VITAE, REVISTA DE LA FACULTAD DE CIENCIAS FARMACÉUTICAS Y ALIMENTARIAS
ISSN 0121-4004 / ISSNe 2145-2660. Volumen 24 número 2, año 2017
Universidad de Antioquia, Medellín, Colombia. págs. 124-131
DOI: http://dx.doi.org/10.17533/udea.vitae.v24n1a05

BIOGUIDED FRACTIONATION FROM *Solanum elaeagnifolium* TO EVALUATE TOXICITY ON CELLULAR LINES AND BREAST TUMOR EXPLANTS

FRACCIONAMIENTO BIODIRIGIDO DE *Solanum elaeagnifolium* PARA EVALUAR LA TOXICIDAD EN LÍNEAS CELULARES Y EXPLANTES DE TUMOR DE MAMA

Leobardo HERNÁNDEZ O.[1], Pilar CARRANZA R.[2], Luis Enrique COBOS P.[1], Lluvia Itzel LÓPEZ L.[1], Juan Alberto ASCASIO V.[1], Sonia Yesenia SILVA B.[1*]

Received: September 27 of 2016. **Approved**: September 20 of 2017

ABSTRACT

Background: Bioactive compounds from the fruit of *S. elaeagnifolium* were isolated since could be highly potential source to develop functional foods or pharmaceutical products. **Objectives:** In this study a bioguided fractionation of the methanolic extract from *S. elaeagnifolium* fruit was carried out to evaluate their cytotoxicity and antitumoral potential on several cell lines and breast tumor explants, respectively. **Methods:** Microdilution method with *A. salina* was used to isolate bioactive compounds. Fractionation was performed by vacuum liquid chromatography, and the monitoring from fractions was done by thin layer chromatography. The effect of the fractions on viability in Vero, HeLa, and MCF-7 cells was assessed using WST-1 assay, whereas in breast tumor explants was evaluated by Alamar blue assay. A qualitative phytochemical analysis was performed to partially identify the compounds contained in the fractions and a spectroscopic characterization by *RP*-HPLC-MS was done to identify the group of compounds responsible for the effect on the cell lines and the mammary explants. **Results:** Several fractions were isolated from the fruit of *S. elaeagnifolim*. Notwithstanding, the FVLC7 showed a higher activity in *A. salina* assay. This fraction reduced the viability at 39 ± 1.67, 15.05 ± 0.09 and 66.10 ± 4 % in Vero, HeLa and MCF-7 cells, at 100 µg/mL, respectively. On the other hand, showed an effect in breast tumor explants obtained from a patient in remission. Qualitative phytochemical analysis showed that FVLC7 contains alkaloids, coumarins, and sesquiterpene lactones. Characterization by *RP*-HPLC-MS detected quinic acid, chlorogenic acid, dicaffeoylquinic acid as well as presence of an alkaloid. **Conclusion:** On this basis, our results suggest that cytotoxic effect of FVLC7 isolated from the fruit of *S. elaeagnifolium* could be mediated by quinic, chlorogenic, and dicaffeoylquinic acids.

Keywords: *Solanum*; fractionation; bioguided; cell line; tumor.

RESUMEN

Antecedentes: Los compuestos bioactivos del fruto de *S. elaeagnifolium* fueron aislados ya que representan compuestos con un alto potencial para el desarrollo de alimentos funcionales o productos farmacéuticos. **Objetivos:** En este estudio se realizó un fraccionamiento biodirigido de un extracto metanólico de frutos de *S. elaeagnifolium* para evaluar la citotoxicidad y el potencial antitumoral en los explantes de tumor de mama.

1 Facultad de Ciencias Químicas, Universidad Autónoma de Coahuila, Boulevard Venustiano Carranza s/n, México. Saltillo, Coahuila, México

2 Centro de Investigación Biomédica del Noreste, IMSS. 2 de Abril Esq. San Luis Potosí, Colonia Independencia, Monterrey Nuevo León, México.

* Corresponding autor: yesenia_silva@uadec.edu.mx

Métodos: Se utilizó el método de microdilución con *A. salina* para aislar los compuestos bioactivos. El fraccionamiento se realizó mediante cromatografía de líquido a vacío, y la monitorización de las fracciones se realizó por cromatografía en capa fina. La viabilidad de las fracciones en las líneas de células Vero, HeLa y MCF-7 se evaluó usando el ensayo WST-1, mientras que en los explantes tumorales de mama se evaluaron mediante el ensayo azul de Alamar. Así mismo, se realizó un análisis fitoquímico cualitativo para identificar parcialmente los compuestos que contenían las fracciones y una caracterización espectroscópica por *RP*-HPLC-MS de los compuestos responsables del efecto sobre las líneas celulares y los explantes mamarios. **Resultados:** De todas las fracciones aisladas de *S. elaeagnifolium*, la fracción FVLC7 (100 μg/mL) tuvo la actividad más alta en el ensayo de *A. salina*. Por otra parte, redujo la viabilidad un 39 ± 1,67, 15,05 ± 0,09 y 66,10 ± 4,44% en las células Vero, HeLa y MCF-7, respectivamente. Esta fracción mostró un efecto en los explantes de tumor de mama obtenidos de un paciente en remisión. El análisis fitoquímico cualitativo reveló que la FVLC7 contiene alcaloides, cumarinas y lactonas sesquiterpénicas. La caracterización por *RP*-HPLC-MS detectó ácido quínico, ácido clorogénico, ácido dicafeoilquínico así como presencia de un alcaloide. **Conclusión:** Nuestros resultados sugieren que el efecto tóxico de la fracción FVLC7 aislada del fruto de *S. elaeagnifolium* podría deberse a los ácidos quínico, clorogénico y dicafeoilquínico.

