006). Quassin has potential use in the control of agricultural pests, as it has insecticidal activity (Gilbert, 2006; Viegas Junior, 2003), shows potential antifertility (Njar et al., 1995) and antimalarial (Raji and Bolarinwa, 1997; Houël et al., 2009; Mishra et al., 2010). Parain was less investigated from a biological point of view.

Considering that medicinal plants have innumerable biologically active chemical constituents, the aim of the present study was to investigate the action of Pau Tenente and its isolated compounds quassin and parain as cytotoxic agents against liver tumor cells of *Rattus norvegicus* (HTC).

2. Materials and Methods

2.1. Cell line

The cells derived from *Rattus norvegicus* (HTC) hepatoma, obtained from the Cell Bank of Rio de Janeiro-RJ-Brazil, were grown in 25 cm^2 culture flasks, containing 10 ml of DMEM culture medium (Dulbecco's Modified Eagle's Medium), supplemented with 10% fetal bovine serum and incubated in a BOD oven at 37 ºC.

2.2. Treatment solution

The treatment solution was the crude ethanolic extract (80%) of the dried bark stems of *Picrasma crenata* Engl. (family Simaroubaceae) (extracted in the dark at room temperature), and the quassin and parain compounds isolated from this plant, provided by Prof. Dr. Cláudio Roberto Novello (Novello et al., 2003, 2008; Cardoso et al., 2009). *P. crenata* was collected in Ortigueira (Paraná, Brazil) and a voucher specimen (HUM 8361) was deposited in the Herbarium of the State University of Maringá (Paraná, Brazil).

The crude extract of Pau Tenente (lyophilized) was dissolved in DMEM culture medium in the following concentrations: 5, 10, 50, 100, 200, 300, 400, 500 and 1000 µg/mL.

Quassin and parain, after lyophilization, were dissolved with culture medium plus dimethyl sulfoxide (DMSO), in the final concentration of 25 µL of DMSO/mL of culture medium for quassin and, 20 µL of DMSO/mL of medium culture for parain. Afterwards, this solution was used to prepare the quassin and parain treatment concentrations (1, 5, 10, 15, 20, 40, 60, 80 and 100 µg/mL of culture medium).

The concentrations tested were chosen in order to perform an evaluation screening, from low to high doses, as proposed by Meerloo et al. (2011), Vendruscolo et al. (2022) and Santos et al. (2023).

2.3. Cytotoxicity test

The MTT [3-(4,5-Dimethilthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] cytotoxicity activity test followed the protocol suggested by Mosmann (1983). 96-well cell culture plates were used, $2.0x10^4$ HTC cells/well were seeded. After 24 hours the culture medium was discarded and 100 µL of new medium was added for the groups: negative control (CO-) (culture medium); positive control (CO+) (cytotoxic agent methyl methane sulfonate (MMS - 500 µM); solvent control (CS) (25 µL of DMSO/mL of culture medium for quassin and 20 µL of DMSO/mL of culture medium for paraína), and treatments with different concentrations of crude extract of Pau Tenente and quassin and parain compounds.

The cells were incubated for 24, 48 and 72 hours and, after this time, the culture medium was replaced by 100 µL of serum-free medium, plus MTT (0.167 mg/mL). The plates were incubated for another 4 hours and, afterwards, the medium containing MTT was discarded and 100 µL of DMSO was added to the wells, for dilution of the formed formazan crystals.

The reading was performed in a micro plate reader at 492 nm and the data were expressed by means of the absorbances obtained in the three biological repetitions. The statistical analysis was performed with the data of the average absorbances, by one-way analysis of variance (ANOVA), followed by the Dunnet tests (α=0.05, $p<0.05$, n=3), to compare the absorbances negative control means with the absorbances of each treated group, at each treatment time, and Tukey (α=0.05, $p<0.05$, n=3), to compare the average absorbances between the concentrations of each treatment, at each time, between each concentration within the different times tested and between the same concentrations, at the same times, for quassin and parain, by the Action Stat Program.

The percentage values of cell viability (VC) were estimated by Equation 1.

$$VC = \left(\frac{A_T}{A_{CO-}} \right) \times 100 \quad (1)$$

Where:
VC: Cell viability [%];
A_T: Absorbance of treatment;
A_{CO-}: Absorbance of the negative control.

2.4. Adjusting polynomials from data

The polynomials adjusted by the empirical equations, which described the behavior of the HTC cells for each tested compound, were constructed from the experimentally obtained data and were valid only within the concentration and time ranges employed. The coefficients of each equation were obtained using the Scilab 6.0.1 software, using the "coeff" command, with a 95% confidence interval.

