Thermodynamic and kinetic modeling of the protection of mammalian cells against ethanol-induced damage by extracts of *Jatropha curcas* (Euphorbiaceae)

Nathalia B. S. Cardoso¹ | Mabel B. Esteves² | Carlos F. S. Bonafe³ | Luis C. M. S. Paulillo² | Jose A. C. Bispo⁴

1Programa de Pós-Graduação em Ciências Farmacêuticas, Universidade Estadual de Feira de Santana (UEFS), Feira de Santana, Brazil
2Programa de Pós-Graduação em Tecnologias Aplicáveis à Bioenergia, Centro Universitário de Tecnologia e Ciências de Salvador (UNITFC), Salvador, Brazil
3Departamento de Bioquímica e Biologia Tecidual, Instituto de Biologia, Universidade Estadual de Campinas (UNICAMP), Campinas, Brazil
4Departamento de Tecnologia, Universidade Estadual de Feira de Santana (UEFS), Feira de Santana, Brazil

Correspondence
Jose Ailton Conceicao Bispo,
Departamento de Tecnologia,
Universidade Estadual de Feira de Santana (UEFS), Av. Transnordestina s/n, Bairro Novo Horizonte, 44036-900, Feira de Santana, BA, Brazil.
Email: jacobispo1@gmail.com

Funding information
Fundação de Amparo à Pesquisa do Estado de São Paulo; Coordenação de Aperfeiçoamento de Pessoal de Nível Superior; Conselho Nacional de Desenvolvimento Científico e Tecnológico

The medicinal plant *Jatropha curcas* exerts beneficial effects against ethanol toxicity in humans. In this work, we investigated the protective effect of three extracts (ethanol [Et], vegetal glycerin [VG], and propylene glycol [PG]) of *J. curcas* leaves against Et-induced damage in human peripheral blood mononuclear cells. The cytotoxicity of the extracts alone or in combination with each other in mononuclear cells was also assessed. A thermodynamic and kinetic analysis of the protection assumed that the cells were in a metastable equilibrium of growth and death, and the mass action of the extracts was used to determine the optimal concentration for each extract. The PG and VG extracts were cytotoxic, with an efficiency index of protection (η) of approximately −20% and −41%, respectively. By contrast, η values of ~220% were observed for Et extracts (0.625% vol/vol) and combined extracts (Et + PG) at concentrations of (0.375 + 0.250)% and (0.5 + 0.5)%, respectively. These results identify *J. curcas* as a promising source of bioactive compounds and show that appropriate modeling can provide a powerful system for determining the optimal conditions for drug action.

**KEYWORDS**

drug development, growth model, *Jatropha curcas*

### 1 INTRODUCTION

*Jatropha curcas* is a tropical medicinal plant with significant anti-inflammatory, antiretroviral, and antibiotic activities¹-⁴ and is grown commercially on farms in areas of low to high precipitation.⁵ *Jatropha curcas* has monoecious flowering
that is characterized by the absence of an articulated peduncle. The subfamily Crotonoideae contains 175 species widely distributed in tropical semiarid regions of Africa, India, Central and South America, and the Caribbean (including the West Indies). The subgenus Curcas, except for J. curcas L. that is found in other regions, is restricted to Mexico, the Sahara desert, Arizona, and Texas.6,7

The name Jatropha is of Greek origin, from the word iatrós, which means doctor or of medical use, and etrophé, meaning food or nutrient. The leaves and latex contain phenolic compounds in higher concentrations (38.8 and 26.0 mg/dL, respectively) than other plant parts; the roots and stem bark have a low phenol content (8.0 and 6.1 mg/dL, respectively). The highest content of flavonoids occurs in latex (16.3 mg/dL), followed by leaves, roots, and stem with values of 1.40, 1.06, and 0.09 mg/dL, respectively. In general, the total saponin content is high in all plant parts, with latex having the highest saponin content (96.7 mg/dL), followed by leaf and root/stem with values of 66.2 and 58.0 mg/dL, respectively.3

A cyclic octapeptide, curcacyclin A, purified from J. curcas latex shows moderate concentration-dependent inhibition of the classical human complement pathway and human T-cell proliferation.8,9 A related octapeptide, curcacyclin B, also modulates immunological activity.10 Other components identified in stem and leaf samples include proteins and amino acids, depsides and depsidones, tannins and phenols, alkaloids, steroids and triterpenes, saponins, and polysaccharides. By contrast, reducing sugars have been detected only in leaf extracts. The presence of these substances indicates the great diversity of functional chemical compounds present in J. curcas.11

Aidah et al12 reported that various extracts of J. curcas were bactericidal and bacteriostatic against Staphylococcus aureus and Escherichia coli, and attributed this activity to organic and fatty acids present in the extracts that probably interacted with the hydrophobic structures of the bacteria. Mujumdar and Misar13 described anti-inflammatory activity after the topical application of root powder in the form of a paste in a model of mouse ear inflammation. Katagi et al14 reported the presence of isoamericanol A (IAA) that has anticancer effects in several human cancer cell lines and inhibits cell proliferation in a concentration-dependent manner. IAA also causes cell cycle arrest between the G2 phase and mitosis and modulates the expression of a variety of genes. These findings suggest that IAA has considerable potential as a new anticancer drug.