Palabras clave: *Solanum*; fraccionamiento biodirigido; línea celular; tumor.

INTRODUCTION

Solanum elaeagnifolium Cavanilles (Solanaceae) is widely distributed in the North of Mexico (1). The plant grows in scrubland, pasture and disturbed areas, accordingly is considered a weed for agricultural crops (1) and is highly toxic to livestock (2). *S. elaeagnifolium* fruit contains an enzymatic complex employed to artisan manufacture Asadero-cheese (3). The extracts are used in Mexican folk medicine and are known to contain solasodine, β-sitosterol, campesterol, stigmasterol and Δ-5-avenasterol (4). The methanol extract contains glycosylated flavones with cytotoxic effect on MCF-7 and HPG-2 cell lines (5). *Solanum* has anti-inflammatory (6), anti-hepatotoxic (7), hypotensive (8), cytotoxic (9), antiviral (10) and antifungal (11) properties. *Solanum* contains steroidal saponins and glycoalkaloids (12) such as solasonine and solamargine (13). These compounds have inhibitory effects on human cancer cell lines such as HT-29, HCT-15 (colon), LNCaP, PC-3 (prostate), T47D, MDA-MB-231 (breast), PLC/PRF/5 and HepG2 (human hepatoma), and JTC-26 (cervical cancer cells) (14). The anticancer potential from *Solanum* is plausible (4) because species allied produce similar metabolites (15, 16). Therefore, compounds isolated from *S. elaeagnifolium* potentially represent a great alternative to develop new anticancer agents (17). *Artemia salina* assay represent a reliable, reproducible, accurate, and economical method to selecting bioactive compounds from several sources (18). This experimental approach allows the selection of purified fractions with biological actions from plants (19, 20). Therefore, in this study a bioguided fractionation of a methanolic extract from *S. elaeagnifolium* fruit was carried out to evaluate its cytotoxicity on human tumoral cell lines and anti-tumoral potential on breast tumor explants.

MATERIALS AND METHODS

Extraction *S. elaeagnifolium* fruit

The fruit *S. elaeagnifolium* Cavanilles was collected in Saltillo, Coahuila, Mexico during November 2014. The plant material was identified and a voucher specimen (026294) was deposited in the herbarium of the Faculty of Biological Sciences of the Autonomous University of Nuevo Leon (21). Ripe fruit was dried in an oven at 28°C and ground in a STAR Tisamatic-mill. The methanolic extraction was performed at 10% w/v under constant stirring for 2 h at 25°C (Hotplate Fisher Scientific), to obtain the crude methanolic extract of *S. elaeagnifolium* fruits (CMESeF). The CMESeF was filtered with Whatman #4 paper and clarified with Whatman G/FA, then was concentrated on a rotary evaporator (Büchi R-120) at 40°C and dried with a lyophilizer (Labconco Freeze 5). Then, the yield Y (%) was calculated by gravimetric analysis (analytical balance Ohaus N1B110 Navigator) (22). The CMESeF was stored at -20°C in a freezer until use.

Bioguided fractionation of *S. elaeagnifolium*

Separation from CMESeF by liquid-liquid partition

The CMESeF (10%) dissolved in MeOH (w/v) was dispersed on Hexane (1:1) under constant stirring (Hotplate Fisher Scientific) with a flow rate of 6 mL/min. Then, the methanolic (MFSe) and hexane phase (HxFSe) were separated by a liquid-liquid partition. The HxFSe was dried in a rotary evaporator (Büchi R-120) at 30°C and determined the Y (%). The MFSe was dispersed into ethyl acetate (EtOAc) in a ratio 1:1 to obtain a homogeneous solution (MFSe/EtOAc). Later MFSe/EtOAc was separated in two phases by adding distilled H_2O in a ratio 2:1 (MFSe/EtOAc:H_2O) and stored for 24 h at -20°C, to achieve separation from MFSe and EtOAc to obtain EtOAcFSe. Then, the phases were dried on a rotary evaporator (Buchi R-120) at 30°C, and calculated the Y (%). The fractions obtained were tested in *A. salina* nauplii to select those with the highest effect and continue the purification process (23). The most active fractions were characterized by qualitative phytochemical tests and fractionated by vacuum liquid chromatography (VLC).

Selection compounds by A. salina assay

This assay was carried out in a 96-well plate, as described by Meyer *et al*, with some modifications (24). *A. salina* cysts were hatched in artificial sea water at 37 g/L at 25°C with aeration and constant light source for 24 h. Concentrations of 50, 100, 250, 500 and 1000 μg/mL from CMESeF as well as each fraction recovered in the different purification steps were tested for 24 h at 25°C. A concentration-response curve with $K_2Cr_2O_7$ at 0, 5, 10, 15, and 20 μg/mL, as well as a negative control (viability control) was performed. LC_{50} was determined with the percent of mortality M (%) by a linear regression analysis.