3. Results and Discussion

3.1. Crude extract

Statistical analysis of the cytotoxicity activity data of HTC cells treated for 24, 48 and 72 hours with the crude ethanol extract (80%) of Pau Tenente (Table 1) show that no concentration of this extract was statistically different from the control negative, in none of the evaluation times (24, 48 and 72 hours), indicating absence of cytotoxic effect. The cell viability of the groups treated with the extract was even greater than 99.80% (24 hours), 102.10% (48 hours) and 65.86% (72 hours). The lowest values of cell viability were at 72 hours, where the lowest concentration (5 µg/mL) showed mean absorbance statistically lower than the absorbances of the highest concentrations (500 and 1000 µg/mL).

These results corroborate those found by Novello et al. (2008), who also confirmed low toxic activity of the hydro alcoholic extract of *P. crenata* in vivo tests. In this experiment, the masses of the body, lungs, liver and heart of rats treated intravenously with 2500 and 5000 mg of the extract/kg remained unchanged. The study by Toma et al. (2002) also demonstrated absence of signs of toxicity and/or death of mice before oral administration (5000 mg/kg) and intraperitoneal (1000 mg/kg) of 70% ethanolic, 100% ethanol, dichloromethane and hexane extracts of *Q. amara*, a plant that has properties similar to the one studied in the present study (*P. crenata*) and which, in turn, did not show a cytotoxic effect to HTC cells with the use of crude ethanolic extract (80%).

However, in general, a slight proliferation of HTC cells can be seen in 48 hours, evidenced by their high cell viability at this time, and also by the fact that their average absorbances that were statistically higher and different from those obtained in times of 24 hours (concentrations of 10, 50, 100, 200, 300, 500 and 1000 µg/mL) and 72 hours (5, 10, 50 µg/mL), by the Tukey test. Maranhão et al. (2014), found similar results evaluating the aqueous extract of *Simarouba amara* Aublet, a medicinal plant also belonging to the Simaroubaceae family, where all concentrations of the extract induced proliferation of hepatocytes from treated rats, possibly due to their inhibitory action on development of free radicals by catechins, explained by the recovery of antioxidant enzymes and the decrease in lipid peroxidation.

However, it is worth noting that the ethanolic extracts of *Picrasma quassinoides*, a species of Picrasma native to Asia, showed cytotoxic effect for SiHa (cervical cancer) (Gong et al., 2020) and HepG2^{G12V} cells (liver cancer) (Xie et al., 2020), indicating an effect dependent on the species, soil and/or climate in which these species grow.

From the adjusted curve of the data obtained for the crude extract of Pau Tenente (Figure 1), it was possible to obtain the polynomial of Equation 2. Its coefficients (a, b and c) (Table 2) with lower and upper limits of 95% of confidence were obtained by the Scilab 6.0.1 software, using the "coeff" command. When the limits of the coefficients do not include zero as a result, it means that the data has been well adjusted, since the null solution (0,0,0) has no physical meaning, nor can there be a saddle point (point where the slope of the surface is null).

Table 1. Percentage of viability of HTC cells (VC) treated with different concentrations of the crude extract of Pau Tenente incubated for 24, 48 and 72 hours.

Groups	VC (%)		
	24h	48h	72h
CO-	100.00	100.00	100.00
CO+	57.16*	45.08*	50.91*
5µg/mL	100.73aB	102.10aB	65.86bB
10µg/mL	103.60aA	110.10aB	74.22aB
50µg/mL	102.36aB	120.80aB	81.63aB
100µg/mL	99.81aB	109.90aB	88.20aA
200µg/mL	100.62aB	105.50aB	89.69aA
300µg/mL	101.01aB	108.30aB	83.21aA
400µg/mL	106.62aA	107.90aA	87.24aA
500µg/mL	103.99aB	115.50aB	91.39bA
1000µg/mL	110.56aB	118.3aB	96.88bA

CO-: Negative Control; CO+: Positive Control.
*: Result statistically different from the negative control (Dunnet test, $p < 0.05$). Averages followed by the same lowercase letter do not differ statistically from each other, when comparing concentrations at the same time of assessment, and upper case, within the same concentration at different times of assessment (Tukey Test, $p < 0.05$).

Table 2. Coefficients of the fitted curve equation for the experiment with the crude extract of Pau Tenente.

Coefficients	Average Value	Minimum Value	Maximum Value
A	0.0181	0.01741	0.01878
B	$1.123.10^{-6}$	$6.029.10^{-7}$	$1.642.10^{-6}$
C	-0.0001877	-0.0001982	-0.0001772

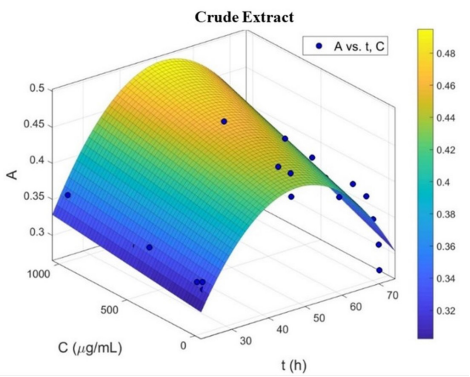

Figure 1. Adjusted curve for absorbance versus concentration versus time data, with crude Pau Tenente extract.