In muscle cells, a J. curcas extract was exerted a powerful antioxidant effect3,5,15 and can be useful in the treatment of conditions such as alcoholism. This beneficial effect is attributed largely to the presence of phenolic compounds, one of the main groups that act as primary antioxidants or free-radical scavengers to reduce the concentration of reactive oxygen species.15 Acute or chronic alcohol consumption alters the cellular components of the innate and adaptive immune systems.16 Alcohol abuse interferes with immune activity, causing splenic and thymic atrophy, in addition to affecting the redistribution of peripheral blood leukocytes by reducing their ability to migrate after injury or infection. Alcoholism also causes functional abnormalities in natural killer, T and B cells, as assessed through cellular and humoral immune responses.17

The aim of this work was to provide an experimental basis for using J. curcas extracts to protect cells against Et-induced damage. Specifically, we report a detailed thermodynamic study of the effects of J. curcas extracts in cell damage and during protection against Et-induced damage. The kinetics of cell viability was assessed by quantifying energy parameters, with the cell population considered to be in metastable equilibrium. Thermodynamic studies similar to that described here have been reported for other systems, including bacterial and yeast growth, for example, Tyson and Diekmann,18 Deenick et al,19 Desmond-Le Quéméner and Bouchez,20 Osella et al,21 and our group.22 We also describe the potential application of this approach for optimizing the experimental conditions.

2 | MATERIALS AND METHODS

2.1 | Raw material

Jatropha curcas leaves were collected in the city of Senhor do Bonfim, in the northeastern Brazilian state of Bahia. Healthy leaves were selected based on their color, normal shape, and absence of parasites and diseases. A reference sample of the botanical material was deposited under reference number 238896/01 in the Herbarium of the State University of Feira de Santana (HUEFS), Feira de Santana, BA, Brazil.

2.2 | Extract preparation

Extracts of J. curcas were prepared using a variation of the protocol described by Brotto et al.23 The leaves were initially dried at 45°C in an air heater for 19 hours, after which 20 g was weighed and added to 100 mL of ethanol (Et),
vegetal glycerin (VG), or propylene glycol (PG) to obtain each extract. The three solutions were then homogenized with magnetic shaking for about 24 hours at room temperature, after which they were centrifuged (3000 rpm for 15 minutes at room temperature) to obtain a supernatant that was aliquoted and stored in sterilized recipients. These stock solutions were then used to provide different volumes of extract (0.05-3.00 μL/well) in a total assay volume of 200 μL/well in 96-well plates. Thus, the addition of, for example, 1.0 μL of extract to the wells would result in a final concentration of 0.5% vol/vol.

2.3 | Total blood samples

Total blood samples were collected by aseptic venipuncture into tubes with EDTA. The blood was obtained from four healthy male and female donors (aged 25-40 years), with no alcohol consumption in the 72 hours preceding collection. This study was approved by the ethics committee of the Centro de Pesquisas Biomédicas, Faculdade de Tecnologia e Ciências, Salvador, BA (registration no. 1449984 CAAE: 53343315.8.0000.5032).

2.4 | Separation of peripheral blood mononuclear cells

Peripheral blood mononuclear cells were obtained from blood samples collected as described earlier. Peripheral blood lymphocytes were obtained by the Ficoll-Hypaque density gradient centrifugation (1500 rpm for 30 minutes at room temperature in a clinical centrifuge). The cell-containing fraction was washed three times with the phosphate-buffered saline (1100 rpm for 7 minutes at room temperature in a clinical centrifuge) and resuspended in RPMI 1640 culture medium containing 25 mM HEPES, penicillin/streptomycin, and 50 mM beta-mercaptoethanol supplemented with 10% fetal bovine serum. The number of cells was counted in a Neubauer chamber and adjusted to 10^6 cells/mL.

2.5 | Cell culture and viability

The lymphocytes (initial cell concentration of 10^6 cells/mL) were incubated in 96-well plates (total volume of 200 μL/well) in the absence or presence of 1.0% Et and/or different concentrations of J. curcas extract. The plates were incubated at 5% CO₂ and 37°C for 6 to 24 hours, and after 6 hours of initial incubation, the cells were counted at 2-hour intervals. Cell viability was assessed using the Trypan blue exclusion method in which the negatively charged chromophore does not penetrate intact cells (blue-stained cells were considered nonviable). After incubation, 50 μL of cell suspension was placed in a polypropylene Eppendorf tube and 10 μL of Trypan blue was added followed by mixing with a micropipette. After a 5-minute incubation at room temperature, the viable cells were counted in a Neubauer chamber using a microscope. Images of the plates were captured with a camera coupled to the microscope.

2.6 | Thermodynamic analysis

The approach used here was an extension of a model previously proposed for yeast duplication and virus dissociation based on a metastable equilibrium state between the initial cells (M) and the cells resulting from growth/death (M*) as follows:

$$ M \leftrightarrow 2M^*.$$

As previously described, the superscript (*) is used to differentiate the starting cells from those arising through cell duplication. Despite the complexity of cell division, the thermodynamics involved in such a system can be understood by examining the preliminary and final concentrations of the main components of the process, while keeping all other conditions constant. It is important to note that the analysis applied here is not a “simplification,” but a direct measure of objective data and yields relevant conclusions and predictions for the system, as shown below. Thermodynamically, the biological process can be considered an entropy-controlled state that requires energy influx to maintain steady-state conditions.
Based on the relationship (1), the “extent of process” parameter can be introduced to characterize the system.\(^{22}\) Thus, the concentration of each species \((c_M\) and \(c_{M'}\)) at time zero \((t_0)\) and any time \((t)\) can be described as

\[
c_M(t_0) = c_{M_0},
\]

\[
c_{M'}(t_0) = 0,
\]

whereas at time \((t)\), the relationship would be

\[
c_M(t) = c_{M_0} - x_M,
\]

\[
c_{M'}(t) = 2x_M,
\]

where \(c_{M_0}\) corresponds to the initial cell concentration and \(x_M\) is the extent of process parameter.

From Equations (4) and (5), the Gibbs free energy of cell growth/death related to Equation (1) becomes

\[
\Delta G_M = -RT \ln \left( \frac{c_{M'}^2}{c_M} \right) = -RT \ln \left( \frac{4x_M^2}{c_{M_0} - x_M} \right),
\]

where \(R\) is the gas constant and \(T\) is the absolute temperature (in kelvin).

