Revista Internacional de Investigación e Innovación Tecnológica

Página principal: www.riiit.com.mx

# Toxicity essays and photoprotective effects of partitions of *Euphorbia tirucalli* L.

# Ensayos de toxicidad y efecto fotoprotector de particiones de *Euphorbia tirucalli* L.

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**Technological innovation:** Pharmacological development of photoprotectors based on medicinal plants.

**Industry application area:** This research focuses on the pharmacological effects for the extracts from *E. tirucalli* L.

Received: june 26th, 2023
Accepted: february 12th, 2024

## Resumen

Desde la antigüedad, las plantas han sido utilizadas en la medicina tradicional por sus diversas propiedades terapéuticas; la producción de medicamentos a partir de ellas y su uso para la prevención, atenuación o curación de enfermedades dieron lugar a la fitoterapia, la cual sentó las bases de la farmacopea actual. El objetivo de este trabajo fue evaluar los efectos fotoprotector de extractos obtenidos de *E. tirucalli* L. así como, sus efectos tóxicos sobre eritrocitos humanos y *Artemia salina*, con el fin de proponer el uso de éstos como potenciales agentes dermoprotectores de origen botánico. Mediante el modelo de *Artemia salina*, se obtuvo que las particiones metanólicas y etanólicas eran moderadamente tóxicas, con una $DL_{50}$ de 144.77 y 189.54 µg $mL^{-1}$, respectivamente. La prueba de toxicidad sobre eritrocitos ($AAPH^{\bullet}$) reveló que son potencialmente citoprotectoras dosis-dependiente, alcanzando el 99% de inhibición de la hemólisis a 1000 µg $mL^{-}$

[1], lo cual se presume está relacionada con su contenido de polifenoles, respectivamente de 3.52 y 19.46 µg EAG $mg^{-1}$ con la partición metanólica y etanólica, respectivamente. La prueba de fotoprotección demostró que poseen este efecto frente a las radiaciones UV-B, alcanzando un FPS > 9 y > 18, respectivamente. Como conclusión, la partición etanólica del extracto de *E. tirucalli* L., mostró la mayor actividad citoprotectora, de contenido fenólico y de fotoprotección, además de mostrar moderada toxicidad sobre organismos pluricelulares, por lo tanto, se sugiere continuar con otros ensayos de letalidad para su empleo como fotoprotectores biológicos de uso tópico.

**Palabras claves**: Antioxidante, Dermoprotector, Hemólisis; UV-B.

## Abstract

Plants have long been recognized in traditional medicine for their therapeutic properties, contributing significantly to the evolution of phytotherapy and modern pharmacopoeia. This study aimed to assess the photoprotective effects of *Euphorbia tirucalli* L. extracts and their toxicity on human erythrocytes and Artemia salina, with a view to proposing them as potential dermoprotective agents.

Using the Artemia salina model, both methanolic and ethanolic partitions exhibited moderate toxicity, with LD50 values of 144.77 µg mL-1 and 189.54 µg mL-1, respectively. Subsequent erythrocyte toxicity tests (AAPH-) indicated dose-dependent cytoprotective effects, reaching 99% inhibition of hemolysis at 1000 µg mL-1, likely attributable to their polyphenol content: 3.52 µg EAG mg-1 and 19.46 µg EAG mg-1 for methanolic and ethanolic partitions, respectively.

Furthermore, the photoprotection test demonstrated significant efficacy against UV-B radiation, with calculated Sun Protection Factor (SPF) values exceeding 9 and 18 for methanolic and ethanolic partitions, respectively.

In conclusion, the ethanolic partition of *Euphorbia tirucalli* L. extract displayed the highest cytoprotective activity, phenolic content, and photoprotection. Despite moderate toxicity on multicellular organisms, further lethality testing is recommended to explore their potential as biological photoprotectors for topical use.

**Keywords**: Antioxidant, Dermoprotective, Hemolysis, UV-B

## 1. Introduction

Various vital functions in humans, such as vitamin D synthesis, vasodilation of the upper layers of vessels, epidermal layer thickness, and melanin secretion, are associated with sunlight exposure. However, excessive exposure to solar radiation poses significant risks, including sunburn, cancer, and the photoaging process.