This adjustment was defined by the coefficient of determination (R^2), which must be the closest to a unit to be the best possible. In this specific case, R^2=0.8895 was reached, which shows that 88.95% of the results are explained by the factors studied, indicating a good relationship between the experimental data and the curve established by the empirical equation found. This result is excellent considering that experimental data do not always follow a pattern, as in the case of Thomas et al. (2006), who prove a good quality of the curve obtained by means of a larger one R^2. And, from these data, it is possible to perceive a greater dependence on absorbance, that is, on cell viability, with the treatment time than with the concentration of the crude extract.

$$A = a.t + b.t.C + c.t^2 \quad (2)$$

Where:
A: Absorbance;
C: Concentration of the tested compound (crude extract of Pau Tenente);
t: Treatment time.

3.2. Quassin

The data in Table 3 shows the results of the cytotoxicity activity test performed with the compound quassin. Statistical analysis indicates that no concentration of quassin, at the evaluation times of 24 and 48 hours, had a cytotoxic effect on the liver tumor cells of rats. In fact, cell viability was greater than 92.96% (24 hours) and 83.58% (48 hours). It is also possible to notice an increase in the absorbance of time from 24 to 48 hours for all concentrations tested, being statistically significant for the lowest concentrations (1, 10, 15, 20 and 60 µg/mL), indicating a proliferation of HTC cells within 48 hours, as well as that observed for the crude ethanolic extract.

The absence of cytotoxicity to quassin against cells derived from cervical cancer (HeLa) was also demonstrated by Fukamiya et al. (2005). Almeida et al. (2007), after 48 hours of treatment, also confirmed that quassin was one of the least efficient against cancer, testing it for the inhibitory effect against the activation of the Epstein-Barr virus antigen (EBV-EA), induced by 12-O-tetradecanoylforbol-13-acetate (TPA), in Raji cells. And, Xu et al. (2016), also showed that none of the quassinoid compounds, isolated from the 95% ethanolic extract of the *Picrasma quassioides* stems, showed cytotoxic activity.

At 72 hours, the highest concentrations (80 and 100 µg/mL) were statistically different from the negative control and cytotoxic to HTC cells, with cell viability of 60.76% (100 µg/mL) and 61.89% (80 µg/mL) (Table 3). This may have occurred because quassin, in high concentrations and treatment time, may have acted as a cytotoxic or antiproliferative agent, as shown by Mata-Greenwood et al. (2002), who assessed the cytotoxicity of the quassinoid brusatol identified the interruption of the cell cycle of tumor cells in phase G1.

This effect can already be seen in the time of 48 hours, where the comparison between the different concentrations tested, in the same evaluation time, already indicated a difference between the lowest concentrations (1, 5, 10, 20 and 60 µg/mL) and the higher concentrations (80 and 100 µg/mL), which showed lower average absorbances and, consequently, lower cell viability of HTC cells. Within 72 hours, this effect was maintained, with the lowest concentrations (1, 5, 10, 15, 20 and 40 µg/mL) also showing absorbances statistically different from the highest concentrations (60, 80 and 100 µg/mL).

From the adjusted curve of the data obtained for quassin (Figure 2), it was possible to obtain the polynomial of Equation 3, and the values of the coefficients of the generated equation are in Table 4. In this case, the R^2 was 0,8908, which is again an optimal value, as 89.08% of the results are explained by the factors studied. In this case, one can perceive, again, a greater dependence on absorbance (cell survival) over time than with concentration.

Table 3. Viability percentages of HTC cells (VC) treated with different concentrations of quassin, incubated for 24, 48 and 72 hours.

Groups	VC (%)		
	24h	48h	72h
CO-	100.00	100.00	100.00
CO+	57.29*	51.11*	45.95*
CS	91.06a	80.93*b	50.10*b
1 µg/mL	98.66aB	105.50bB	79.93bA
5 µg/mL	100.33aA	104.40bA	91.78bA
10 µg/mL	99.85aB	111.50bB	90.38bA
15 µg/mL	100.45aB	107.20bB	90.20bA
20 µg/mL	97.75aB	106.70bB	83.36bA
40 µg/mL	102.19aA	97.29aA	81.78bA
60 µg/mL	96.92aB	103.30bB	71.45bB
80 µg/mL	95.57aA	89.14bA	61.89*bA
100 µg/mL	92.96aA	83.58bA	60.76*bA

CO-: Negative Control; CO+: Positive Control; CS: Solvent Control. *: Result statistically different from the negative control (Dunnet test, $p<0.05$). Means followed by the same lowercase letter do not differ statistically from each other, when comparing the concentrations at the same evaluation time, and upper case, within the same concentration at the different evaluation times, using the Tukey test ($p<0.05$).

