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PHOTOPROTECTIVE PROPERTIES OF *LIPPIA TRIPHYLLA* HYDROALCOHOLIC EXTRACT AND *IN VITRO* SAFETY EVALUATION

Mancheno Moncayo, M. J.[1]; Acosta León, K. L.[1]; Vinueza Tapia, D. R.[1*]

[1]Polytechnic School of Chimborazo, [ESPOCH], Panamericana Sur km 1 ½, Riobamba-Ecuador

*drvinueza@espoch.edu.ec

Abstract

The aim of this research was to perform the assessment of the photoprotective effect of the hydroalcoholic extract of leaves of *Lippia triphylla* through ultraviolet B radiation (UV-B) induced cell death model on a strain of *Escherichia coli* ATCC 25922 and Mansur equation apply in order to determine the *in vitro* sun protection factor (SPF).

Hydroalcoholic extract of the dry powered leaves of *L. triphylla* was obtained by cold maceration method and was subjected to preliminary phytochemical screening. The photoprotective effect of the plant extract at three concentration levels of 20, 200 and 2000 µg/mL and controls 2-ethylhexyl 4-methoxycinnamate (OMC) and 2-ethylhexyl 4-(dimethylamino) benzoate (PAMIDATE O) at the same concentration levels of sample were carried out using UV-B chamber and suitable quartz cells, determining the survivor bacteria number versus time. The safety of plant extract was evaluated by hen´s egg coriallantoic membrane irritation test (HET-CAM) and genotoxicity assay (micronuclei). Furthermore, total phenolic content was estimated by using Folin-Ciocalteu reagent, total flavonoid content was evaluated through of AlCl3 complexation method and 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was used to determine the in vitro radical scavenging capacity of plant extract.

Phytochemical analysis of extract revealed the presence of major classes of phytochemicals such as flavonoids, tannins, triterpenes, steroids, etc.; but no alkaloids were detected. The hydroalcoholic extract showed an appreciable phenolics and flavonoids content, even though exhibited a mild antioxidant activity. The UV-B photoprotective activity of the plant extract was significant in comparison with the standard photoprotective chemicals, OMC and PAMIDATE O; due it does not show a statistical difference ($p<0.05$) when comparing the lower concentration of the hydroalcoholic extract (20 µg/mL) v.s. OMC and PAMIDATE O (20 µg/mL). The photoprotective effect of the plant extract was directly proportional to the concentration.

Based on the results of this preliminary study, it can be concluded that *L. triphylla* is a very interesting source of natural photoprotective compounds which can be used to prevent many chronic disorders derived from UV-B radiation exposition through its inclusion in cosmetic formulations. Further detailed phytochemical studies are needed to identified the chemical compounds responsible of the photoprotective activity showed, although it could be phenylpropanoid derivatives of vebarcoside type, a noted chemical marker of the Verbenaceae and Scrophulariaceae families, of which has been reported a wide range of biological activities including anti-inflammatory, antioxidant, anti-bacterial, anti-tumor, anti-fungal, photoprotective as well as chelating effects.

Keywords: *Lippia triphylla, photoprotection, UV-B.*

Introduction

The World Health Organization (WHO) report that UV levels are higher closer to the equator. At the equator level the sun's rays have a shorter distance to travel through the atmosphere and therefore less of the harmful UV radiation can be absorbed. With increasing altitude less atmosphere is available to absorb UV radiation. With every 1000 m in altitude, UV levels increase by approximately 10%. In addition, WHO established the UV index (UVI) like a measure of the level of UV radiation. The values of the index range from zero upward - the higher the UVI, the greater the potential for damage to the skin and eye, and the less time it takes for harm to occur. The UVI is an important vehicle to alert people about the need to use sun protection (1). The maximum UVI value for humans, accordingly to the World Health Organization is 11 and it is catalogued like extreme. On the Ecuador country, the Ecuadorian Civil Space Agency inform through its Hyperion report, that at Guayaquil and Quito cities at noon the UVI averages 14 and 24, respectively, such values are alarming because there is an increased risk of skin and ocular lesions (2).