The adverse effects of solar radiation stem from its ionizing effect within the ultraviolet (UV) spectrum, which comprises UV-C (< 280 nm), UV-B (280-320 nm), and UV-A (320-400 nm). Despite UV-B radiation constituting only 5% of total solar radiation reaching the Earth's surface, studies have linked its exposure to neoplasias, mutations, photoaging, immunosuppression, cataracts, and photosensitivity.

Mexico, situated in a warm tropical zone with intense UV radiation, experiences UV indices ranging from 6 to 12 throughout the year, leading to sunburn, erythema, and desquamation. Consequently, the use of sunscreens becomes imperative.

Despite the increasing availability of sunscreen products containing compounds capable of filtering solar radiation (e.g., p-aminobenzoic acid, salicylates, benzophenones), skin cancer diagnoses persist, with fatalities attributed to malignant melanoma, particularly in sun-exposed areas such as the face, neck, head, and back of hands. The limited protective efficacy of these compounds and their potential to exacerbate dermatitis risk highlight the need for alternative solutions, including biological sunscreens with enhanced protection and compatibility with the body's natural defense mechanisms. Given antioxidants' ability to mitigate solar radiation's harmful effects, there is growing interest in developing botanical-based products with antioxidant properties.

Furthermore, synthetic photoprotectors degrade over prolonged sun exposure, diminishing their protective efficacy. Given that UV radiation induces free radical production, products capable of providing both photoprotection and cytoprotection are crucial to bolstering the body's natural defense mechanisms.

*Euphorbia tirucalli* L., a shrubby plant indigenous to Africa and colloquially known as pencil fingers, has a history of use in traditional medicine against tumors, excrescences, nodules, and abscesses. Studies have demonstrated its antimicrobial activity against various pathogens and its potential in promoting burn healing.

This study aims to evaluate the photoprotective effects of *E. tirucalli* L. extracts and their toxicity on human erythrocytes and Artemia salina, with the intention of proposing them as botanical dermoprotective agents.

## 2. Materials and methods

*Obtaining plant material*

*Euphorbia tirucalli* L. specimens were collected from the northern region of San Nicolás de los Garza municipality, Nuevo León, Mexico. Taxonomic classification was conducted at the Department of Botany, Faculty of Biological Sciences, Universidad Autónoma de Nuevo León, and registered in the herbarium with folio number 029755. The plant material underwent thorough washing with distilled water and was subsequently dried at 40°C using a 150W white light lamp. Following drying, the material was ground using a manual grinder to facilitate the extraction process. The resulting ground material was stored at room temperature away from light until further use.

*Preparation of Organic E. tirucalli L. Partitions*

The dried and ground plant material underwent extraction using various solvents (hexane, chloroform, ethanol, and methanol) in a Soxhlet apparatus. The partitions obtained from the extraction process were recovered, and solvents were removed under reduced pressure using a Yamato rotary evaporator (mod. RE200, Japan). The partitions were subsequently dried in an oven (Rios-Rocha, HS-33, Mexico) at temperatures not exceeding 40°C. Once solvent-free, the four partitions were stored at room temperature (20°C) until required for experimentation.

*Human Erythrocyte Toxicity Test*

The toxicity of the partitions on human erythrocytes was evaluated following

protocols with modifications from Alvarez-Suárez et al. and Botta et al,.[14] In brief, stock solutions of the partitions were prepared by dissolving 50 mg of the partition in 1 mL of methanol and sonicating the mixture by sonication (Ney ULTRAsonik, 19H, USA).

*Erythrocyte Solution*

Human blood samples were acquired through venipuncture from willing donors and carefully collected in EDTA tubes to prevent coagulation. To maintain sample integrity, the collected blood was promptly stored at 4°C for a period of 24 hours. Following the storage period, the blood underwent centrifugation at 1000 rpm for 10 minutes to separate its components. The resultant cell pellet underwent a meticulous washing process utilizing a phosphate buffer solution. This solution, formulated with precise proportions, included 8 g NaCl, 0.2 g KCl, 1.15 g Na2HPO4, 0.20 g KH2PO4, 0.10 g CaCl2, and 0.10 g MgCl2•6H2O per 100 mL, adjusted to pH 7.4 (CTR Scientific, México). After three rounds of thorough washing, the cell pellet was retrieved via centrifugation (CLAY ADAMS, Sero-Fuge 2001, USA). Subsequently, two separate solutions were prepared utilizing the recovered cell pellet. The first solution comprised a 5% suspension of erythrocytes in phosphate buffer solution (PBS), intended for the hemolysis test. The second solution, a 10% suspension, was designated for the cytoprotection test. Notably, both suspensions were freshly prepared immediately prior to each test. By adhering to this meticulously standardized procedure, the integrity and suitability of the blood samples were preserved, ensuring reliable outcomes in subsequent laboratory analyses.