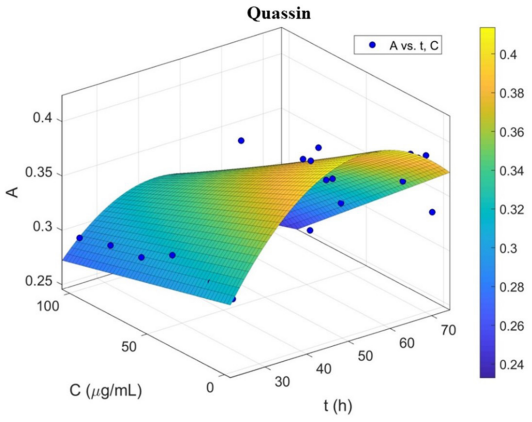

Figure 2. Adjusted curve for absorbance versus concentration versus time data, with quassin isolated from Pau Tenente.

These data can be confirmed by the statistical analysis of the cytotoxicity test (Table 3), since only for higher concentrations and times the absorbance declines with more intensity, demonstrating the cellular unfeasibility and the existing cytotoxicity.

$$A = a + b.t + c.t.C + d.t^2 \quad (3)$$

Where:
A: Absorbance;
C: Concentration of the tested compound (Pau Tenente quassin);
t: Treatment time.

3.4. Parain

The data in Table 5 shows the results of the parain cytotoxicity activity test. Statistical analysis indicates that no concentration of parain was statistically different from the negative control in the evaluation times of 24 and 48 hours, with no cytotoxic effect, similar to that found with crude extract and quassin. Cell viability in these cases was greater than 96.84% (24 hours) and 94.39% (48 hours). It can also be noted an increase in absorbance from 24 to 48 hours, statistically significant ($p \leq 0.05$), for concentrations of 5, 10, 15 and 20 µg/mL, meaning a proliferation behavior of HTC cells within 48 hours, similar to that observed with the crude extract and the quassin compound.

At 72 hours, the concentrations of 1, 5, 20, 40, 60, 80 and 100 µg/mL of parain were statistically different from the negative control, promoting the mortality of HTC tumor cells, with cell viabilities below 72.74% and reaching 53.40% (100 µg/mL) (Table 5). Only concentrations of 10 and 15 µg/mL were not cytotoxic at 72 hours, as well as being statistically different from the concentration of 100 µg/mL. The presence of a ketocarbonyl group in the in the C ring of isoparain resulted in low cell viability for SH-SY5Y cells co-treated with this compound and H_2O_2, by the MTT method (Zhao et al., 2019). Therefore, the chemical structures of the quassinoids isolated from *P. crenata* can influence their biological activities.

These results are relevant, because Guo et al. (2005) already highlighted the importance of the search for new natural sources of quassinoids as anticancer drugs. The cytotoxic activity found in the present study, with parain, may have been due to the inhibition of NADH oxidase of cell membranes, an effect proven by the glaucarubolone quassinoid in HeLa cells (Morré et al., 1998).

From the adjusted curve of the data obtained for parain (Figure 3), it was possible to obtain the polynomial of Equation 4, and the coefficients of the equation are plotted in Table 6. In this case, the R^2 was 0,7633, which is also a value well, considering that experimental data does not always follow a pattern perfectly and that biological systems are very sensitive. The similarity between Equations 3 and 4 is observed, but the values of the coefficients change. This means that time has a greater influence on absorbance (cell viability), since the longer the time, the lower the absorbance and, consequently, the lower the viability of these cells.

Table 4. Coefficients of the fitted curve equation for the experiment with quassin from Pau Tenente.

Coefficients	Average Value	Minimum Value	Maximum Value
A	0.1322	0.08566	0.1788
B	0.0104	0.008192	0.01261
C	$-1.721.10^{-5}$	$-2.075.10^{-5}$	$-1.367.10^{-5}$
D	$-9.732.10^{-5}$	-0.00012	$-7.461.10^{-5}$

Table 5. Percentage of viability of HTC cells (VC) treated with different concentrations of parain, incubated for 24, 48 and 72 hours.

Groups	VC (%)		
	24h	48h	72h
CO-	100.00	100.00	100.00
CO+	57.13*	51.53*	51.55*
CS	98.41a	90.74b	70.00*a
1 µg/mL	105.05aA	106.40aA	64.12*aA
5 µg/mL	98.49aB	108.00aB	64.53*aA
10 µg/mL	100.96aB	109.70bB	77.74Ba
15 µg/mL	99.38aB	113.70bB	81.25Ba
20 µg/mL	98.53aB	109.00aB	72.74*aA
40 µg/mL	102.87aA	104.30aA	70.72*aA
60 µg/mL	98.74aB	106.00aB	65.62*aA
80 µg/mL	96.84aA	98.85aA	60.86*aA

CO-: Negative Control; CO+: Positive Control; CS: Solvent Control. *: Result statistically different from the negative control (Dunnet test, $p<0.05$). Means followed by the same lowercase letter do not differ statistically from each other, when comparing the concentrations at the same evaluation time, and upper case, within the same concentration at the different evaluation times, using the Tukey test ($p<0.05$).