Equation (6) allows calculation of the Gibbs free energy of cell growth during the process of growth. Thus, the degree of cell growth/death \((\alpha_M)\) for an initial cell concentration \((c_{M_0})\) can be expressed as

\[
\alpha_M = \frac{x_M}{c_{M_0}}.
\]

Introducing Equation (7) into Equation (5) yields a relationship that correlates the parameter \(\alpha_M\) with the Gibbs free energy of growth/death:

\[
\Delta G_M = -RT \ln \left( \frac{2^2c_{M_0}^2\alpha_M^2}{c_{M_0}(1 - \alpha_M)} \right) = -RT \ln \left( \frac{2^2c_{M_0}^2\alpha_M^2}{(1 - \alpha_M)^2} \right).
\]

As in previous studies,\(^{25,28}\) this equation shows that a change in the energy of reaction is obtained from the degree of growth/death, the initial species concentration, and the specified stoichiometry of reaction. In these conditions, differentiation of the degree of growth/death with respect to time also furnishes the respective velocity of cell growth/death \((v_M)\) in arbitrary units:

\[
v_M = \left( \frac{\partial \alpha}{\partial t} \right).
\]

An additional parameter to quantify the efficiency of such processes is provided by the efficiency index \((\eta)\).\(^{29}\) Thus, rewriting this parameter for the extract effect \((\eta_{extr})\), we obtain

\[
\eta_{extr} = \left( \frac{[M^*]_t - [M^*]_0}{[M^*]_0} \right) \times 100,
\]

where \([M^*]_t\) is the number of control cells at time \(t\) with harmful effect induced by Et and \(s\) \(\mu\)L of extract solution and \([M^*]_0\) is the number of cells at time \(t\) in the presence of Et alone.

Thus, if \(\eta_{extr} = 0\), then the extract does not affect the cell viability, whereas if \(\eta_{extr} \neq 0\), its value indicates the percentage at which the cell viability is increased \((\eta_{extr} > 0)\) or decreased \((\eta_{extr} < 0)\). A complete application of these procedures is performed below to exemplify the improvement reached by the present approach. For these cases, the fitting function used \((F)\) was the following:

\[
F = \sum_{i=1}^{n-1} f_i(y_{i+1} - y_i) + y_n,
\]
where $y$ is the $i$th experimental data, $n$ is the total number of experimental points, and $f_i$ corresponds to the relative species fraction in solution, given by

$$f_i = \frac{1}{1 + \exp((t - a_i)/b_i)}, \quad (13)$$

where $t$ is the experimental time of the $i$th data point and $a_i$ and $b_i$ are fitting parameters.

### 3 RESULTS AND DISCUSSION

The experiments were done in triplicate for each condition analyzed. Curve fitting was done as previously described and yielded determination coefficients ($R^2 > 0.95$ and a random residual distribution for all processes.

The effects of the extracts on cells not exposed to Et are shown in Figures 1, 3A,C,E, and 4A,C,E. The control conditions for the respective effect of protection are shown in Figures 2,3B,D,F, and 4B,D,F. Figure 1A shows the results obtained for the peripheral blood lymphocyte concentration vs time at various concentrations of Et extract. The lines show the curves fitted to the experimental data. Figure 1B shows the respective residuals of these nonlinear adjustments. As indicated in Figure 1A, cell viability decreased over time, even in the absence of Et. The cytotoxicity of the Et extracts was particularly pronounced after ~18 hours for volumes >0.75 μL. With average adjustment of the fitting parameters, it was possible to generate the three-dimensional (3D) surfaces of these experimental and theoretical results (Figure 1C). This procedure has been described in detail for kinetic systems involving the Michaelis-Menten equation. When this mathematical method is combined with a physicochemical hypothesis, it can be used to describe models such as those of Monod, Monod et al., and others, including that of the present investigation (Equation 1).

The 3D plot shown in Figure 1C provides a better understanding and visualization of the interactive process between cells and extracts and allows the observation of regions of biochemical events, as well as the surrounding conditions. In this case, the greater toxicity observed after 18 hours with extract values >0.75 μL appears as a fast depression in the surface, whereas low extract values lead to a smooth progressive decrease. This behavior is partially related to changes in the medium nutritional composition, but also suggests some time-dependent toxicity for extract volumes >0.75 μL. From cuts in the z-axis of Figure 1C, it is possible to obtain the level lines as a function of time and extract addition, as shown in Figure 1D. This figure shows at least three points of higher depression, one between 10 and 12 hours and two others at about 17 and 21 hours, indicating a greater change in cell behavior. For times <10 hours, there were no significant differences between the samples with and without Et extract. An accurate analysis of this figure revealed three very distinct regions. In the first and second parts (left and bottom portions of Figure 1D), the extract changes from a state that does not affect the viability (0-11 hours) to a state of moderate cell death (11-17 hours), whereas in the third part (top right and middle portions of Figure 1D), the cell death becomes accentuated, especially for Et extract volumes of 1 and >1.5 μL.

The corresponding experiment done using cells in 1% Et allowed assessment of the protective effect of *J. curcas* extract against Et-induced damage (Figure 2). Figure 2A shows the data and the fitted curves, while the respective residuals of the fit are shown in Figure 2B. The cell viability of the entire series decreased from initial values of ~130 x 10^6 cells/mL to ~70 x 10^6 cells/mL in the presence of 1% Et after 6 hours (Figure 1A). At all concentrations, the Et extract of *J. curcas* protected the cells against damage by 1% Et (Figures 1C and 2C). Figure 2A shows that the plot of residuals was similar to that of Figure 1A and this similarity allowed comparison of the respective surface plots (Figures 1C and 2C). Examination of the surface response in the presence of Et (Figure 2C) revealed a convex region where the protective effect was more marked—the viability was 70 to 80 x 10^6 cells/mL with the addition of 0.75 to 1.25 μL of Et extract. In a more detailed view, Figure 2D shows that the previous region of higher cell death between 1.5 and 3 μL of Et extract and an elapsed time >28 hours were still present, although the viability increased slightly from 45 x 10^6 cells/mL to 53 x 10^6 cells/mL, indicating that at this point, the addition of Et somehow improved the cell viability. Figure 2D also shows that the higher viability bands are predominantly horizontal, whereas those in Figure 1D are vertical, corresponding to a time-dependent character of cell inactivation in the absence of 1% Et compared with the response in the presence of Et.