*Hemolysis Test*

Samples were prepared from the stock solution of the partition under analysis (50 mg mL-1) by pipetting volumes of 25, 20, 15, 10, 5, and 2.5 µL into 1.5 mL conical tubes. To each tube, the appropriate volume of phosphate-buffered saline (PBS) was added to achieve a final volume of 1 mL. Subsequently, 250 µL of the 5% erythrocyte suspension (ERS 5%) was added to each tube, resulting in final partition concentrations of 1000, 800, 600, 400, 200, and 100 µg mL-1, respectively. Each assay was performed in triplicate.

Blanks were prepared by mixing the corresponding partition with PBS solution without the addition of ERS 5%. Furthermore, solvent inactivation checks were conducted by replacing partition volumes with equivalent solvent volumes and adjusting with PBS to a final volume of 1250 µL. Distilled water was employed as a positive control, while PBS served as the negative control.

Following preparation, each system mixture was incubated at room temperature for 30 minutes and then centrifuged at 13,500 rpm for 5 minutes at 4 °C (Select spin, 17T, USA). Subsequently, 200 µL of the supernatant from each system was transferred to a 96-well microplate, and absorbance was measured at λ = 550 nm using a TS Absorbance Reader (Biotek elx800, USA). Hemolysis percentage (%H) was determined using the formula 1[14]:

$$\%H = \frac{[Abs(x) - Abs(-)]}{[Abs(+) - Abs(-)]} * 100$$

**Formula 1.** Percentage of hemolysis (%H).

Abs (x): individual absorbance of the sample, obtained at 540 nm.
Abs (-): absorbance of the blank at 540 nm.
Abs (+): absorbance of the positive control at 540 nm.

*Toxicity test on Artemia salina*

The bioassay was conducted following the protocol outlined by Leos-Rivas et al13.

Initially, 10 nauplii (larval stage of Artemia salina) were introduced into each well of a 96-well microplate containing 100 µL of saline solution (Costar, Corning, USA). To evaluate the toxicity of the partitions under study (methanolic and ethanolic), 100 µL of the partition at an initial concentration of 1900 ppm was added, followed by serial dilutions to achieve concentrations ranging from 475 to 0.232 ppm, with each dilution performed in triplicate. Following a 24-hour exposure period to the partitions, deceased nauplii were enumerated using a stereoscope (Lieder, MC-720X, USA). Statistical determination of the Lethal Dose 50 (LD50) was conducted via linear regression analysis using the PROBIT method, facilitated by the SPSS V 20® software (IBM, USA).

*Cytoprotection test*

The antihemolytic activity of the ethanolic and methanolic partitions extracted from *Euphorbia tirucalli* L. was assessed using the AAPH• assay, following the methodology described by Botta et al[14]Similar to the hemolysis test, mixing systems were prepared with volumes adjusted accordingly. The 10% erythrocyte suspension (ERS) was exposed to the AAPH• radical (CTR Scientific, México) in the presence of varying concentrations of the partition under evaluation. The mixture was then incubated at 37°C for 150 minutes with constant agitation (Luzeren, THZ 100, México).

Following incubation, the mixture underwent centrifugation at 13,500 rpm for 5 minutes at 4°C, and the resulting supernatant (200 µL) was transferred to a 96-well microplate. Absorbance was measured at 540 nm using a Bio-Tek ELx800 spectrophotometer (USA). A positive control comprising a mixture of 10% ERS, PBS, and AAPH• was utilized, while a negative control consisted of a mixture of PBS and 10% ERS.

Given that the partitions were dissolved in methanol, an additional test was conducted by exposing erythrocytes to the AAPH• reagent in the presence of methanol volumes proportional to the partition concentrations. Blanks with extracts were prepared using a mixture of PBS and the respective partition, yielding final concentrations ranging from 100 to 1000 µg mL-1. Each assay was carried out in triplicate.

The percentage of cytoprotection was calculated using the following formula [14]:

$$\%C = \left[1 - \frac{[Abs(x) - Abs(-)]}{[Abs(+) - Abs(-)]}\right] * 100$$

**Formula 2.** Percentage of cytoprotection.

Abs (x): individual absorbance of the sample, obtained at 540 nm.
Abs (-): absorbance of the blank at 540 nm.
Abs (+): absorbance of the positive control at 540 nm.