Sunlight is the primary source of energy of living organisms. The sun radiation that reaches the earth is divided into UV, visible light, infrared and radio frequencies. The UV spectrum comprises UVC (100–280 nm), UVB (280–315 nm) and UVA (315–400 nm) wavelengths (3). Ultraviolet-B (UVB) radiation usually affects the epidermal basal layer of skin. UVB radiation causes various biological effects that result in erythema, inflammation, photoaging, cell death and others. UVB radiation induces transitions in pyrimidine sites that give rise to the singular cyclobutane pyrimidine dimers (CPD), which are associated with DNA damage. The induced damages on the DNA by effect of UVB radiation subsequently cause apoptotic cell death. The skin damage derived by UVB radiation on the skin cells, promotes hot spot mutations at the p53 gene that can ultimately trigger cell death (4).

Methods

Drugs and chemicals

All reagents were of analytical or cosmetic grade. Trypan blue and orcein were purchased from Sigma-Aldrich Company (St. Louis, USA). 2-ethylhexyl 4-methoxycinnamate (OMC) and 2-ethylhexyl 4-(dimethylamino) benzoate (PAMIDATE) were purchased from Ningbo Hi-Tech Zone Yefeng New Materials Technology Co., Ltd. (China). All other reagents were purchased from Merck (Darmstadt, Germany).

Collection of plant material

Lippia triphylla, Verbenaceae, was collected at Ecuador, Chimborazo province, Riobamba town, sector S 01° 40' 10.254" W 078° 40' 22.508", at 2895 meters above sea level. The plant material was taxonomically identified by the botanist Jorge Caranqui at Polytechnic School of Chimborazo and a specimen was deposited at Herbarium. *L. triphylla* leaves were collected, dried at 50 °C in a forced convection oven for 8 h, and it were ground in a knife mill until particle size of 2-3 mm. This collection and study were done under the Research Agreement to Access to Genetic Resources MAE-DNB-CM-2018-0086, approved by Ministerio del Ambiente del Ecuador.

Extraction of the plant material

The dried powdered flowers (100 g each) were extracted by maceration with 1000 mL 70% v/v ethanol for 72 h at room temperature with occasional shaking. Then, the extracts were filtered and the process was repeated on the marc until material were exhausted. The collected filtrates were polled and evaporated under reduced pressure (50°C, -0.5 bar) to yield the dry extract (2.48 %). The obtained solids were stored at 4°C and vacuum until use.

Preliminary phytochemical screening

Phytochemical screening of the freshly prepared crude extracts of flowers was carried out to investigate the presence of secondary metabolites such as flavonoids, alkaloids, terpenoids, saponins, and tannins using standard procedure (5) (6).

Total flavonoid determination

The total flavonoids were measured by a colorimetric assay modified by Boukhris et al. (2013). 1 mL aliquot of the diluted sample or standard solution of quercetin (20, 40, 60, 80 and 100 mg/L) was added to a 10 mL volumetric flask containing 4

mL of H2O. At zero time, 0.3 mL of NaNO2 (5%, w/w) was added to the flask. After 5 min, 0.3 mL AlCl3 (10% w/w) was added. At 6 min, 2 mL of NaOH (1 M) was added to the mixture. Immediately, the reaction flask was diluted to volume with the addition of 2.4 mL of of H2O and thoroughly mixed. The absorbance of the mixture, characterized by a pink colour, was determined at 510 nm compared to a water control (7). Total flavonoids were expressed as mg quercetin equivalents (QE)/100 g of hydroalcoholic extract.