The PG and VG extracts were used to compare the protective effect among extracts as the biomolecules responsible for protection probably have distinct different solubilities in different solvents. Figure 3 shows the results for the PG extract (control—Figure 3A,C,E and in the presence of 1% Et—Figure 3B,D,F). This extract caused a similar effect to the Et extract when added in volumes <1 μL. However, cell viability decreased from ~135 x 10^6 cells/mL when using Et extracts
The influence of Et extract on human peripheral blood lymphocytes. Control cells were not exposed to ethanol. A, Cell viability \( [M^*] \) as a function of incubation time (in hours). The lines were fitted using the function described in Equation (12). B, Residual plot for the data fitted to the lines in panel A. C, Surface. D, Contour plots of cell concentration as a function of the incubation time (in hours) and volume of ethanol extract (in microliters). The experiments were done in a total volume of 200 μL/well. Et, ethanol

(Figure 1A) to \( \sim 120 \times 10^6 \) cells/mL in the first 6 hours for PG extracts. The addition of PG extract in volumes \( \geq 1 \) μL markedly reduced the cell viability in the initial 6 hours of exposure followed by a slight recovery and a second cycle of cell death after 16 hours; overall, the toxicity was greater than for the Et extract. Figure 3B shows that the greatest toxicity was associated with extract additions \( > 1 \) μL and a duration of exposure to the extract \( > 18 \) hours.

The greater cytotoxicity of the PG extracts reflected not only the polarity or viscosity and miscibility of the plant components but also differences in diffusion and the presence of a greater number of harmful biomolecules retained in the PG phase. This conclusion was strengthened by the high level of cell damage observed at shorter incubation times and at higher concentrations of PG extract (Figure 3C). Figure 3E makes this clear by showing the increase in damage at these early times, with viability reaching \( 83 \times 10^6 \) cells/mL after 14 hours, and \( 38 \times 10^6 \) cells/mL at 22 to 24 hours. However, for incubation times \( > 16 \) hours and PG extract additions \( < 1 \) μL, the cell viability was better than with the Et
FIGURE 2  Protective action of Et extract in relation to the concentration of human peripheral blood lymphocytes in the presence of 1% ethanol. A, Cell viability \([M^*]\) as a function of incubation time (in hours). B, Residual plot for the data fitted to the lines in panel A. C, Surface. D, Contour plots of cell concentration as a function of the incubation time (in hours) and volume of ethanol extract (in microliters) in a total volume of 200 \(\mu\)L. Et, ethanol

extract (Figure 1D), for which the lowest level of cell viability was \(62.1 \times 10^6\) cells/mL compared with \(77.5 \times 10^6\) cells/mL for the PG extract (Figure 3E).

Comparison of the results obtained with the PG extract (Figure 3B) and 1% Et indicated that the addition of Et to the medium reduced the cell viability to levels below those observed in the absence of PG extract. Thus, for the time range of 6 to 10 hours and additions of 1.25, 1.5, and 1.75 \(\mu\)L, the PG extract acted as an additional harmful compound in the medium (Figure 3B). In this case, the finding that after 10 hours, the viability of the cells was higher than in the absence of the PG extract indicated that after the initial contact of the cells with Et, the remaining cells underwent enhanced proliferation in the presence of PG extract, while in the absence of this extract, there was a progressive decrease to death.

Figure 3D showed the results obtained for 1.75 \(\mu\)L of extract in which the addition of Et reduced the time dependence of the cell viability indicated in Figure 3A. Figure 3D shows the surface generated from the results of Figure 3B.
FIGURE 3  The influence of PG extract on human peripheral blood lymphocytes in the absence (A, C, and E) and presence (B, D, and F) of 1% ethanol. A, Cell viability [M*] as a function of incubation time (in hours). The lines were fitted as described in Figure 1. B, Surface. C) Contour plots of cell concentration as a function of incubation time. D, Cell viability [M*] as a function of incubation time (in hours) in cells damaged by 1% ethanol in the presence of specified volumes (in microliters) of PG extract (total assay volume of 200 μL). E, Surface. F, Contour plots for PG extract in the presence of 1% ethanol. PG, propylene glycol.
This analysis revealed three peaks of higher viability and a region near 1.5 μL of PG extract of very low viability, similar to and at the same position as that in Figure 3C. The simultaneous analysis of Figure 3E,F indicated that the addition of an extract volume >1.0 μL resulted in harmful effects. In addition, the increase in the number of level lines near 22 hours and with the addition of ∼1.5 μL PG extract suggested that for both situations (Figure 3B,C), some biochemical compound was causing cell death, independently of the extract type (Figure 2D).