*Sun Protection Factor of crude partitions of E. tirucalli* L.

The Sun Protection Factor (SPF) was determined following the method outlined by Mansur et al[19]. Initially, stock solutions of each partition (methanolic and ethanolic) were prepared at a concentration of 1 mg mL-1. Subsequently, dilutions were made to achieve concentrations of 0.25, 0.2, 0.15, 0.1, and 0.05 mg mL-1. The absorbance of each system was measured within the UV-B wavelength range of 290 to 320 nm using a scanning spectrophotometer (UNICO, SQ-2800), with 5 nm increments. Methanol served as the blank, while benzophenone (Sigma-Aldrich) was utilized as the positive control. All assays were conducted in triplicate to ensure accuracy and reproducibility. The SPF was then calculated using the following formula:

$$\text{FPS}= CF \sum_{290}^{320} EE(\lambda) * I(\lambda) * Abs(\lambda)$$

**Formula 3.** Sun Protection Factor.

Where, SPF= Sun Protection Factor, CF (correction factor) = 10, EE (λ)= Erythemogenic effect of radiation of wavelength λ. I (λ) = Intensity of the sun at wavelength λ; Abs (λ)= Absorbance of the solution at wavelength λ (Table 1).

**Table 1.** EE (λ)* I (λ) constants defined by Mansur et al 19 for SPF calculation.

| λ | 290 | 295 | 300 | 305 | 310 | 315 | 320 | Total |
|---|---|---|---|---|---|---|---|---|
| $EE(\lambda) * I(\lambda)$ | 0.0150 | 0.0817 | 0.2875 | 0.3278 | 0.1864 | 0.0839 | 0.0180 | 1 |

**Table 2.** Phytochemical tests.

| Compounds and functional groups | Test | Test development | Interpreting |
|---|---|---|---|
| **Carboxyl group** | $NaHCO_3$ | Add 100 µL of sodium bicarbonate (10 %, w/w) to the solution*. | The test is positive if carbon dioxide bubbles are released. |
| Carbonyl group | | Add 100 µL of the reagent 2,4-DNFH (2,4-dinitrophenylhydrazine) to the solution*. | The test is positive if a yellow, red or orange precipitate is formed. |
| Tannins | $FeCl_3$ | Add 100 µL of reagent (5% w/w in ethanol) to the solution*. | The test is positive if a dark green or black coloration is observed. |
| Sterols and terpenes | Liebermann-Burchard | 100 µL of Liebermann-Burchard reagent, consisting of a mixture of acetic anhydride (1 mL), chloroform (1 mL) and concentrated sulfuric acid (0.1 mL), was added to the solution*. | A red coloration indicates the presence of terpenes, while a blue coloration indicates the presence of sterols. |
| Carbohydrates | Antrona | Suspend the partition (1 mg) in water and add a few drops of an anthrone solution (0.2%, v/v) in concentrated sulfuric acid. | The test is positive if a blue-green or violet ring appears on the interface. |
| Flavonoids | Shinoda | Add 100 µL of concentrated hydrochloric acid and one or two magnesium filings. | Appearance of deep red color: presence of flavonoids, while green/blue color: presence of flavonones, flavonones, flavonols, flavonols or xanthones. |
| Alkaloids | Mayer | Add a few drops of hydrochloric acid to the solution*. | Blue coloration indicates the presence of alkaloids. |
| Sesquiterpenes | Baljet | Add a few drops of solution A and B to 3 mg of dissolved partition in 1 mL of solvent. | Orange or dark red coloration indicates the presence of sesquiterpenes. |
| Saponins | Foams | Dissolve each partition in 1mL of water and shake. | The appearance of persistent foams indicates the presence of saponins. |