Table 6. Coefficients of the fitted curve equation for the experiment with parain from Pau Tenente.

Coefficients	Average Value	Minimum Value	Maximum Value
A	0.1442	0.08068	0.2078
B	0.009209	0.006197	0.01222
C	$-1.103.10^{-5}$	$-1.585.10^{-5}$	$-6.199.10^{-6}$
D	$-8.15.10^{-5}$	-0.0001125	$-5.05.10^{-5}$

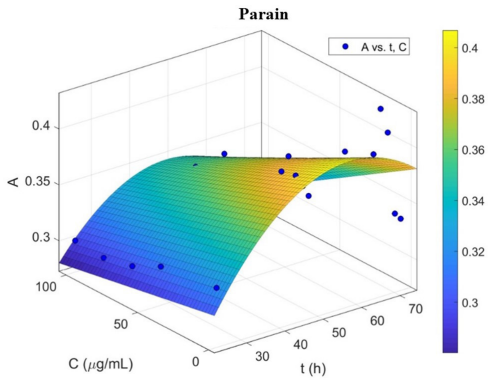

Figure 3. Adjusted curve for absorbance versus concentration versus time data, with parain isolated from Pau Tenente.

In addition, this equation and its coefficients corroborate with the statistical analyzes, which show cytotoxicity of parain from the lowest concentrations tested, indicating that the inducing effect of apoptosis or cell death occurs due to the presence of the compound, regardless of the concentration tested, but influenced by exposure time.

$$A = a + b.t + c.t.C + d.t^2 \quad (4)$$

Where:
A: Absorbance;
C: Concentration of the tested compound (Pau Tenente parain);
t: Treatment time.

4. Conclusions

Based on the experimental results, we highlight the isolated compound parain, which under experimental conditions, showed cytotoxic activity against the tested HTC cells, with a good extraction yield (0.132%), when compared to quassin (0.046%), and potential for future research and applications in the pharmaceutical industry. Although the highest extraction yield was from the crude ethanolic extract (4.5%), it did not show any cytotoxic activity. Thus, the results prove the cytotoxic activity of quassin and parain, being of interest to the general population as important information, since data and activities in the literature are scarce.

Acknowledgements

Universidade Tecnológica Federal do Paraná for the TCC scholarship.

References

ALMEIDA, M.M.B., ARRIAGA, A.M.C., SANTOS, A.K.L., LEMOS, T.L.G., BRAZ-FILHO, R. and VIEIRA, I.J.C., 2007. Ocorrência e atividade biológica de quassinóides da última década. *Quimica Nova*, vol. 30, no. 4, pp. 935-951. http://dx.doi.org/10.1590/S0100-40422007000400033.

ALVARENGA, C.F., LIMA, K.M.N., MOLLICA, L.R., AZEREDO, L.O. and CARVALHO, C., 2017. Uso de plantas medicinais para o tratamento do Diabetes mellitus no Vale do Paraíba – SP. *Revista Ciencias de la Salud*, vol. 2, no. 2, pp. 36-44.

ALVES, I.A.B.S., MIRANDA, H.M., SOARES, L.A.L. and RANDAU, K.P., 2014. Simaroubaceae family: botany, chemical composition and biological activities. *Revista Brasileira de Farmacognosia,* vol. 24, pp. 481-501. http://dx.doi.org/10.1016/j.bjp.2014.07.021

BACH, H.G., WAGNER, M.L., RICCO, R.A. and FORTUNATO, R.H., 2014. Sale of medicinal herbs in pharmacies and herbal stores in Hurlingham district, Buenos Aires, Argentina. *Revista Brasileira de Farmacognosia*, vol. 24, no. 2, pp. 258-264. http://dx.doi.org/10.1016/j.bjp.2014.04.001.

BOENO, S.I.S., PASSOS, M.S., FÉLIX, M., VIEIRA, M.G.C., CARVALHO JÚNIOR, A.R., BRAZ-FILHO, R. and VIEIRA, I.J.C., 2021. Biological activity and 13C NMR spectral data of skeleton type C20 quassinoids (1985-2019). In: A. RAHMAN, ed. *Studies in natural products chemistry*. USA: Elsevier, chap. 4, vol. 68, pp. 97-172. http://dx.doi.org/10.1016/B978-0-12-819485-0.00011-6.