Figure 4 shows the results for the VG extract. With the exception of an extract volume of 1.5 μL, all the other results remained close to 70 × 10^6 cells/mL and showed little change from 6 to 24 hours (Figure 4A). The addition of VG extract to the medium reduced the cell concentration from 140 × 10^6 cells/mL to about half of this value in all experiments, except for the volume of 1.5 μL. VG extract protected the cells against Et toxicity at volumes up to 1 μL. Overall, the results indicated a protective effect at extract volumes <1.0 μL and for times >10 hours. Only the Et and PG extracts provided protection starting 6 hours after contact with 1% Et. By contrast, the VG extract drastically reduced the initial viability (Figure 4A) and the presence of Et enhanced this decrease, especially at volumes >1 μL and incubation times >20 hours (Figure 4C). The presence of a harmful biochemical compound or array of compounds in the VG extract could explain the effects observed here, as the cell viability was lower than for the PG and Et extracts (∼30 × 10^6 cells/mL for VG extract—Figure 4E vs ∼36 and 62 × 10^6 cells/mL for the latter two extracts, respectively—Figures 3E and 1D). This result indicates a low polarity for the compound(s) involved and a higher affinity for the PG and VG phases.

After assessing the effects of the individual extracts, combinations of the extracts were screened for a possible potentiating action. As high volumes of extract resulted in cytotoxicity, the limits established were 1.25 μL for the Et extract and up to 1 μL for the PG and VG extracts. Figure 5 shows the action of the PG extract in the presence of 0.75 to 1.25 μL of Et extract. In this figure, the isolated line with symbols on the left side of the surfaces indicates the survival of control cells in the absence of Et extract (Figure 5A). The addition of 0.75 μL of Et extract and up to 1 μL of PG extract indicated that the combined extracts markedly enhanced the cell protection compared with that observed in the absence of Et extract (isolated line). The effect observed was even more marked at higher concentrations of Et extract (Figure 5C,E). Figure 5B shows the region of greater protection between the additions of 0.2 and 0.8 μL of PG extract, with maximum protection occurring at 0.5 μL of PG extract after 12 hours. The lowest survival occurred with ∼0.07 to 0.3 μL of PG extract and incubation times >18 hours, although even under these conditions, cell protection was still observed. The addition of 1.0 μL of Et extract (Figure 5C,D) yielded similar results. Cell viability decreased with additions of PG extract up to 0.5 μL and increased from 0.5 to 1.0 μL. This profile of cell viability was comparable with that of control samples in the absence of Et (Figure 1A). Increasing the volume of Et extract to 1.25 μL made the medium more toxic compared with the previous results (Figure 5E,F). The latter panels also show that an increase in PG extract did not enhance the protection of the cells, whereas the addition of 1.25 μL of Et extract reduced the sensitivity of cells in the presence of PG extract.

Figure 6 shows the correlation between the toxic effect of Et and the corresponding protection by J. curcas extracts based on microscopic images of peripheral blood lymphocytes. This figure compares cells in the absence and presence of 1% Et and in the presence of 1% Et in combination with 1.25 μL of Et extract and the combination of 1.25 μL of Et extract and 0.1 μL of PG extract. The presence of 1% Et markedly enhanced the cell damage, leading to significant cell death (dark points) after 24 hours (Figure 6B) compared with control cells (Figure 6A). The addition of 1.25 μL of Et extract prior to Et damage (Figure 6C) reduced the extent of cell damage, with some alterations in cell morphology, indicating partial protection. When combinations of extracts were used (Figure 6D), the cell intactness was essentially preserved, with results similar to those observed for control cells (Figure 6A). This finding agreed with the results presented in Figures 1-5.

As described in Equation 11, the efficiency index ($\eta_{extr}$) quantifies very adequately the protective action of the extracts because the calculation considers the number of viable cells at each time interval $t$ in the control. The percentage obtained for each experimental point reflects the number of viable cells. Figure 7 shows the results obtained for this parameter for the PG extract and the mixture of Et and PG extracts. These findings indicate that for Et extract additions of up to 1.0 μL, the efficiency of protection reached values of ∼48% in the first 9 hours (Figure 7A). After 24 hours, the efficiency increased to $\eta_{extr} = ∼225%$. Figure 7B shows a minimum efficiency index value of +25.85%, indicating that the Et extract provided protection at any volume used. By contrast, the corresponding results for the PG extract (Figure 7C,D) indicated that this extract did not protect in all conditions ($\eta_{extr} < 0$). Indeed, for PG extract volumes of 0.9 to 1.5 μL and up to 10 hours of incubation, there was a negative $\eta_{extr}$ of −18.58% and −9.22% (Figure 7D). According to Equation 11, $[M*]_{extr} = (1 + \eta_{extr}/100) \times [M*]_0$, both cell death and cell division are used in computing the $\eta_{extr}$. The combination of Et and PG extracts (Figure 7E,F) yielded high $\eta_{extr}$ values, similar to those observed with Et extract alone (Figure 7B), although the entire surface response provided a better efficiency index (Figure 7F). The minimum and maximum efficiency indices were, respectively, 0.5 μL after 8 hours and 0.9 to 1.0 μL after 24 hours.
FIGURE 4  The influence of VG extract on human peripheral blood lymphocytes in the absence (A, C, and E) and presence (B, D, and F) of 1% ethanol. A, Cell viability \([M^*]\) as a function of incubation time (in hours). The lines were fitted as described in Figure 1. B, Surface. C, Contour plots of cell concentration as a function of incubation time. D, Cell viability \([M^*]\) as a function of incubation time (in hours) in cells damaged by 1% ethanol in the presence of specified volumes (in microliters) of VG extract (total assay volume of 200 μL). E, Surface. F, Contour plots for VG extracts in the presence of 1% ethanol. VG, vegetal glycerin.
FIGURE 5  Protective effect of PG extract on human peripheral blood lymphocytes exposed to 1% ethanol. The PG extract was tested in the absence and presence of 0.75 μL (A, B), 1.0 μL (C, D), and 1.25 μL (E, F) of Et extract. A, C, E, Surface plots. B, D, F, Contour plots of cell concentration as a function of the incubation time and volume of PG extract added (total assay volume of 200 μL). The isolated lines and symbols in the surface plots (A, C, and E) correspond to the condition in absence of Et extract. Et, ethanol; PG, propylene glycol
To understand the kinetics of cell protection, we examined fluctuations in the velocity of growth in the presence of Et and Et + PG extracts (Figure 8). Equation 10 furnishes the rate of cell division/death in specific experimental conditions, with positive velocities indicating cell division and negative velocities indicating cell death. Most kinetic models, such as that of Monod, 31 involve hyperbolic or sigmoidal curves for growth rate. Our results revealed a very complex behavior that made it unfeasible to characterize the process by fitting specific parameters. For this analysis, we therefore considered the system to be in a metastable equilibrium state, as described by Equation 9. Figure 8A shows that most of the range observed had negative values with regions of higher death rates near −0.1 A.U./h. The number of cell deaths/hour corresponded to 0.1 multiplied by the maximum cell concentration observed for the Et extract experiments (∼98 × 10⁶ cells/mL, Figure 2A) that yielded 9.8 cell deaths/hour/mL. The surface response for cell death (Figure 8B) was a more accurate indicator of the rate values and helped in visualizing details related to metabolic processes, nutritional factors, and the presence of harmful compounds. The use of combinations of extracts (Figure 8C,D) reduced the heterogeneity and fluctuations in the kinetic behavior. The rates cycled between high and low values at 6-hour intervals, with lower velocities at 9, 15, and 21 hours, were possibly related to cyclic fluctuations in the above-mentioned factors (Figure 8D).