***Phytochemical profile:*** Verde-Star *et al*[21]

Total phenol content

The total phenolic content in the partitions was assessed following the protocol outlined by Shahidi and Naczk[22]. The experiments were conducted in triplicate using a macrodilution system. Initially, a calibration curve was constructed using gallic acid as the standard, covering a concentration range of 2 to 20 µg mL-1. For the analysis of each partition, 250 µL of a partition stock solution (0.2 mg/mL) was combined with 750 µL of methanol to achieve a final concentration of 0.05 mg mL-1. To this mixture, 6 mL of methanol (CTR Scientific, México) was added, followed by 500 µL of Folin-Ciocalteu reagent (MERCK, Germany). The solution was incubated for 5 minutes in darkness. Subsequently, 1.5 mL of sodium carbonate solution ($Na_2CO3$, 20%, CTR Scientific, México) was added to adjust the pH to 10. The resulting solution was transferred to a 10 mL volumetric flask and kept in darkness at room temperature. After 2 hours, the absorbance was measured at a wavelength of 760 nm using a Beckman Coulter DU®650 Spectrophotometer. A mixture of methanol, Folin-Ciocalteu reagent, and sodium carbonate served as the blank. The total

phenolic content was expressed in micrograms of gallic acid equivalents per gram of extract (µg GAE mg-1 E).

Quantification of total phenols was performed using a gallic acid calibration curve as a reference, which is cited below:

$$Abs = 0.0171 Con.gal.ac. - 0.0042$$

**Formula 4.** Total phenol content.

Where, "Abs" is the absorbance and "Con.gal.ac" is the concentration of gallic acid.

*Statistics*

The data obtained were analyzed using the SPSS V 20® (IBM, USA) software, employing analysis of variance (ANOVA) and Tukey's test to assess significant differences between treatments and to compare means for treatment homogeneity, respectively. Each assay was conducted in triplicate. Treatment responses were deemed statistically significant at $p \leq 0.05$.

## 3. Results and discussions

*Toxicity test on human erythrocytes*

The hemolysis assay involved exposing human erythrocytes to various partitions of *Euphorbia tirucalli* L. as illustrated in Figure 1, the hemolytic effect of these partitions exhibited a dose-dependent relationship. At concentrations equal to or below 100 µg mL-1, minimal hemolytic effects were observed across all partitions. However, distinctions in hemolytic potency became more pronounced beyond 600 µg mL-1. For instance, at 1000 µg mL-1, the hexanolic partition demonstrated the highest hemolytic effect (~50%), followed by the chloroformic partition with a 10% effect; conversely, the methanolic and ethanolic partitions exhibited minimal hemolytic effects (<5%) at this concentration. Statistical analysis via ANOVA ($\alpha \leq 0.05$) confirmed significant differences in hemolytic effects across varying concentrations. Furthermore, Tukey's test ($p \leq 0.05$) indicated homogeneity among the effects of partitioning treatments (i.e., chloroformic, methanolic, and ethanolic) and controls (i.e., negative and methanol) at concentrations equal to or below 600 µg mL-1. This suggests that the beneficial biological effects of these partitions (i.e., ethanolic, methanolic, and chloroformic) could be harnessed in human applications without posing a significant risk. Notably, as per Sylwia Cyboran et al[23] and Lux et al[24], the cytotoxicity test conducted on human erythrocytes provides valuable insights, indicating that damage to their cell membrane may correlate with susceptibility in more complex cells with a greater number of organelles.