Total phenolics determination

The Folin-Ciocalteau method for determination of total phenolics was used in accordance with the description of Waterman and Mole (8). To 200 mL of deionized water were added 50 g of Na2WO4, 6.13 g of H3PMo12O40, 25 mL of concentrated HCl, and 12.5 mL of 85% o-H3PO4, and the solution was refluxed for 10 h. A few drops of Br2(liq) was added, and the final volume was adjusted to 250 mL. Sample (200 µL) was vortexed with about 10 mL of destilled water and 1 mL of Folin-Ciocalteu reagent. After 1 min and before 8 min, 3.75 mL of a 20 g/100 mL Na2CO3 solution was added, and time was recorded as time zero. The volume was made up to 20 mL with distilled water, and the solution was vortexed three to four times during the next 2 h. After exactly 2 h, the absorbance was recorded at 760 nm (9). Total phenols were expressed as mg gallic acid equivalents (GAE)/100 g of hydroalcoholic extract, using a calibration curve of a freshly prepared gallic acid solution.

Radical scavenging capacity

Solutions of the *L. triphylla* hydroalcoholic extract (leaves) in ethanol 70% v/v were prepared at concentrations in the range of 10-1000 µg/mL. 100 µL of each concentration was mixed with 3.9 mL of 60 µM DPPH in methanol, at room temperature. The samples were kept in the dark for 30 min, and only after that was the absorbance measured at 515 nm in UV-vis spectrophotometer (10). The blank solution was composed by methanol. The negative control solution was prepared by mixing 3.9 mL of 60 µM DPPH solution with 100 µL of ethanol 70% v/v. Similar solutions in the same medium (ethanol 70% v/v) of gallic acid in range of (10-100 µg/mL) were prepared and tested for scavenging activity. The experiments were repeated 3 times to confirm the reproducibility of the data. The antioxidant activity was expressed as the percentage of DPPH radical inhibition. The IC50 was calculated by means of logarithmic regression of the curves obtained by plotting the results of percentage the DPPH inhibition (11). On these plots, the abscissa represents the concentration of *L. triphylla* hydroalcoholic extract (leaves) and the ordinate represents the antioxidant activity.

Sun protection factor (SPF) of the crude extract

The hydroalcoholic extract was dissolved in ethanol 70% v/v (12) to a final concentration of 20, 200 and 2000 µg/mL. The SPF model used in this research was according to the methodology described by (13). The absorbance of samples was measured in UV-B wavelength range (290-320 nm), with 5-nm increments and three determinations by each point. The SPF was calculated by applying the Mansur equation:

$$SPF(spectrometry) = CF \times \sum_{290}^{320} EE(\lambda) \times I(\lambda) \times abs(\lambda)$$

Where: CF (correction factor) = 10; EE(λ) is the erythemal efficiency spectrum; I(λ) is the solar intensity spectrum; abs(λ) is the absorbance of the solution. The values of EE(λ) x I(λ) are constant according (14), and are exposed in Table 1.

Photoprotection using a bacterial model

A strain of *Escherichia coli* (ATCC 25922) was grown in heart–brain broth until the culture reached a concentration of 10^7–10^8 cells/mL (O.D.≈ 0.3 read at 550 nm). The bacteria were centrifuged by 10 min at 6000 rev/min, suspended in Ringer PBS (pH 7.0), and transferred into quartz cuvettes. The extract was dissolved in ethanol 70% v/v (20, 200 and 2000 µg/mL) and placed in a quartz cuvette. A cuvette containing bacteria was placed behind the cuvette containing the photoprotective substance, thus forming one experimental unit. The experimental units were irradiated with a UVB lamp (290–315 nm, Philips TL 40W/12 RS SLV/25 UVB Broadband, AT) and an irradiation dose of 0.60 J/cm^2. The number of surviving bacteria was detected in accordance with the dilution method at different time periods (15).