Based on the findings discussed above, we conclude that the physicochemical model and the corresponding mathematical approach described here provide powerful tools for modeling and optimizing cell proliferation and death. The possibility of calculating the Gibbs free energy of reaction (Equation 9) allows the spontaneity of the process to be studied and, more importantly, permits the estimation of additional parameters such as volume change and entropy, 25 the stoichiometry of effectors such as protons, 33,34 heat capacity, and others. This is possible if the parameter analyzed is studied in relation to specific conditions. For instance, the assessment of volume change and entropy requires experiments done at different pressures and temperatures, respectively. For proton stoichiometry, the variable should be pH.

Figure 9 shows the free energy of the survival process for Et (Figure 9A,B) and a combination of Et and PG extracts (Figure 9C,D). Figure 9A,B shows that the surface presents predominantly as a plane, but with a depression in the center, corresponding to the addition of 1.25 μL of Et extract and an elapsed time of ∼12.5 hours. The corresponding Gibbs free energy was −4041.97 J/mol for a predominantly positive Gibbs free energy in the surface plot. This energy minimum corresponds to the optimal point for the addition of Et extract. Such data are of practical importance, as they should save considerable working time because they provide direct access to optimization. Analysis of the effects of combinations

**Figure 6** Images of peripheral blood lymphocytes stained with Trypan blue dye. A, Control cells (negative control). Cells exposed to 1% ethanol (1%) alone (B), 1.25 μL of Et extract (C), or 1.0 μL of Et extract plus 1.0 μL of PG extract (D). The final assay volume was 200 μL in all experiments. Et, ethanol; PG, propylene glycol.
FIGURE 7  Protective action of Et (A, B), PG (C, D), and PG extracts on human peripheral blood lymphocytes in the presence of 1 μL of Et after exposure to 1% ethanol. Surface (A, C, and E) and contour (B, D, and F) plots of the extract efficiency index ($\eta_{extr}$) as a function of the incubation time and volume of extract tested (total assay volume of 200 μL). Et, ethanol; PG, propylene glycol.
FIGURE 8  Protective action of Et (A, B) and PG extracts on human peripheral blood lymphocytes in the presence of 1 μL of Et extract (C, D) after exposure to 1% ethanol. Surface (A, C) and contour (B, D) plots of rate of cell growth/death ($v_M$) in A.U. as a function of the incubation time and volume of extract added (total assay volume of 200 μL). A.U., arbitrary units; Et, ethanol; PG, propylene glycol.
FIGURE 9 Protective action of Et (A, B) and PG extracts on human peripheral blood lymphocytes in the presence of 1 μL of Et extract (C, D) after exposure to 1% ethanol. Surface (A, C) and contour (B, D) plots of the Gibbs free energy of cell growth/death ($\Delta G_M$) (in joules per mole) as a function of the incubation time and volume of extract added (total assay volume of 200 μL). Et, ethanol; PG, propylene glycol

of the Et and PG extracts identified a distinct low free energy profile (Figure 9C,D) and showed that optimal conditions required PG extract volumes >1 μL. There was a general reduction in these values when PG extract was added. This can be appreciated by comparing the minimum of $-6559.1$ J/mol and maximum of $9287.2$ J/mol in Figure 9D with their respective minimum and maximum for the same range of extract concentrations in Figure 9B.

This work investigated the effect of extracts from a plant with known protective properties against Et-induced cell damage. It is currently unclear whether alcohols affect proteins directly through specific binding sites or indirectly through interactions in the solvent. Et stabilizes secondary structure and destabilizes tertiary structure, probably via hydrophobic interactions and hydrogen bonds related to carbon chains and hydroxyl groups, respectively. At a cellular level, such interactions include the binding of Et to open ion channels. The results obtained here were similar to the antioxidant effects of extracts from this same plant. A very large number of plants used in traditional medicine exert therapeutic effects, the mechanisms of which have yet to be explored at the molecular level and this represents a formidable challenge for investigators. The theoretical approach described in this work could provide a powerful tool to assist in the isolation and characterization of plant products with relevant biological activity. The general method is applicable to different systems used to investigate chemical effects in biological materials, even in those with a high degree of complexity.