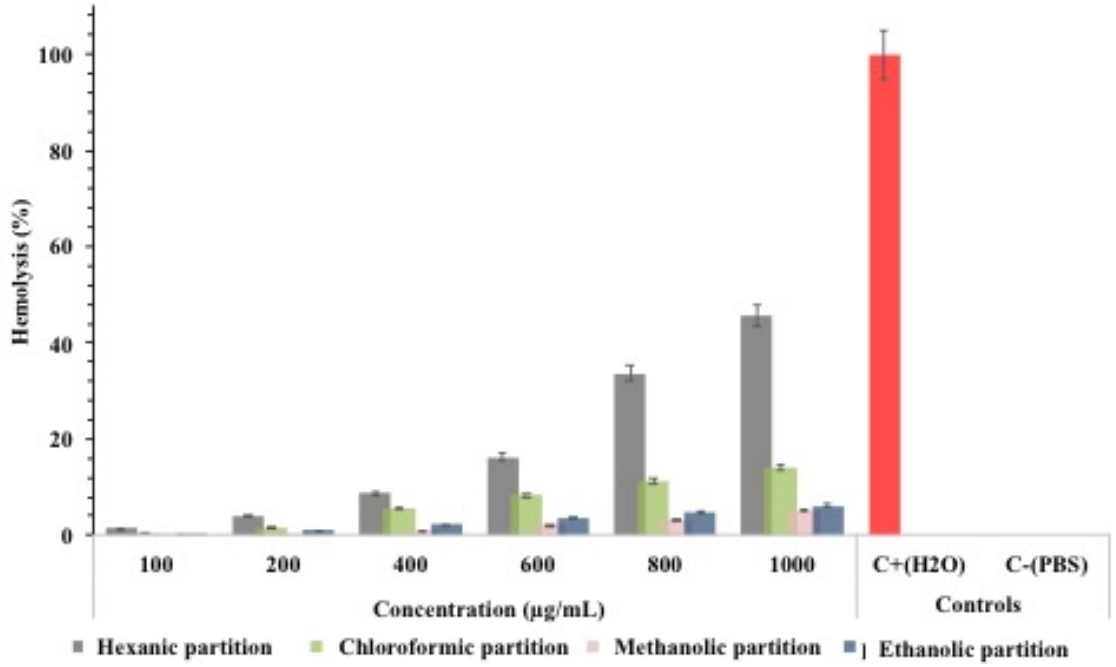


**Figure 1.** Hemolytic effect of the partitions obtained from *E. tirucalli* L. as a function of concentration (*n=3*).

*Toxicity on A. salina*

The regression analysis in PROBIT revealed that partitions containing methanol and ethanol were classified as "moderately toxic" according to CYTED 25, with LD50 values of 189.54 and 144.77 µg mL-1, respectively. This observation may be attributed to the absence of alkaloids in the extracts investigated in this study, which are often associated with the toxicity of plant extracts[26]. The moderate toxicity observed in the methanolic and ethanolic partitions towards *A. salina* suggests that their pharmacological potential could be harnessed for human therapeutic applications[27], pending further comprehensive toxicological evaluations

*Cytoprotection test*

Following the observation of the methanolic and ethanolic partitions' low hemolytic percentages, their cytoprotective effects on human erythrocytes against AAPH• radical-induced hemolysis were subsequently assessed. The erythrocyte suspension (10%) was subjected to AAPH• exposure for 2 hours and 30 minutes at 37°C under moderate agitation. As depicted in Figure 2, both partitions demonstrated an effective cytoprotective effect at concentrations ≥ 200 µg mL-1, completely preventing hemolysis. However, above 100 µg mL-1, a decline in cytoprotective efficacy was noted for both partitions, with values of 55% and 35% observed for the ethanolic and methanolic partitions, respectively. In contrast, the positive control (ERS + PBS + AAPH•) exhibited 100% hemolysis induced by AAPH•, while the negative control (ERS + PBS) showed no hemolysis. ANOVA analysis indicated a significant difference ($\alpha \leq 0.05$) between treatments (i.e., partitions and controls), while Tukey's test revealed homogeneity in the effects of treatments from 200 to 1000 µg mL-1 (cytoprotection > 95%). This cytoprotective effect could be attributed to the presence of tannins and flavonoids in the partitions, as previous studies have highlighted the antioxidant potential of polyphenols[11,28-30]. The observed lower cytoprotection at 100 µg mL-1 may be attributed to the lower treatment concentration[30].