The M (%) was determined by the follow equation:

$$M(\%) = (LN * 100)/(DN + LN) \quad \text{(Equation 1)}$$

where: LN= live nauplii, DN= dead nauplii

Qualitative phytochemical characterization

The sulphuric acid test was used to identify flavones, chalcones, and quinones. The Shinoda test was used to determine flavonoids while the Baljet test was applied to detect sesquiterpen lactones. The Dragendorff, Wager, and Mayer tests were used to determine alkaloids. The Liebermann-Burchard test was used to identify triterpenes and steroid compounds while Molisch test was utilized to detect carbohydrates. The sodium hydroxide test was used to determine coumarins (25). In this way, both CMESeF and the fractions were characterized on 12-well porcelain plates using a solution at 2000 μg/mL of each sample.

Chromatographic separation from CMESeF

The eluents employed in the separation of CMESeF (2000 μg/mL) by VLC were pre-selected on Merck 60 (1.6 x 5 cm) TLC plates. The eluent was selected with retention factor values (Rf) ≥ 0.1 between fractions (26). Fractions were observed at 254 and 365 nm with ultraviolet light. The eluents used were: EtOAc-MeOH-H_2O (100:13.5:10), EtOAc-MeOH (90:10), EtOAc-MeOH (60:20) Hex-$CHCl_3$-AcOH (45:45:10), $CHCl_3$-MeOH-AcOH (47.5:47.5:5), $CHCl_3$-An (4:1) $CHCl_3$-MeOH (9:1), $C_2H_5OC_2H_5$-$CHCl_3$ (1:4), $CHCl_3$-EtOAc (1:1), Hex-AcOEt (3:1), where EtOAc= ethyl acetate, MeOH= methanol, H_2O= distilled water, Hex= hexane, $CHCl_3$= chloroform, AcOH= glacial acetic acid, $C_2H_5OC_2H_5$= ethyl ether, An= acetone.

On this way, the MFSe showed a greater effect on *A. salina*. In consequence was fractionated by VLC. For this, 1 g of MFSe was eluted within a glass column (Pyrex, 3 cm of diameter) pre-packed with G silica gel (Fluka) (27). The elution was carried out by connecting the column to a negative pressure source of 20 psi (Felisa F-1500L vacuum pump), using a flow rate of 2 mL/min. The elution gradient used as mobile phase was 50 mL of $CHCl_3$-MeOH (100:0 → 10:90). Ten fractions were obtained: FVLC1, FVLC2, FVLC3, FVLC4, FVLC5, FVLC6, FVLC7, FVLC8, FVLC9, and FVLC10. Fractions were dried and the Y (%) was determined as described above.

Evaluation toxicity on cellular lines and breast tumor explants

Viability assay

Vero, HeLa, and MCF-7 cell lines were donated by the Northeast Biomedical Research Center (CIBNOR-IMSS). Those fractions with higher activity on *A. salina* were tested on cell lines to assess viability by WST-1 technique. Briefly, 5 x 10^3 cells/well from each cell line were cultured in DMEM/F12 supplemented with penicillin-streptomycin (0.1%), glucose (25 mM) and inactivated fetal bovine serum (FBS, 10%) at 37°C, a humidified atmosphere and CO_2 (5%) (NAPCO-6200 CO_2 incubator). FVLC7 (10 and 100 μg/mL), two negative controls (10% ethanol v/v-3.8% DMSO v/v, and medium culture) and a positive control (Triton 1X). Then, WST-1 (10 μL), was added to each well and incubated during 2 h. Spectrophotometric reading was performed at 450 nm in a microplate reader using Gen 5 software and cellular morphology was observed by optic microscopy. Results were expressed as percent of the viability V (%).

Evaluation from antitumor effect

The antitumor effect was evaluated using Alamar blue bioassay on breast tumor explants obtained from a patient in remission following ethics codes of the Helsinki Declaration (28). Tumor was cultured in trypticase-yeast-iron (TYI) culture medium. The TYI medium was supplemented with inactivated FBS (10%), vitamins (2.26%), sodium selenite insulin transferrin (1%), D-glucose (1 M), L-glutamine (200 mM) and sodium pyruvate (100 mM). Then, the tumor was cut in 2 explants (Brendel Viton, Viton Inc, Tucson Arizona, USA) using a Krebs-Henseleit buffer and each explant subdivided in explants with a uniform diameter (UDE). The assay was carried out with 2 UDE´s/well in a 6-well plate pre-filled with TYI medium at 37°C, humidified atmosphere, CO_2 (5 %) and shaking at 25 rpm. FVLC7 (10 μg/mL) and a negative control (TYI medium) were evaluated. Supernatant from each treatment was placed in a new well-containing DMEM/F12 (200 μL) and Alamar blue (1:10), then, incubation realized for 3-4 h. Fluorescence values were read using a multimode microplate reader (Synergy BioTek HT) at 530 nm excitation/590 nm emission wavelengths. The viability percentage regarding control was calculated using the software AbD Serotec.

Characterization by RP-HPLC-MS

FVLC7 was characterized by Reversed-Phase High Performance Liquid Chromatography coupled to Mass Spectroscopy (RP-HPLC-MS). An electrospray ion detector (ESI-MS) and an ion trap (IT Varian 500-MS Mass Spectrometer, USA) source were used. Samples were analyzed in full scan mode, and all experiments were examined in negative mode $[M\text{-}H]^{-1}$ using a range 50-2000 m/z. Samples were injected in mass spectrometer at a flow rate of 50 mL/min for 7 min. Nitrogen was used as nebulizing gas and helium as damping gas. The parameters utilized for ion source were: electrospray voltage (5.0 kV), capillary voltage (90.0 V) and temperature (350°C). Data were analyzed using MS Workstation software V 6.9.