A very important effect of plant extracts that is of medical relevance is their antitumor activity. The toxicity of chemotherapeutic candidate drugs, alone or in combination, in normal and tumor cells is a very important aspect in the design of new anticancer drugs. A compound that offers pharmacological protection to normal cells can allow the use of a higher concentration of chemotherapeutic drugs, thereby providing a more effective treatment. The approach described
here provides a simple means for determining the optimal conditions for drug combinations that would otherwise demand numerous experimental conditions. Other systems, such as the effects of chemicals on nonbiological materials, may benefit from the model described here. The optimization of processes that require chemicals from distinct sources could also be investigated using this approach.

4 CONCLUSION

The results described here indicate that PG and Et extracts of J. curcas can protect cells against Et-induced damage. The thermodynamic modeling used allowed a detailed characterization of the complex processes of cell death and growth. The calculation of performance parameters such as the efficiency index (η) and Gibbs free energy (ΔG) provided a detailed understanding for optimizing the action of biochemical compounds. The kinetic approach used here allowed determination of the extent of reaction and thermodynamic parameters in a metastable equilibrium. Under these conditions, it was possible to assess the energy changes in the system over time. Overall, the approach used here involving physicochemical considerations and the mathematical treatment of surface plots provided advantages in data analysis and should be useful in developing drugs and equipment and in biomolecule prospection. This approach should be applicable to a variety of systems and allow the optimization of different processes.

ACKNOWLEDGMENTS

This work was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), and Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), Brazil. The authors thank Dr. Stephen Hyslop for editing the English of the manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ORCID

Jose A. C. Bispo https://orcid.org/0000-0002-9554-0856

REFERENCES

1. Abdelgadir HA, Van Staden J. Ethnobotany, ethnopharmacology and toxicity of Jatropha curcas L. (Euphorbiaceae): a review. South Afr J Bot. 2013;88:204-218. https://doi.org/10.1016/j.sajb.2013.07.021.
2. Gübitz GM, Mittelbach M, Trabi M. Exploitation of the tropical oil seed plant Jatropha curcas L. Bioresour Technol. 1999;67:73-82. https://doi.org/10.1016/S0960-8524(99)00069-3.
3. Oskoueian E, Abdullah N, Saad WZ, et al. Antioxidant, anti-inflammatory and anticancer activities of methanolic extracts from Jatropha curcas Linn. J Med Plants Res. 2011;5:49-57.
4. Pabón LC, Hernández-Rodríguez P. Importancia química de Jatropha curcas y sus aplicaciones biológicas, farmacológicas e industriales. Rev Cuba Plantas Med. 2012;17:1028-4796.
5. Paullillo LC, Mo C, Isaacson J, et al. Jatropha curcas: from biodiesel generation to medicinal applications. Rec Pat Biotechnol. 2012;6:192-199. https://doi.org/10.2174/1872208311206030192.
6. Gusmão CAG. Desempenho do pinhão-manso (Jatropha curcas L.) de segundo ano submetido a diferentes doses e relações NPK (master thesis). Montes Claros, MG, Brazil; 2010.
7. Hirota BCK, Trevisan RR, Dias JFG, Miguel MD, Miguel OG. Fitoquímica e atividades biológicas do gênero Jatropha: mini-revisão. Visão Acadêmica. 2010;11:103-112. https://doi.org/10.5380/acd.v11i12.21374.
8. van den Berg AJJ, Horsten SFAJ, den Bosch JJK, et al. Curcacycline A—a novel cyclic octapeptide isolated from the latex of Jatropha curcas L. FEBS Lett. 1995;358:215-218. https://doi.org/10.1016/0014-5793(94)01405-P.
9. Dwijayanti DR, Rifa’i M. Gynura procumbens ethanolic extract promotes lymphocyte activation and regulatory T cell generation in vitro. J Trop Life Sci. 2015;5:14-19.
10. Auvin C, Baraguey C, Blond A, Lezenven F, Pousset JL, Bodo B. Curcacycline B, a cyclic nonapeptide from Jatropha curcas enhancing rotamase activity of cyclphilin. Tetrahedron Lett. 1997;38:2845-2848. https://doi.org/10.1016/S0040-4039(97)00495-4.
11. Beserra FP, Aguiar RWS, Carvalho EEN, Borges JCM, do Vale BN. Jatropha curcas L. (Euphorbiaceae) como novo bioinseticida: análise fitoquímica preliminar e atividade larvicida contra Aedes aegypti (Diptera: culicidae). Amaz. Sci. Health. 2014;2:17-25.
12. Aidah N, Abdullah N, Oskoueian E, Sioe CC, Saad WZ. Membrane-active antibacterial compounds in methanolic extracts of Jatropha curcas and their mode of action against Staphylococcus aureus S1434 and Escherichia coli E216. Int J Agric Biol. 2014;16:723-730.
13. Mujumdar AM, Misar AV. Anti-inflammatory activity of Jatropha curcas roots in mice and rats. J Ethnopharmacol. 2004;90:11-15. https://doi.org/10.1016/j.jep.2003.09.019.
14. Katagi A, Sui L, Kamitori K, et al. Inhibitory effect of isoamericanol a from Jatropha curcas seeds on the growth of MCF-7 human breast cancer cell line by G2/M cell cycle arrest. *Heliyon*. 2016;2:1-17. https://doi.org/10.1016/j.heliyon.2015.e00055.

15. Igbinosa OO, Igbinosa IH, Chigor VN, et al. Polyphenolic contents and antioxidant potential of stem bark extracts from *Jatropha curcas* (Linn). *Int J Mol Sci*. 2011;12:2958-2971. https://doi.org/10.3390/ijms12052958.

16. Nelson S, Kolls JK. Alcohol, host defence and society. *Nat Rev Immunol*. 2002;2:205-209. https://doi.org/10.1038/nri744.

17. Cook RT. Alcohol abuse, alcoholism, and damage to the immune system—a review. *Alcohol Clin Exp Res*. 1998;22:1927-1942. https://doi.org/10.1111/j.1460-3140.1998.tb17291.x.