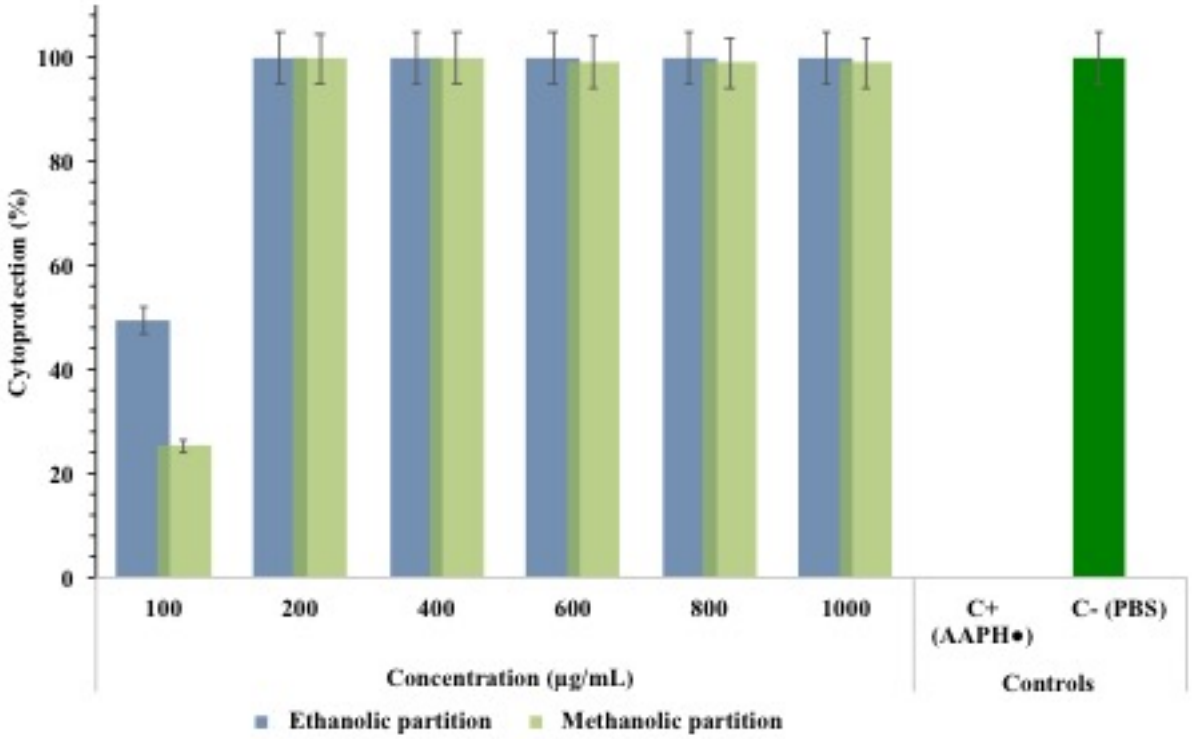


**Figure 2.** Cytoprotective effect on human erythrocytes of ethanolic and methanolic partitions of *E. tirucalli* L. as a function of concentration (*n=3*).

*Sun Protection Factor*

Extrinsic factors like pollution and chemicals pose threats to skin health, but UV radiation stands out as the primary culprit behind skin cancer and cellular aging[31]. Solar radiation, particularly UV-B rays, induces mutagenesis, carcinogenesis, and cellular senescence by directly affecting cellular DNA[32]. Recognizing these risks, our study delved into the photoprotective potential of methanolic and ethanolic partitions extracted from *E. tirucalli* L. using the in vitro method outlined by Mansur[19], with benzophenone from Sigma-Aldrich serving as the positive control.

As depicted in Figure 3, both partitions exhibited significant photoprotective effects against UV-B radiation at a concentration of 250 µg mL-1. The Sun Protection Factor (SPF) was calculated as 9.25 for the methanolic partition and an impressive 18.42 for the ethanolic partition, surpassing the SPF of 5.4 observed for benzophenone. ANOVA analysis confirmed significant differences among the three treatments at a significance level of ≥ 95%. Notably, the ethanolic extract demonstrated the highest SPF, offering over three times the protection compared to benzophenone (at concentrations ranging from 100 to 250 µg mL-1). Moreover, the methanolic extract exhibited a higher SPF compared to the positive control.

Ramos and Ivonne[33] suggested that the photoprotective potential of these extracts may stem from their polyphenol content, as evidenced in their phytochemical profile. The presence of these compounds likely contributes to the observed variations in SPF values between the ethanolic and methanolic partitions. Specifically, the ethanolic partition boasted a higher total polyphenol content (19.46 µg EAG mL-1) compared to the methanolic partition (3.52 µg EAG mL-1).