Statistical analysis

All data were reported as a mean value ± standard error (EE). The LC_{50} data from *A. salina* obtained with CMESeF (N= 24), MFSe (N= 9), EtOAcFSe (N= 3), HxFSe (N= 9) fractions isolated by VLC (N= 3), positive control (N= 9), and negative control were analyzed by ANOVA and Dunn test. *A. salina* data was compared against $K_2Cr_2O_7$, while cell lines data with Triton 1X. Breast tumor explants results were analyzed using Student's T-test (N= 3) and compared with the negative control. All statistical analysis were performed in GraphPad Prism 5© software. Statistical significance was accepted with values of $p \leq 0.05$. The assays with *A. salina*, cell lines, and breast tumor explants performed in triplicate.

RESULTS

Bioguided fractionation of *S. elaeagnifolium*

Separation from CMESeF by liquid-liquid partition

A yield of 10.90 ± 2.82% of CMESeF was extracted from *S. elaeagnifolium* fruit while with the liquid-liquid partition the yield was of 2.340, 13.519 and 61.522% of the fractions HxFSe, EtOAcFSe and MFSe, respectively.

Selection of isolated fractions with biological activity (A. salina assay)

The positive control ($K_2Cr_2O_7$) induced toxic effect in *A. salina* with high potency (LC_{50}= 13.90 ± 0.29 μg/mL). In contrast, the CMESeF and MFSe had the same effect than the control although with less potency since 40-fold higher concentrations (LC_{50}= 567.0 ± 12.1 μg/mL and 565.1 ± 49.9 μg/mL, respectively) were used, whereas with EtOAcFSe and HxFSe fractions 72-fold higher concentrations (LC_{50} > 1000 μg/mL) were required. Similar results were obtained with fractions separated by VLC since LC_{50} of FVLC2, FVLC4, and FVLC9 were 518 ± 26.0, 840.1 ± 86.6, and 450 ± 0.0 μg/mL respectively, while the LC_{50} for FVLC3, FVLC5, FVLC6, FVLC8, and FVLC10 was > 1000 μg/mL. FVLC1 exhibited a toxic effect similar to the positive control but with 7-fold higher concentration (LC_{50}= 123.5 ± 0.2 μg/mL). Interestingly, FVLC7 (LC_{50}= 16.0 ± 0.0 μg/mL) had the same toxic effect than the control positive (Figure 1). The negative control had no toxic effect.

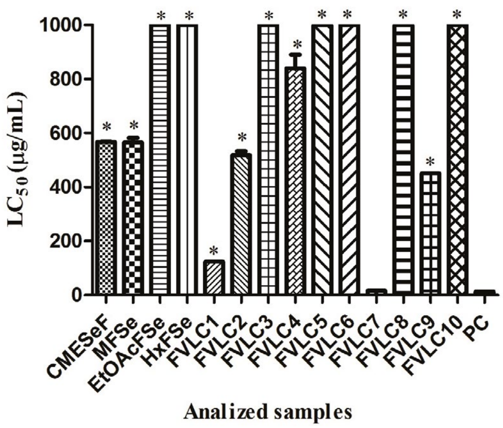

Figure 1. Median lethal concentration in *A. salina* of CMESeF and its isolated fractions. All comparison were performed against positive control (PC). *, $p \leq 0.05$.

Qualitative phytochemical characterization

Sesquiterpene lactones, alkaloids and coumarins were identified in the CMESeF, MFSe and EtOAcFSe but not in HxFSe. In FVLC1 and FVLC9, sesquiterpene lactones and alkaloids but not coumarins were detected while in FVLC2, FVLC8 and FVLC10, alkaloids and coumarins, but not sesquiterpene lactones, were found. In FVLC3 and FVLC6, sesquiterpene lactones, alkaloids, coumarins, and lactones were identified. In FVLC4, sesquiterpene lactones and alkaloids were found. In FVLC5 and FVLC7, sesquiterpene lactones, alkaloids, and coumarins were identified.

Chromatographic separation from CMESeF

Chromatographic profile showed that only four eluents did separate the compounds of CMESeF. With $CHCl_3$:MeOH:AcOH (47.5:47.5:5) three fractions with different Rf´s (Rf_1= 0.68, Rf_2= 0.80 and Rf_3= 0.90) were separated. With EtOAc:MeOH:H_2O (100:13.5:10) four fractions with various Rf´s (Rf_1= 0.03, Rf_2= 0.05, Rf_3= 0.13 and Rf_4= 0.23) were obtained. With EtOAc:MeOH (60:20) and $CHCl_3$:MeOH eluent (9:1), a fraction (Rf_1= 0.08) and three fractions (Rf_1= 0.08, Rf_2= 0.13 and Rf_3= 0.50) were separated. The fractionation by VLC showed higher yield (%) of FVLC5 (36.941%) than the rest of the isolated fractions of MFSe. These results were calculated considering the yield as 100% of the sample used for this fraction (21.652 g) (Table 1).

FVLC7 (100 µg/mL) reduced the viability of all cell lines at 39 ± 1.67, 15.05 ± 0.09 and 66.10 ± 4.44% of Vero, HeLa, and MCF-7 cells. Similar results were observed microscopically since in all cases, FVLC7 decreased cell density (Figure 2).

Table 1. Yield of the fractions obtained by vacuum liquid chromatography (FVLC) from MFSe.