The positive controls were 2-ethylhexyl 4-methoxycinnamate (OMC) and 2-ethylhexyl 4-(dimethylamino) benzoate (PAMIDATE) (at the same concentration levels of sample), and the negative control was ethanol 70% v/v (2). The photoprotective activities of all substances were evaluated in parallel. Tests were repeated in three independent experiments, and the assays were performed in triplicate. The results are expressed by plotting the logarithm of the number of survivors versus the irradiation time. The mortality rate (K) was calculated by linear regression analysis (3).

Safety of the photoprotective extracts: irritant potential evaluation

Hydroalcoholic extract was evaluated regarding its safety (16). To verify the safety of the extract, it was dissolved in phosphate buffer saline (PBS) pH 7.4. Three different methods were performed: HET-CAM test, CAM-TBS test (17) and Genotoxicity test (3).

In vitro eye irritation tests (HET-CAM)

The irritating potential of the hydroalcoholic extract was performed using HET-CAM (hen's egg test-chorioallantoic membrane) test (18). The assay employs the CAM of a 10-day-old fertilized hen's eggs. The CAM, a membrane which surrounds the developing chick embryo, is highly vascularized (19), and is regarded as being insensitive to pain (20). For this test, the hydroalcoholic extract of *L. triphylla* was dissolved at concentrations of 500, 250, 125, and 62.5 µg/mL. The fertilized chicken eggs were obtained from commercial source. On the 10th day of incubation, the egg shell was removed around the chamber air, showing the shell membrane. After CAM was exposed, 300 µL of each extract concentration were applied. The positive control (NaOH 0.1 M), and negative control (distilled water) were performed to demonstrate the validity of the test. For each concentration and controls, three eggs were used. After the application of the extracts, the membrane and blood vessels were examined for 5 min. The time of appearance, measured in seconds, of each irritant effect (haemorrhage, lysis and coagulation) was recorded. The following formula is used to generate an irritation score (IS):

$$\mathrm{IS} = \frac{(301-H)\times 5}{300} + \frac{(301-L)\times 7}{300} + \frac{(301-C)\times 9}{300}$$

Where H = the time taken to start the hemorrhage reactions; L = time taken to start of vessel lysis; C = time taken to start coagulation.

The average score was calculated for each extract, and the extracts were classified into four categories: non-irritant (IS < 1), low irritant (1 ≤ IS < 5), Moderate irritant (5 ≤ IS < 9) and irritant (IS≥ 9) (21).

Chorioallantoic Membrane-Trypan Blue Staining (CAM-TBS) test

CAM-TBS is a quantitative assay for toxicity assessment of formulations (22) (23). In this test, trypan blue dye is used as an injury indicator of the chorioallantoic membrane (CAM), and the method shows a good correlation with the in vivo Draize eye irritation test (24) (25). The methodology is developed similarly to the HET-CAM test, but after the removal of the sample, 500 µL of a PBS and 0.1% of trypan blue staining (TBS) were added to the CAM on the surface limited by an 18 mm diameter silicone ring. TBS to excess was rinsed off with distilled water, the CAM limited by the silicone ring was extracted and transferred into a tube containing 5 mL of N,N-dimethylformamide, that later was vortexes and centrifugation. The solution absorbance was measured by spectrophotometry at 595 nm. The trypan blue that entered to the cells could be correlated to the injury caused by the formulation to the CAM. Each formulation was classified according to the mean value of four eggs based on the HET-CAM scores: 0–4.99 corresponding to no irritant/slightly irritant (NOI/SLI); 5.00–8.99 corresponding to moderately irritant (MOI); and 9.00–21.00 corresponding to (severe irritant) SEI. The score (d) for each formulation was assessed using the following equation (23).

$$TBS\ concentration = d \times \frac{5}{1000} \times 10^{9} nmol$$

As a negative control four eggs were submitted to the same procedure, without formulation was added (17).