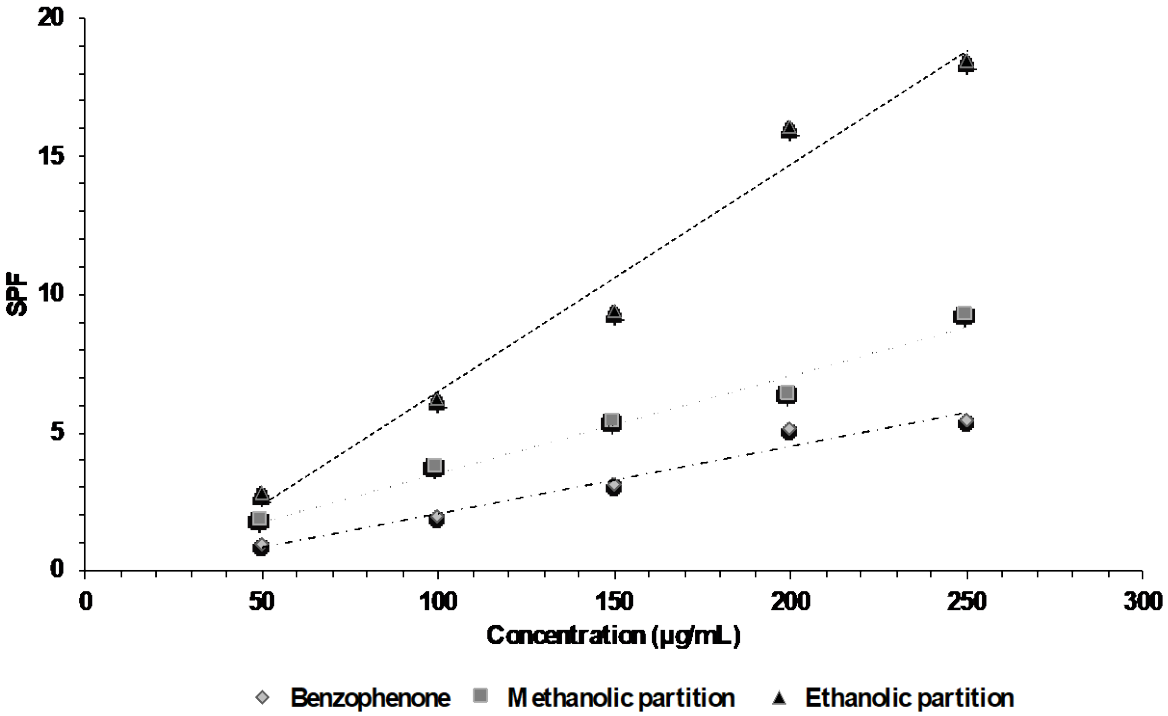


**Figure 3.** Sun protection factor (SPF) against UV-B radiation (290-320 nm) of the methanolic and ethanolic partitions, using benzophenone as a positive control at different concentrations (50-250 µg $mL^{-1}$) with respect to the extracts (*n=3*).

*Phytochemical profile*

The phytochemical analysis unveiled distinct compounds across the partitions: sterols and triterpenes in the hexane partition, tannins, flavonoids, sterols, and sesquiterpenlactones in the chloroformic partition, carboxyl groups, tannins, sterols, carbohydrates, flavonoids, and saponins in the ethanolic partition, and carboxyl groups, tannins, sterols, triterpenes, carbohydrates, flavonoids, sesquiterpenlactones, and saponins in the methanolic partition[21]. Previous studies have corroborated the presence of various compounds in the aerial parts of *E. tirucalli* L., including sterols (campesterol, stigmasterol, beta-sitosterol, isofucosterol, and cycloartenol), triterpenes (taraxerane and cycloeuphordenol), tannins (*tirucallin* A), polyphenols (*tirucallin* B, euphorbine A, euphorbine F), and sesquiterpenes (tirucadalenone)[34].

*Total phenols*

The results revealed that the ethanolic partition exhibited a notably higher content of total polyphenols, quantified at 19.4588 µg EAG/mg of extract, compared to the methanolic partition, which yielded 3.52 µg EAG/mg of extract. This discrepancy may be attributed to the presence of tannins (specifically *tirucallin* A) and flavonoids in the ethanolic partition, whereas the methanolic partition contained only flavonoids, serving as a reference to polyphenols. These compounds are widely recognized for their antioxidant properties, capable of scavenging superoxide anions and hydroxyl radicals. Notably, as highlighted by Aisah et al [17], the tannins identified in the ethanolic extract of E. *tirucalli* L. possess remarkable wound-healing potential, representing an additional positive biological effect of the partitions suitable for skin application.

## 4. Conclusions

Tests conducted on human erythrocytes demonstrated that both the methanolic and ethanolic partitions exhibited non-hemolytic properties and displayed potential dose-dependent cytoprotective effects. Moreover, in terms of photoprotective activity, both partitions surpassed the sun protection factor of the positive control (Benzophenone). These combined biological activities suggest that the topical application of *E. tirucalli* L. partitions could potentially support the natural renewal system of the human body owing to their cytoprotective effects. These activities complement the previously reported benefits of *E. tirucalli* L. extracts, such as their antimicrobial properties and promotion of collagen synthesis. However, given the findings from the *A. salina* model, further toxicity testing is warranted to mitigate any potential adverse effects associated with topical application.

## 5. Acknowledgments

Thanks to Dr. Eduardo Sánchez García for the technical facilities provided.

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