FVLC	Y (%)
1	0.105
2	1.182
3	9.571
4	23.063
5	36.941
6	7.846
7	3.318
8	2.287
9	2.174
10	1.883

Evaluation toxicity on cellular lines and breast tumor explants

Viability assay

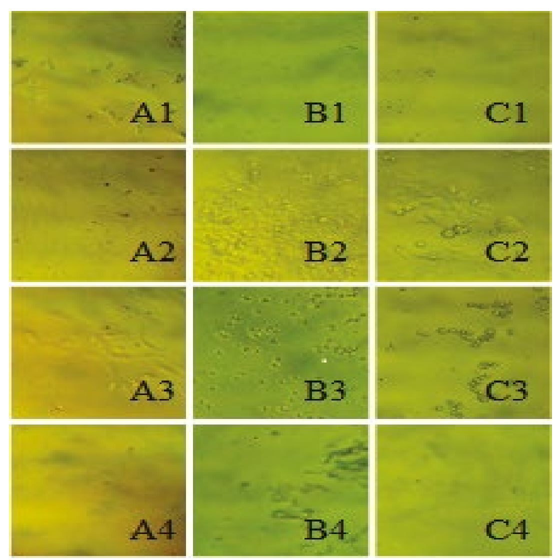

Figure 2. Microscopic observations of Vero, HeLa, and MCF-7 cell lines against FVLC7 (100 µg/mL) after 48 h of exposure. The images include treatments described below: A1= cell line Vero + positive control (Triton 1X), A2= cell line Vero + negative control with culture medium, A3= cell line Vero + negative control 10% ethanol and 3.8% DMSO, A4= cell line Vero + 100 µg/mL FVLC7. B1= cell line HeLa + positive control (Triton 1X), B2= cell line HeLa + negative control with culture medium, B3= cell line HeLa + negative control 10% ethanol and 3.8% DMSO, B4= cell line HeLa + 100 µg/mL FVLC7. C1= cell line MCF-7 + positive control (Triton 1X), C2= cell line MCF-7 + negative control with culture medium, C3= cell line MCF-7 + negative control 10% ethanol and 3.8% DMSO, C4= cell line MCF-7 + 100 µg/mL FVLC7.

Evaluation from antitumor effect

FVLC7 (10 µg/mL) decreased 26.23 ± 9.6% the viability of tumor explants.

Characterization by RP-HPLC-MS

Mass spectra obtained from FVLC7 fraction detected traces of quinic acid (**1**) (ESI m/z 191 $[M-H]^{-1}$), chlorogenic acid (**2**) (ESI m/z 353 $[M-H]^{-1}$), dicaffeoylquinic acid (**3**) (ESI m/z 515 $[M-H]^{-1}$). The signal (ESI m/z 395 $[M-H]^{-1}$) might correspond to an alkaloid (ESI m/z 395 $[M-H]^{-1}$) (Figure 3).

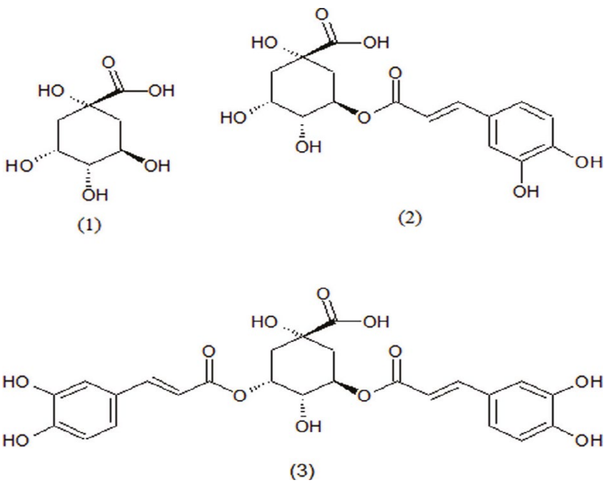

Figure 3. Quinic acid (1), chlorogenic acid (2) and dicaffeoylquinic acid (3) identificated on FVLC7.

DISCUSSION

Yield of CMESeF is higher than reported in *S. elaeagnifolium* leaf under same extraction conditions (29). This difference in Y could be explained by the number of compounds with the same chemical nature found in the fruit or leaves, because they interact through a dipole moment with methanol, which allows the intermolecular forces that keep together are overcome to extract them (30). The eluent with the best profile fractionation was $CHCl_3$:MeOH:AcOH (47.5:47.5:5) (31). However, using MFSe we found a better yield probably due to substances with solvated dipole moments (32). Assays with *A. salina* revealed that LC_{50} of CMESeF is similar those reported for crude methanolic extracts from other solanaceae in ranges of 107.2 to ≥ 1000 µg/mL (33, 34). The positive control had a LC_{50}= 13.90 ± 0.29 µg/mL comparable with those obtained in other works (35). In order to determine the relationship between the effect presented by CMESeF and the fractions we follow the next: elevated lethality= 0.1-100 µg/mL, moderate lethality= 100-300 µg/mL, low lethality= 300 - 640 µg/mL and minimum lethality= ≥ 640 µg/mL (37). On this way, CMESeF exhibited a low lethality while EtOAcFSe y HxFSe exhibited a minimum lethality similarly to several researches (36).