CARDOSO, M.L.C., KAMEI, M.S., NUNES, R.F., LAZERI, N.S., NETO, J.R.S., NOVELLO, C.R. and BRUSCHI, M.L., 2009. Development and validation of an HPLC method for analysis of *Picrasma crenata*. *Journal of Liquid Chromatography & Related Technologies*, vol. 32, no. 1, pp. 72-79. http://dx.doi.org/10.1080/10826070802548663.

DEBENEDETTI, S., MUSCHIETTI, L.M., BAREN, C.V., CLAVIN, M., BROUSSALIS, A., MARTINO, V. and STEELE, J., 2002. In vitro antiplasmodial activity of extracts of Argentinian plants. *Journal of Ethnopharmacology*, vol. 80, no. 2-3, pp. 163-166. http://dx.doi.org/10.1016/S0378-8741(02)00024-7. PMid:12007706.

FUKAMIYA, N., LEE, K.H., MUHAMMAD, I., MURAKAMI, C., OKANO, M., HARVEY, I. and PELLETIER, J., 2005. Structure–activity relationships of quassinoids for eukaryotic protein synthesis. *Cancer Letters*, vol. 220, no. 1, pp. 37-48. http://dx.doi.org/10.1016/j.canlet.2004.04.023.

GILBERT, B., 2006. Produtos naturais industrializáveis da Amazônia. *Revista Fitos*, vol. 2, no. 3, pp. 30-38. http://dx.doi.org/10.32712/2446-4775.2006.57.

GOMES, M.A., PRIOLLI, D.G., TRALHÃO, J.G. and BOTELHO, M.F., 2013. Carcinoma hepatocelular: epidemiologia, biologia, diagnóstico e terapias. *Revista da Associação Médica Brasileira*, vol. 59, no. 5, pp. 514-524. http://dx.doi.org/10.1016/j.ramb.2013.03.005.

GOMES, P.R.M., FIRMO, W.C.A. and VILANOVA, C.M., 2014. Estudo etnobotânico de plantas medicinais hipoglicemiantes no Bairro Maracanã no Município de São Luís, Maranhão, Brasil. *Scientia Plena*, vol. 10, no. 9, pp. 1-11.

GONG, Y.X., LIU, Y., JIN, Y.H., JIN, M.H., HAN, Y.H., LI, J., SHEN, G., XIE, D., REN, C., YU, L., LEE, D., KIM, J., JO, Y., KWON, J., LEE, J., PARK, J.H., KWON, T., CUI, Y. and SUN, H., 2020. *Picrasma quassioides* extract elevates the cervical cancer cell apoptosis through ROS-mitochondrial axis activated p38 mapk signaling pathway. *In Vivo*, vol. 34, pp. 1813-1823. http://dx.doi.org/10.21873/invivo.11977.

GUO, Z., VANGAPANDU, S., SINDELAR, R.W., WALKER, L.A. and SINDELAR, R.D., 2005. Biologically active quassinoids and their chemistry: potential leads for drug design. *Current Medicinal Chemistry*, vol. 12, no. 2, pp. 173-190. http://dx.doi.org/10.2174/0929867053363351.

HAN, J.L., LV, T.M., SONG, S.J. and HUANG, X.X., 2023. β-carboline alkaloids in Picrasma quassioides and their chemotaxonomic significance. *Biochemical Systematics and Ecology*, vol. 107, pp. 104614. http://dx.doi.org/10.1016/j.bse.2023.104614.

HOUËL, E., BERTANI, S., BOURDY, G., DEHARO, E., JULLIAN, V., VALENTIN, A. and STIEN, D., 2009. Quassinoid constituents of *Quassia amara* L. leaf herbal tea. Impact on its antimalarial activity and cytotoxicity. *Journal of Ethnopharmacology*, vol. 126, no. 1, pp. 114-118. http://dx.doi.org/10.1016/j.jep.2009.07.037. PMid:19665539.

KUJAWSKA, M. and SCHMEDA-HIRSCHMANN, G., 2022. The use of medicinal plants by Paraguayan migrants in the Atlantic Forest of Misiones, Argentina, is based on Guaraní tradition, colonial and current plant knowledge. *Journal of Ethnopharmacology*, vol. 283, pp. 114702. http://dx.doi.org/10.1016/j.jep.2021.114702. PMid:34627987.

LAPA, A.J., SOUCCAR, C., LIMA-LANDMAN, M.T.R., GODINHO, R.O. and NOGUEIRA, T.C.M.L., 2004. Farmacologia e toxicologia de produtos naturais. In C.M.O. Simões, E.P. Schenkel, G. Gosmann, J.C.P. Mello, L.A. Mentz and P.R. Petrovick, eds. *Farmacognosia: da planta ao medicamento*. Porto Alegre, RS: Editora da UFRGS/ Editora da UFSC, p. 247-262.