Genotoxicity assay (micronucleus test)

The genotoxicity was determined according to the method of (26). *Vicia faba* var. minor seeds were used for this study. Dry seeds of *V. faba* were soaked for 24 h in distilled water, the seed coats were removed, and the seedlings were allowed to germinate between two layers of moist cotton. After seven days, the primary roots (approximately 2 cm in length) were selected randomly, and 4 seeds were used per treatment. The growing roots were treated for 12, 24, or 48 h with the extract at various concentrations (0.1, 0.2 and 0.4% w/v), followed by a 48 h recovery period. Tap water was used as a negative control, and ethanol was used as a positive control. The exposure time was 48 h for the negative and positive controls. Each batch was incubated at 22 °C. At the termination of the exposure time for each treatment, meristematic and F1 cell regions were prepared from each root tip separately by simply cutting the meristem section (first millimeter behind the root cap) and F1 section. Root development is initiated at the apex of the root tip by mitotic divisions in the meristematic region (about 1 mm in length above the root cap) and the F1 cells (about 1 mm) are moved upward to lengthen the root structure (3).

Micronucleus (MCN) formation is the result of acentric fragments or laggards being excluded from the nucleus proper during mitosis. The MCN are revealed in the subsequent generation in the interphase or prophase cells, in the F1 cells. The meristematic region was used to determine the mitotic index, and the F1 region for the score of MCN. The roots were placed into a Farmer solution (1:3 acetic acid and absolute MeOH) at 4 °C for 24 h. The roots were subsequently hydrolyzed in 1 N HCl at 55 °C to break the bonds of the plant cell wall. The samples were rinsed three times with distilled water to remove the HCl and placed in 70% EtOH at 4 °C. The roots were macerated in 45% acetic acid and were then stained for 5 min with 1% orcein acetate for observation via an optical microscope (CX-31, Olympus, USA). Three thousand cells per experimental group were scored to determine the mean frequency of micronuclei and the mitotic index. Each experiment was repeated three times (3). To calculate the genotoxicity, the following equations were used:

$$Mitotic\ index\ (MI) total = number\ of\ cells\ of\ each\ phase \times \frac{100}{1000}$$

and

$$Micronuclei\ (MCN) = number\ of\ interphase\ cells\ with\ MCN \times \frac{100}{1000}$$

Data Analysis

All values were expressed as mean values±SD (standard desviation) and data were analyzed by applying an analysis of variance (ANOVA) followed by Tukey test to compare treatments v.s. standard. The results were considered statistically significant if $P<0.05$.

Results

Phytochemical screening

According to the results of preliminary phytochemical screening the extract showed the presence of flavonoids, phenolic compounds, terpenoids, steroids, carbohydrates, glycosides and saponins. Alkaloids, proteins and aminoacids were absent in the extract (see Table 2).

Total flavonoids and total phenolics determination

The total flavonoids content (TFC) and total phenolics (TPC) content of the extract expressed in terms of quercetin equivalents and gallic acid equivalents, respectively; as well yield (% w/w) are presented in Table 3. The TFC were calculated using the following linear regression equation based on the calibration curve of quercetin $A=0.0014C+0.0144$; $R^2=0.9994$. Where A is absorbance and C is amount of quercetin in µg/mL. The TPC were calculated using the following linear regression equation based on the calibration curve of gallic acid $A=0.0013C-0.0276$; $R^2=0.9992$. Where A is absorbance and C is amount of gallic acid in µg/mL. The extract was found to contain appreciable amounts of flavonoid and phenolic compounds.

Radical scavenging capacity

The scavenging capacity of different concentration of extract on the DPPH free radical

was compared with standard antioxidant, gallic acid. The results are expressed as % inhibition and are shown in the Table 4. The extract showed a dose dependent scavenging activity and it exhibited 17.30% inhibition of free radicals at 200 µg/mL whereas at the same concentration gallic acid showed 90.48% inhibition. The scavenging ability of the extract was found to be non-significant (P>0.05) in comparison to gallic acid.