These results revealed that MFSe and CMESeF contain the main bioactive compounds probably due to its high polarity. Compounds detected in *S. elaeagnifolium* such as flavonoids, alkaloids, steroids, saponins, triterpenes and tannins have been reported in *Solanum* plants (37). Therefore, the chemical composition found in CMESeF could be related to conditions of collection, storage capacity, synthesis (in each organ), geographical region, time season, and phenological age of plant (37). It has been proposed that alkaloids and coumarins are responsible for *A. salina* toxicity of CMESeF, because there are a close relationship between toxicity and alkaloids in other plants (38). The absence of these compounds by qualitative phytochemical analysis in the HxFSe was due to the polarity. The fractionation by VLC increased the Y (%) of CMESeF when the proportion of methanol was rised during the separation process, whereby a larger amount was obtained with $CHCl_3$:MeOH (60:40). Therefore, the Y (%) each fraction depends on the equilibrium between mobile and stationary phases, as well as their distribution coefficient and relative retention (39).

FVLC5, FVLC8, and FVLC10, had no toxic effect on *A. salina* (40), whereas FVLC7 (100 µg/mL) had toxic effect rather than the rest of the fractions tested (36). This fraction also decreased the viability of Vero, HeLa, and MCF-7 cells. However, HeLa cell line was more susceptible than Vero and MCF-7 cells. Furthermore, FVLC7 produced morphological changes in the size of HeLa and MCF-7 cells (41). Here, we speculate that the fraction permeates into the cells and trigger molecular interactions associated with the respiratory and metabolic activity in the cell membrane or cytoplasm (42). Interestingly, the Vero cells did not present morphological changes after treatment with FVLC7 which is an advantage because it is a healthy cell line (43). FVLC7 (10 µg/mL) induced a slight toxicity on the breast tumor explants, this effect could be increased with a higher dose (44, 45). We believe that this response may be due that the explants have more than one cellular interaction, since a complex net of different components from extracellular matrix that together with tumor microenvironment protects neoplastic cells from the cytotoxic agents (46). A difference on the effect of FVLC7 in the viability of the explants compared with cell lines is that both have different anatomical and chemical composition, and that one or more inducers or enzyme inhibitors could participate in the transformation of Alamar blue (47). The bioactive compounds identified in *S. elaeagnifolium* are similar to those reported in other *Solanum* species, in this regard, species such as *Solanum tuberosum* L. contain quinic, dicafeoylquinic and chlorogenic acids (48). *Solanum melongena* L. also contain chlorogenic acid (49). Although the chemical structure of the alkaloid found in *S. elaeagnifolium* is unknown, it may be mediating the toxicity of S. *elaeagnifolium* since compounds wich contain an alkaloid structure reduce the growth of several cancer cell lines (49). The identification of this alkaloid structure guarantees future investigations.

CONCLUSIONS

In this study we develop methods to separation of bioactive compounds from *S. elaeagnifolium*. Of this way, the FVLC7 reduced the viability of HeLa and MCF-7 rather than Vero cells indicating a selective response of this fraction. The reduction of the viability of breast tumor explants suggests that FLVC7 could represent a new source of antitumor agents. Chemical analysis of this fraction has revealed that quinic, chlorogenic and dicaffeoylquinic acids may be mediating the toxic effect of FVLC7 in HeLa and MCF-7 cells and in breast explants.

CONFLICT OF INTEREST

The authors declare no competing financial interest.

ACKNOWLEDGEMENTS

SYSB acknowledge to CONACYT-Mexico for the support with a doctoral fellowship (492213) to LHO which strengthens the program in Science and Technology of Food as well as to academic research team (UCOAH-CA-27-PRODEP).

AUTHORS´ CONTRIBUTIONS

SYSB designed the experiments performed in this work (compounds separation by VLC, *A. salina* assays and qualitative phytochemical characterization). LECP, SYSB, and PCR supervised the cell lines assays. PCR conceived the explants assays. LILL and JAAV jointly directed the chemical characterization by HPLC. LHO designed, performed experiments and analyzed data. All authors wrote the paper. LECP made correction and style of the document. All authors approved the final version of the manuscript.