MARANHÃO, H.M., VASCONCELOS, C.F., ROLIM, L.A., ROLIM NETO, P.J., SILVA FILHO, R.C., FERNANDES, M.P., COSTA-SILVA, J.H., ARAÚJO, A.V. and WANDERLEY, A.G., 2014. Hepatoprotective effect of the aqueous extract of *Simarouba amara* Aublet (Simaroubaceae) stem bark against carbon tetrachloride (CCl_4)-induced hepatic damage in rats. *Molecules (Basel, Switzerland)*, vol. 19, no. 11, pp. 17735-17746. http://dx.doi.org/10.3390/molecules191117735. PMid:25365298.

MARTÍNEZ, M.L., POSER, G.V., HENRIQUES, A., GATUSSO, M. and ROSSINI, C., 2013. Simaroubaceae and Picramniaceae as potential sources of botanical pesticides. *Industrial Crops and Products*, vol. 44, pp. 600-602. http://dx.doi.org/10.1016/j.indcrop.2012.09.015.

MATA-GREENWOOD, E., CUENDET, M., SHER, D., GUSTIN, D., STOCK, W. and PEZZUTO, J.M., 2002. Brusatol-mediated induction of leukemic cell differentiation and G(1) arrest is associated with down-regulation of c-myc. *Leukemia*, vol. 16, no. 11, pp. 2275-2284. http://dx.doi.org/10.1038/sj.leu.2402696. PMid:12399973.

MEERLOO, J.V., KASPERS, G.J.L. and CLOOS, J., 2011. Cell sensitivity assays: the MTT assay. *Methods in Molecular Biology (Clifton, N.J.)*, vol. 731, pp. 237-245. http://dx.doi.org/10.1007/978-1-61779-080-5_20. PMid:21516412.

MISHRA, K., CHAKRABORTY, D., PAL, A. and DEY, N., 2010. *Plasmodium falciparum*: *in vitro* interaction of quassin and neo-quassin with artesunate, a hemisuccinate derivative of artemisinin. *Experimental Parasitology*, vol. 124, no. 4, pp. 421-427. http://dx.doi.org/10.1016/j.exppara.2009.12.007. PMid:20036657.

MONTEIRO, V.F.F., MATHIAS, L., VIEIRA, I.J.C., SCHRIPSEMA, J. and BRAZ-FILHO, R., 2002. Prenylated coumarins, chalcone and new cinnamic acid and dihydrocinnamic acid derivatives from *Brosimum gaudichaudii*. *Journal of the Brazilian Chemical Society*, vol. 13, no. 2, pp. 281-287. http://dx.doi.org/10.1590/S0103-50532002000200023.

MORRÉ, D.J., GRIECO, P.A. and MORRÉ, D.M., 1998. Mode of action of the anticancer quassinoids - inhibition of the plasma membrane NADH oxidase. *Life Sciences*, vol. 63, no. 7, pp. 595-604. http://dx.doi.org/10.1016/S0024-3205(98)00310-5. PMid:9718085.

MOSMANN, T., 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *Journal of Immunological Methods*, vol. 65, no. 1-2, pp. 55-63. http://dx.doi.org/10.1016/0022-1759(83)90303-4. PMid:6606682.

NJAR, V.C., ALAO, T.O., OKOGUN, J.I., RAJI, Y., BOLARINWA, A.F. and NDUKA, E.U., 1995. Antifertility activity of *Quassia amara*: quassin inhibits the setroidogenesis in rat Leydig cells *in vitro*. *Planta Medica*, vol. 61, no. 2, pp. 180-182. http://dx.doi.org/10.1055/s-2006-958044. PMid:7753928.

NOVELLO, C.R., FERREIRA, A.G., MARQUES, L.C. and CORTEZ, D.A.G., 2003. Quassinoids from *Picrasma crenata*. *Natural Product Research*, vol. 17, no. 3, pp. 145-148. http://dx.doi.org/10.1080/1478641031000104082. PMid:12737395.

NOVELLO, C.R., BAZOTTE, R.B., BERSANI-AMADO, C.A., MARQUES, L.C. and CORTEZ, D.A., 2008. Toxicological and pharmacological studies of *Picrasma crenata* (Vell.) Engler (Simaroubaceae) in mice and rats. *Latin American Journal of Pharmacy*, vol. 27, no. 3, pp. 345-348.

OLIVEIRA, F., AKISUE, G. and AKISUE, M.K., 2005. *Farmacognosia*. São Paulo: Editora Atheneu.

OLIVEIRA, F.A., CONDE, B.E., GOMES, F.T., FONSECA, A.S. and CAMPOS, B.C., 2015. Potencial de cura e segurança do uso de plantas medicinais na utilização do método da biodigital como tratamento alternativo no grupo de medicina alternativa em Juiz de Fora, MG. *Perspectivas Online: Biológicas & Saúde*, vol. 17, no. 5, pp. 47-63. http://dx.doi.org/10.25242/88685172015634.