18. Tyson JJ, Diekmann O. Sloppy size control of the cell division cycle. *J Theor Biol*. 1986;118:405-426. https://doi.org/10.1016/S0022-5193(86)80162-X.

19. Deenick EK, Gett AV, Hodgkin PD. Stochastic model of T cell proliferation: a calculus revealing IL-2 regulation of precursor frequencies, cell cycle time, and survival. *J Immunol*. 2003;170:4963-4972.

20. Desmond-Le Quéméner E, Bouchez T. A thermodynamic theory of microbial growth. *ISME J*. 2014;8:1747-1751. https://doi.org/10.1038/ismej.2014.7.

21. Osella M, Nugent E, Cosentino Lagomarsino M. Concerted control of *Escherichia coli* cell division. *Proc Natl Acad Sci USA*. 2014;111:3431-3435. https://doi.org/10.1073/pnas.1313715111.

22. Amorim TS, Lopes SB, Bispo JAC, Bonafe CFS, Carvalho GBM, Martinez EA. Influence of aceralo pulp concentration on mead production by *Saccharomyces cerevisiae* AWRI 796. *Lwt-Food Sci Technol*. 2018;97:561-569. https://doi.org/10.1016/j.lwt.2018.07.009.

23. Broetto L, Paulillo L, Lopes E, et al. Pinhão-Manso, a native *Jatropha curcas* from Brazil, protects muscle cells from ethanol toxicity and improves muscle function. *Biophys J*. 2011;100:288-288.

24. English D, Andersen BR. Single-step separation of red blood cells. Granulocytes and mononuclear leukocytes on discontinuous density gradients of Ficoll-Hypaque. *J Immunol Methods*. 1974;5:249-252.

25. Bispo JAC, Bonafe CFS, Joekes I, Martínez EA, Carvalho GBM, Norberto DR. Entropy and volume change of dissociation in tobacco mosaic virus probed by high pressure. *J Phys Chem B*. 2012;116:14817-14828. https://doi.org/10.1021/jp310219k.

26. Lucia U, Grisolia G. Constructall law and ion transfer in normal and cancer cells. *Proc Rom Acad Series A*. 2017;9:270-275.

27. Lucia U, Grisolia G. Second law efficiency for living cells. *Front Biosc Sch*. 2004;1:98-114.

28. Bispo JAC, Bonafe CFS, Santana KMOV, Santos ECA. A comparison of drying kinetics based on the degree of hydration and moisture gradientsof Ficoll-Hypaque. *J Immunol Methods*. 2015;400:192-198. https://doi.org/10.1016/j.jim.2014.07.014.

29. Carnot S. *Reflections on the Motive Power of Fire: A Critical Edition with the Surviving Scientific Manuscripts*. Manchester, UK: Manchester University Press; 1899.

30. Monod J. *The growth of bacterial cultures*. 1949;3:371-394. https://doi.org/10.1146/annurev.mi.03.100149.002103.

31. Santos JLR, Aparicio R, Joekes I, Silva JL, Bispo JAC, Bonafe CFS. Different urea stoichiometries between the dissociation and denaturation of tobacco mosaic virus as probed by hydrostatic pressure. *Biophys Chem*. 2008;134:214-224. https://doi.org/10.1016/j.bpc.2008.02.010.

32. Santos JLR, Bispo JAC, Landini GF, Bonafe CFS. Proton dependence of tobacco mosaic virus dissociation by pressure. *Biophys Chem*. 2004;111:53-61. https://doi.org/10.1016/j.bpc.2004.04.003.

33. Jain R, Sharma D, Kumar R. Effects of alcohols on the stability and low-frequency local motions that control the slow changes in structural dynamics of ferrocytochrome c. *J Biochem*. 2013;154:341-354. https://doi.org/10.1093/jb/mvt059.

34. Perham R, Makowski L, Schlatter S. Chemical properties of alcohols and their protein binding sites. *CMLS-Cell Mol Life Sci*. 2000;57:265-275.

35. Perham R, Liao J, Wittung-Stafshede P. Differential effects of alcohols on conformational switchovers in a-helical and b-sheet protein models. *Biochemistry*. 2006;45:7740-7749.

36. Dwyer DS, Bradley RJ. Chemical properties of alcohols and their protein binding sites. *CMLS-Cell Mol Life Sci*. 2000;57:265-275.

37. Perham R, Liao J, Wittung-Stafshede P. Differential effects of alcohols on conformational switchovers in a-helical and b-sheet protein models. *Biochemistry*. 2006;45:7740-7749.

38. Atanasov AG, Waltenberger B, Pferschy-Wenzig E-M, et al. Discovery and resupply of pharmacologically active plant-derived natural products: a review. *Biotecnol Adv*. 2015;33:1582-1614. https://doi.org/10.1016/j.biotechadv.2015.08.001.

39. Cragg GM, Newman DJ. Plants as a source of anti-cancer agents. *J Ethnopharmacol*. 2005;100:72-79. https://doi.org/10.1016/j.jep.2005.05.011.

40. Greenwell M, Rahman PKSM. Medicinal plants: their use in anticancer treatment. *Int J Pharm Sci Res*. 2015;6:4103-4112. https://doi.org/10.13040/IJPSR.0975-8232.6(10).4103-12.

---

**How to cite this article:** Cardoso NBS, Esteves MB, Bonafe CFS, Paulillo LCMS, Bispo JAC. Thermodynamic and kinetic modeling of the protection of mammalian cells against ethanol-induced damage by extracts of *Jatropha curcas* (Euphorbiaceae). *Engineering Reports*. 2019;1:e12077. [https://doi.org/10.1002/eng2.12077](https://doi.org/10.1002/eng2.12077)