Sun protection factor

The photoprotective effect of different concentration of extract in terms of sun protection factor (SPF) are summarized in the Table 5. The doses of extract showed a significant photoprotective effect, directly proportional to the concentration. The photoprotective activity of the extract at all of the concentration levels was not comparable to the standards 2-ethylhexyl 4-methoxycinnamate (OMC) and 2-ethylhexyl 4-(dimethylamino) benzoate (PAMIDATE), reference photoprotective chemicals. Significant differences in the photoprotective activity was observed.

Photoprotection using a bacterial model

The photoprotection results of *L. triphylla* were highly close in comparison to standards. The *L. triphylla* extract at all of tested concentrations showed a significant increase in bacterial survivor number compared to blank.

According to results, the bacterial population (5.5×10^7 cells/mL) without protection reached cell death at 6.9 min approximately that is comparable to that obtained by García-Bores et al. (3), with a mortality rate (K) of –1.2106. The bacterial population protected by the L. triphylla did not reach cell death until 90 min of irradiation with UVB and had a mortality rate of K=–0.0419.

K indicates the range of inactivation of *E. coli* caused by UVB. The exposure of a bacterial culture to UVB produces a rapid decline in the population due to damage to the DNA (3). The K and time to death of the population treated with the *L. triphylla* were comparable than those obtained to 2-ethylhexyl 4-methoxycinnamate and 2-ethylhexyl 4-(dimethylamino) benzoate, the active compounds of commercial sunscreens (90 min; K=–0.0438 and 90 min; K=–0.0373). Likewise, under the experimental conditions, the maximum time in which surviving bacteria could be verified due to the protective action of these conventional photoprotectors was 90 min. The *L. triphylla* hydroalcoholic extract K (–0.0419) was 30-fold below the K without protection (–1.253).

Safety of the photoprotective extracts: irritant potential evaluation

The results of HET-CAM and CAM-TBS tests are showed at Table 6. According to the classification by scores, for the *L. triphylla* was classified as non-irritant (NOI), suggesting that it is safe to be applied on the skin. Both tests are related to ocular irritation, which can be associated to cosmetic application on the face, near to the ocular mucous membrane (17).

Discussion

In the present study, the photoprotective effect of three concentrations of hydroalcoholic extract of *L. tryphilla* was evaluated on *Escherichia coli* ATCC 25922. The results indicate that *L. tryphilla* at all concentration levels (20, 200 and 2000 µg/mL) significantly increased bacterial survival in a manner directly proportional to the concentration over a period of 120 min, demonstrating a photoprotective effect on bacteria population against UVB radiation. The co-extracted substances in the crude extract could improve the *L. tryphilla* photoprotective properties (27). Therefore, good photoprotective activity was observed among bacteria treated with OMC and *L. tryphilla* extract at all concentration, except 20 µg/mL concentration, wherein a less survivor time was observed (90 min). The photoprotective activities of the extract were found to be highly potent when compared to the controls OMC and PAMIDATE O. It showed that the extract and controls actions were time and concentration dependent. This can be explained by kinetic differences of the active principle presence in the extract. However, significant differences in SPF values assess through Mansur equation were observed, between the *L. tryphilla* extract and the controls (OMC and PAMIDATE O), which could be related with method shortcomings. The natural compounds present in *L. tryphilla* that growth in Ecuador has not been studied, but an appreciable flavonoid and phenolics content which could be

responsible for the various properties, due to the high content of phenylpropanoids in the extract, this could be related to photoprotective activity because according to the literature these compounds provide chemical defense against UV radiation in *L. tryphilla*, because similar substances in some other plant species fulfill such functions (15). Furthermore, the photoprotective effect of the *L. thryphilla* leaves could be attributed to the presence of biocomplexes phytochemical groups such as flavonoids, terpenoids and saponins since they provide synergic effects in conjunction with phenylpropanoids (28). Therefore, there is a need of further research to find out the active principles (or biocomplex) responsible for the photoprotective activities.