PIRANI, J.R., 1997. *Simaroubáceas*. Santa Catarina: Flora Ilustrada Catarinense.

RAJASEKARAN, S., SIVAGNANAM, K. and SUBRAMANIAN, S., 2005. Antioxidant effect of *Aloe vera* gel extract in streptozotocin-induced diabetes in rats. *Pharmacological Reports*, vol. 57, no. 1, pp. 90-96. PMid:15849382.

RAJI, Y. and BOLARINWA, A.F., 1997. Antifertility activity of *Quassia amara* in male rats - *in vivo* study. *Life Sciences*, vol. 61, no. 11, pp. 1067-1074. http://dx.doi.org/10.1016/S0024-3205(97)00615-2. PMid:9307052.

SANTOS, V.A.M., BRESSIANI, P.A., ZANOTTO, A.W., ALMEIDA, I.V., BERTI, A.P., LUNKES, A.M., VICENTINI, V.E.P. and DUSMAN, E. 2023. Cytotoxicity of capsaicin and its analogs in vitro. *Journal of Biology = Revista Brasileira de Biologia*, vol. 83, pp. e268941. https://doi.org/10.1590/1519-6984.268941.

TEIXEIRA, G.S., FREIRE, R.A., FONSECA, M.I.L. and BIESKI, I.G.C., 2014. Plantas medicinais, fitoterápicos e/ou nutracêuticos utilizados no controle da obesidade. *FLOVET*, vol. 1, no. 6, pp. 27-42.

THOMAS, C., ANDRADE, C.M., SCHNEIDER, P.R. and FINGER, C.A.G., 2006. Comparação de equações volumétricas ajustadas com dados de cubagem e análise de tronco. *Ciência Florestal*, vol. 16, no. 3, pp. 319-327. http://dx.doi.org/10.5902/198050981911.

TOMA, W., GRACIOSO, J.S., ANDRADE, F.D., HIRUMA-LIMA, C.A., VILEGAS, W. and BRITO, A.R.S., 2002. Antiulcerogenic activity of four extracts obtained from the bark wood of *Quassia amara* L. (Simaroubaceae). *Biological & Pharmaceutical Bulletin*, vol. 25, no. 9, pp. 1151-1155. http://dx.doi.org/10.1248/bpb.25.1151.

VENDRUSCOLO, I., VENTURELLA, S.R.T., BRESSIANI, P.A., MARCO, I.G., NOVELLO, C.R., ALMEIDA, I.V., VICENTINI, V.E.P., MELLO, J.C.P. and DÜSMAN, E., 2022. Cytotoxicity of extracts and compounds isolated from Croton echioides in animal tumor cell (HTC). *Brazilian Journal of Biology = Revista Brasileira de Biologia*, vol. 82, pp. e264356. http://dx.doi.org/10.1590/1519-6984.264356. PMid:36169527.

VIEGAS JUNIOR, C., 2003. Terpenos com atividade inseticida: uma alternativa para o controle químico de insetos. *Quimica Nova*, vol. 26, no. 3, pp. 390-400. http://dx.doi.org/10.1590/S0100-40422003000300017.

XIE, D.P., GONG, Y.X., JIN, Y.H., REN, C.X. and SUN, H.N., 2020. Anti-tumor properties of *Picrasma quassioides* extracts in H-Rasg12v liver cancer are mediated through ROS-dependent mitochondrial dysfunction. *Anticancer Research*, vol. 40, no. 7, pp. 3819-3830. http://dx.doi.org/10.21873/anticanres.14371. PMid:32620621.

XU, J., XIAO, D., SONG, W.W., CHEN, L., LIU, W.Y., XIE, N. and QU, W., 2016. Quassinoids from the stems of *Picrasma quassioides* and their cytotoxic and NO production-inhibitory activities. *Fitoterapia*, vol. 110, pp. 13-19. http://dx.doi.org/10.1016/j.fitote.2016.02.004. PMid:26851344.

XU, W.H., LIANG, Z.S., SU, X.M., HE, R.X. and LIANG, Q., 2021. Genus Picrasma: a comprehensive review on its ethnopharmacology, phytochemistry and bioactivities. *Journal of Ethnopharmacology*, vol. 280, pp. 114441. http://dx.doi.org/10.1016/j.jep.2021.114441. PMid:34302942.

ZHAO, W.Y., SONG, X.Y., ZHAO, L., ZOU, C.X., ZHOU, W.Y., LIN, B., YAO, G.D., HUANG, X.X. and SONG, S.J., 2019. Quassinoids from Picrasma quassioides and their neuroprotective effects. *Journal of Natural Products*, vol. 82, no. 4, pp. 714-723. http://dx.doi.org/10.1021/acs.jnatprod.8b00470. PMid:30917277.