REFERENCES

1. Calderón de Rzendowski G, Rzendowski J, Acosta Castellanos S, Aguilar Rodríguez S, Aguilar Santelices R, Lerner De Scheinvar LA, et al. Flora fanerogámica del Valle de México. 2nd ed. México: Instituto de Ecología, A.C. y Comisión Nacional para el Conocimiento y Uso de la Biodiversidad; 2005. 1406 p.
2. Boyd JW, Murray DS, Tyrl RJ. Silverleaf Nightshade, *Solanum elaeagnifolium*, origin, distribution, and relation to man. Econ. Bot. 1984;38(2):210-7.
3. Gutiérrez-Méndez N, Chávez-Garay DR, Jiménez-Campos H. Exploring the milk-clotting properties of a plant coagulant from the berries of *S. elaeagnifolium* var. Cavanilles. J. Food Sci. 2012;77(1):C89-94.
4. Feki H, Koubaa I, Jaber H, Makni J, Damak M. Characteristics and chemical composition of *Solanum elaeagnifolium* seed oil. J. Eng. Appl. Sci. (Asian Res. Publ. Netw.). 2013;8(9):708-12.
5. Radwan MM, Badawy A, Zayed R, Hassanin H, ElSohly MA, Ahmed SA. Cytotoxic flavone glycosides from *Solanum elaeagnifolium*. Med. Chem. Res. 2015;24:1326-30.
6. Emmanuel S, Ignacimuthu S, Perumalsamy R, Amalraj T. Antiinflammatory activity of *Solanum trilobatum*. Fitoterapia. 2006;77(7-8):611-2.
7. Akanitapichat P, Phraibung K, Nuchklang K, Prompitakkul S. Antioxidant and hepatoprotective activities of five eggplant varieties. Food Chem. Toxicol. 2010;48(10):3017-21.
8. lbarrola DA, Hellión-lbarrola MC, Montalbetti Y, Heinichen O, Alvarenga N, Figueredo A, et al. Isolation of hypotensive compounds from *Solanum sisymbriifolium* Lam. J. Ethnopharmacol. 2000;70(3):301-7.
9. Arthan D, Svasti J, Kittakoop P, Pittayakhachonwut D, Tanticharoen M, Thebtaranonth Y. Antiviral isoflavonoid sulfate and steroidal glycosides from the fruits of *Solanum torvum*. Phytochemistry. 2002;59(4):459-63.
10. Rashed K, Sahuc M-E, Deloison G, Calland N, Brodin P, Rouillé Y, et al. Potent antiviral activity of *Solanum rantonnetii* and the isolated compounds against hepatitis C virus *in vitro*. J Funct Foods. 2014;11:185-91.
11. Rowan DD, Macdonald PE, Skipp RA. Antifungal stress metabolites from *Solanum aviculare*. Phytochemistry. 1983;22(9):2102-4.
12. Schirmer Pigatto AG, Mentz LA, Gonçalves Soares GL. Chemotaxonomic characterization and chemical similarity of tribes/genera of the Solanoideae subfamily (Solanaceae) based on occurrence of withanolides. Biochem. Syst. Ecol. 2014;54:40-7.
13. Blankemeyer JT, McWilliams ML, Rayburn JR, Weissenberg M, Friedman M. Developmental toxicology of solamargine and solasonine glycoalkaloids in frog embryos. Food Chem. Toxicol. 1998;36:383-9.
14. Lee K-R, Kozukue N, Han J-S, Park J-H, Chang E-y, Baek E-J, et al. Glycoalkaloids and Metabolites Inhibit the Growth of Human Colon (HT29) and Liver (HepG2) Cancer Cells. J. Agric. Food. Chem. 2004;52(10):2832-9.