The safety level of *L. tryphilla* hydroalcoholic extract in terms of irritation and genotoxicity was zero and very low, respectively; thus guaranteeing its safe use in subsequent applications.

This study confirms the photoprotective activity of the hydroalcoholic extract of *L. tryphilla* (leaves) during the measurement period of the study (120 min). However, further studies are recommended for explaining the mechanism of photoprotective activity and acute as well as chronic dermal toxicity.

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λ (nm)	E (λ) I (λ)
290	0.0150
295	0.0817
300	0.2874
305	0.3278
310	0.1864
315	0.0839
320	0.0180

Table 1. Correlation between the erythemogenic effect (EE) and the radiation intensity at each wavelength (I) (Mansur et al., 1986).

Phytochemical test	Name of the test	*Lippia triphylla leaves*
Tannins	$FeCl_3$ test, Lead acetate test	+
Steroids	Salkowski test	+
Flavonoids	Ammonia test, Alkaline reagent test	+
Saponins	Frothing test	+
Proteins and aminoacids	Ninhydrin test	-
Alkaloids	Dragendorff's, Hager's, Meyer's and Wagner's test	-
Carbohydrates	Molisch's test	+
Glycosides	Nitroprusside test	+
Cardiac glycosides	Keller Killiani test	-
Terpenoids	Salkowski test (modified)	+

Table 2. Results of phytochemical analysis of *L. triphylla* leaves extract. +: present, -: absent.

Extract	Yield (% w/w)	Total flavonoid content mg of QE/g of extract	Total phenolics content mg of GAE/g of extract
Leaves	2.48%	4.86 ±0.05	16.48 ±0.70

Table 3. Yield (% w/w), Total Flavonoids Content and Total Phenolics Content of *L. triphylla* leaf extract expressed in terms of Quercetin equivalents and Gallic acid equivalents, respectively. Values are expressed as mean±SD, n=3.

Concentration (µg/mL)	Inhibition of DPPH (%)	
	***Lippia triphylla* leaf extract**	**Gallic acid**
10	6.50±0.52	22.55±2.78
20	7.15±0.46	34.73±5.41
50	9.41±0.17	76.86±5.59
100	11.97±1.08	89.59±0.79
200	17.30±0.48	90.48±0.44
500	34.42±3.24	91.29±0.15
1000	73.59±3.34	92.17±0.76
IC_{50}	709.66±41.03	23.27±1.64

Table 4. Percentage inhibition of DPPH free radical by *L. triphylla* leaf extract and gallic acid at 515 nm. Values are mean±SD, n=3.

Standards/Sample	20 µg/mL	200 µg/mL	2000 µg/mL
2-ethylhexyl 4-methoxycinnamate	12.5±0.15	14.33±0.17	15.63±0.45
2-ethylhexyl 4-(dimethylamino) benzoate	17.81±0.21	18.49±0.14	19.01±0.34
L. triphylla hydroalcoholic extract	1.68±0.18*	7.67±0.24*	9.55±0.22*

Table 5. Sun protection factor SPF of *L. triphylla* leaf extract at 20, 200 and 2000 µg/mL. SPF values are mean±SD, n=3. * Significant difference at P<0.05 with respect to standars.

***L. triphylla* hydroalcoholic extract (µg/mL)**	**HET-CAM**	**CAM-TBS**	**Classification**
500	0	0.0046±0.003	NOI
250	0	0.0090±0.004	NOI
125	0	0.0093±0.003	NOI
62.5	0	0.0100±0.004	NOI

Table 6. HET-CAM and CAM-TBS results for *L. triphylla* hydroalcoholic extract (dissolved in PBS pH 7.4) with their classification based on the irritant effects and denaturation index. Values are mean±SD. NOI = No irritant

Experimental group	Micronucleus (MCN)	Mitotic Index (MI)
Distilled water	0	27.25±4.50**
Ethanol	2.25±1.25*	2.75±1.70
Lippia thyphilla (0.1% w/v)	0	28.50 ± 4.50**
Lippia thyphilla (0.2% w/v)	0	29.50 ± 1.73**
Lippia thyphilla (0.4% w/v)	0	30.00 ± 2.94**

Table 7. Genotoxicity of extract of *Lippia thyphilla* on *Vicia faba* cells root tips. Values are mean±SD. * Significant differences with respect to all treatments (p<0.05), ** Significant differences with respect to ethanol treatment (p< 0.05).

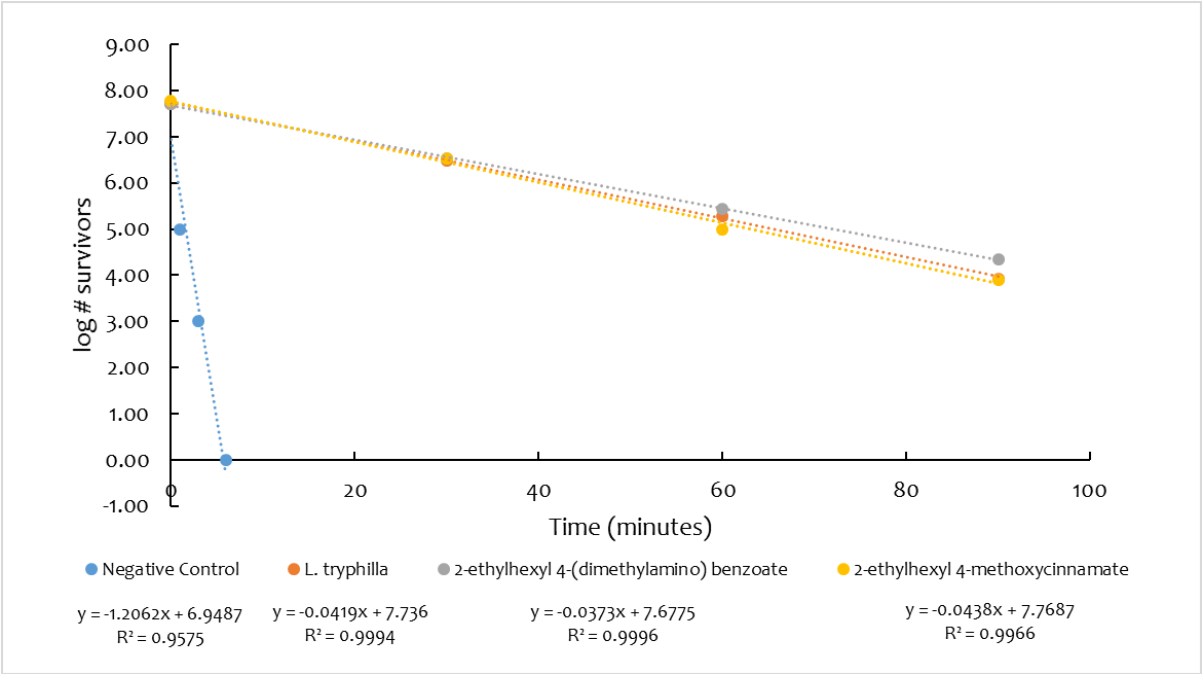

Figure 1. Protective effect against ultraviolet (UV-B) induced cell death of *Escherichia coli*. Ethanol 70% v/v: Negative control without protection (K=−1.206, R^2=0.9575); with protection 2-ethylhexyl 4-methoxycinnamate: (K=−0.0438, R^2=0.9966), 2-ethylhexyl 4-(dimethylamino) benzoate: (K=−0.0373, R^2=0.9996), hydroalcoholic extract of *Lippia trypilla* (K=−0.0419, R^2=0.9994). Where: y – Log of bacteria survivors, x - exposition time of UVB Radiation, K - mortality rate, and R^2 - coefficient of determination