15. Ludwiczuk A, Asakawa Y. Fingerprinting of Secondary Metabolites of Liverworts: Chemosystematic Approach. J. AOAC Int. 2014;97(5):1234-43.
16. He C, Peng B, Dan Y, Peng Y, Xiao P. Chemical taxonomy of tree peony species from China based on root cortex metabolic fingerprinting. Phytochemistry. 2014;107:69-79.
17. Houda M, Derbré S, Jedy A, Tlili N, Legault J, Richomme P, et al. Combined anti-ages and antioxidant activities of different solvent extracts of *Solanum elaeagnifolium* Cav (Solanacea) fruits during ripening and related to their phytochemical compositions. EXCLI J. 2014;13:1029-42.
18. Prakash S, Ramasubburayan R, Ramkumar VS, Kannapiran E, Palavesam A, Immanuel G. In vitro-Scientific evaluation on antimicrobial, antioxidant, cytotoxic properties and phytochemical constituents of traditional coastal medicinal plants. Biomed. Pharmacother. 2016;83:648-57.
19. Rodríguez-López V, Aguirre-Crespo F, Salazar L, Estrada-Soto S. Identification of fatty acid esters and hydrocarbon derivatives from Cyrtocarpa procera Kunth by GC-MS. Nat. Prod. Res. 2006;20(1):1-7.
20. Karchesy YM, Kelsey RG, Constantine G, Karchesy JJ. Biological screening of selected Pacific Northwest forest plants using the brine shrimp (*Artemia salina*) toxicity bioassay. Springerplus. 2016;5(1):510.
21. do Nascimento GE, Hamm LA, Baggio CH, Werner MFDP, Iacomini M, Cordeiro LMC. Structure of a galactoarabinoglucuronoxylan from tamarillo (*Solanum betaceum*), a tropical exotic fruit, and its biological activity. Food Chem. 2013;141:510-6.
22. Aguilar-Santamaría L, Herrera-Arellano A, Zamilpa A, Alonso-Cortés D, Jiménez-Ferrer E, Tortoriello J, et al. Toxicology, genotoxicity, and cytotoxicity of three extracts of *Solanum chrysotrichum*. J. Ethnopharmacol. 2013;150:275-9.
23. Moon JY, Mosaddik A, Kim H, Cho M, Choi H-K, Kim YS, et al. The chloroform fraction of guava (*Psidium cattleianum sabine*) leaf extract inhibits human gastric cancer cell proliferation via induction of apoptosis. Food Chem. 2011;125(2):369-75.
24. Meyer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols DE, McLaughlin JL. Brine Shrimp: A Convenient General Bioassay for Active Plant Constituents. Planta Med. 1982;45:31-4.
25. Pereira Cabrera S, Vega Torres D, Almeida Saavedra M, Morales Torres G. Tamizaje fitoquímico de los extractos alcohólico, etéreo y acuoso de las hojas de *Trichilia hirta* L. QViva. 2009;8(3):192-9.
26. Wagner H, Bladt S. Plant drug analysis a thin layer chromatography atlas. 2nd ed. New York: Springer; 2001. 384 p.
27. Coll JC, Bowden BF. The Application of Vacuum Liquid Chromatography to the Separation of Terpene Mixtures. J. Nat. Prod. 1986;49(5):934-6.
28. Association WM. World Medical Association Declaration of Helsinki Ethical principles for medical research involving human subjects. J. Am. Med. Assoc. 2013;310(20):2191-4.
29. Silva Belmares SY, González Zavala MA, De la Cruz Galicia MG, Macías López MA, Muñoz FF, Guevara MG. Actividad citotóxica de *Solanum marginatum* y *Solanum elaeagnifolium*. Rumbo a una sociedad de conocimiento. Estancias académicas 2012-2013. México: Plaza y Valdes; 2013. 85-94 p.
30. Azmir J, Zaidul ISM, Rahman MM, Sharif KM, Mohamed A, Sahena F, et al. Techniques for extraction of bioactive compounds from plant materials: A review. J. Food Eng. 2013;117:426-36.
31. Weber P, Hamburger M, Schafroth N, Potterat O. Flash chromatography on cartridges for the separation of plant extracts: Rules for the selection of chromatographic conditions and comparison with medium pressure liquid chromatography. Fitoterapia. 2011;82(2):155-61.
32. Alzeer J, Vummidi BR, Arafeh R, Rimawi W, Saleem H, W. Luedtke N. The influence of extraction solvents on the anticancer activities of Palestinian medicinal plants. J. Med. Plant Res. 2014;8(8):408-15.
33. McLaughlin JL, Rogers LL. The use of biological assays to evaluate botanicals. Drug Inf. J. 1998;32:513-24.
34. Silva TMS, Nascimento RJB, Batista MM, Agra MF, Camara CA. Brine shrimp bioassay of some species of Solanum from Northestern Brazil. Braz. J. Pharmacog. 2007;17(1):35-8.
35. González Pérez Y, Aportela Gilling P. Determinación de la toxicidad aguda del dicromato de potasio de larvas de *Artemia salina*. Anu Toxicol. 2001;1(1):104-8.
36. Sanabria-Galindo A, López SI, Gualdrón R. Estudio fitoquímico preliminar y letalidad sobre *Artemia salina* de plantas colombianas. Rev. colomb. cienc. quim. farm. 1997;26(15-19).
37. Shanker K, Gupta S, Srivastava P, Srivastava SK, Singh SC, Gupta MM. Simultaneous determination of three steroidal glycoalkaloids in *Solanum xanthocarpum* by high performance thin layer chromatography. J. Pharm. Biomed. Anal. 2011;54:497-502.
38. Triana J, Eiroa JL, Morales M, Pérez FJ, Brouard I, Marrero MT, et al. A chemotaxonomic study of endemic species of genus Tanacetum from the Canary Islands. Phytochemistry. 2013;92:87-104.
39. Cela R, Lorenzo RA, Casis MdC. Técnicas de separación en química analítica. España: Editorial Síntesis; 2003. 640 p.
40. González AM, Presa M, Latorre MG, Lurá MC. Detección de metabolitos fúngicos con actividad tóxica mediante bioensayo sobre *Artemia salina*. Rev. Iberoam. Micol. 2007;24(1):59-61.
41. Ding X, Zhu F, Gao S. Purification, antitumour and immunomodulatory activity of water-extractable and alkali-extractable polysaccharides from *Solanum nigrum* L. Food Chem. 2012;131:677-84.
42. Stockert JC, Blázquez-Castro A, Cañete M, Horobin RW, Villanueva Á. MTT assay for cell viability: Intracellular localization of the formazan product is in lipid droplets. Acta Histochem. 2012;114(8):785-96.
43. García-Huertas P, Pabón A, Arias C, Blair S. Evaluación del efecto citotóxico y del daño genético de extractos estandarizados de *Solanum nudum* con actividad anti-Plasmodium. Biomédica. 2013;33(1):78-87.
44. de Graaf IAM, Olinga P, de Jager MH, Merema MT, de Kanter R, van de Kerkhof EG, et al. Preparation and incubation of precision-cut liver and intestinal slices for application in drug metabolism and toxicity studies. Nat. Protocols. 2010;5(9):1540-51.
45. Carranza-Rosales P, Santiago-Mauricio MG, Guzmán-Delgado NE, Vargas-Villarreal J, Lozano-Garza G, Ventura-Juárez J, et al. Precision-cut hamster liver slices as an ex vivo model to study amoebic liver abscess. Exp. Parasitol. 2010;126(2):117-25.
46. Morin PJ. Drug resistance and the microenvironment: nature and nurture. Drug Resist Updat. 2003;6(4):169-72.
47. Hamid R, Rotshteyn Y, Rabadi L, Parikh R, Bullock P. Comparison of alamar blue and MTT assays for high through-put screening. Toxicol. In Vitro. 2004;18(5):703-10.
48. López-Cobo A, Gómez-Caravaca AM, Cerretani L, Segura-Carretero A, Fernández-Gutiérrez A. Distribution of phenolic compounds and other polar compounds in the tuber of *Solanum tuberosum* L. by HPLC-DAD-q-TOF and study of their antioxidant activity. J. Food Comp. Anal. 2014;36(1–2):1-11.
49. Zaro MJ, Ortiz LC, Keunchkarian S, Chaves AR, Vicente AR, Concellón A. Chlorogenic acid retention in white and purple eggplant after processing and cooking. LWT-FOOD SCI TECHNOL. 2015;64(2):802-8.