

VITAE, REVISTA DE LA FACULTAD DE CIENCIAS FARMACÉUTICAS Y ALIMENTARIAS
ISSN 0121-4004 / ISSN 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

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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. elaeagnifolium*. 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 $66.10 \pm 4\%$ 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.

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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 $39 \pm 1,67$, $15,05 \pm 0,09$ y $66,10 \pm 4,44\%$ 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 flavonoids 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 H₂O in a ratio 2:1 (MFSe/EtOAc:H₂O) 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 (Büchi 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 K₂Cr₂O₇ at 0, 5, 10, 15, and 20 µg/mL, as well as a negative control (viability control) was performed. LC₅₀ was determined with the percent of mortality M (%) by a linear regression analysis.

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

$$M (\%) = (LN * 100) / (DN + LN),$$

where: LN = live nauplii, DN = (Equation 1)
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 characteri-

zed 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-H₂O (100:13.5:10), EtOAc-MeOH (90:10), EtOAc-MeOH (60:20) Hex-CHCl₃-AcOH (45:45:10), CHCl₃-MeOH-AcOH (47.5:47.5:5), CHCl₃-An (4:1) CHCl₃-MeOH (9:1), C₂H₅OC₂H₅-CHCl₃ (1:4), CHCl₃-EtOAc (1:1), Hex-AcOEt (3:1), where EtOAc = ethyl acetate, MeOH = methanol, H₂O = distilled water, Hex = hexane, CHCl₃ = chloroform, AcOH = glacial acetic acid, C₂H₅OC₂H₅ = 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 CHCl₃-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 10³ 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 CO₂ (5%) (NAPCO-6200 CO₂ 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 μ L), 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, CO₂ (5 %) and shaking at 25 rpm. FVLC7 (10 μ g/mL) and a negative control (TYI medium) were evaluated. Supernatant from each treatment was placed in a new well-containing DMEM/F12 (200 μ L) 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 $[M-H]^{-1}$ 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 \pm standard error (EE). The LC₅₀ 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 K₂Cr₂O₇, 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 $10.90 \pm 2.82\%$ 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 (K₂Cr₂O₇) induced toxic effect in *A. salina* with high potency (LC₅₀ = $13.90 \pm 0.29 \mu$ g/mL). In contrast, the CMESeF and MFSe had the same effect than the control although with less potency since 40-fold higher concentrations (LC₅₀ = $567.0 \pm 12.1 \mu$ g/mL and $565.1 \pm 49.9 \mu$ g/mL, respectively) were used, whereas with EtOAcFSe and HxFSe fractions 72-fold higher concentrations (LC₅₀ > 1000 μ g/mL) were required. Similar results were obtained with fractions separated by VLC since LC₅₀ of FVLC2, FVLC4, and FVLC9 were 518 ± 26.0 , 840.1 ± 86.6 , and $450 \pm 0.0 \mu$ g/mL respectively, while the LC₅₀ for FVLC3, FVLC5, FVLC6, FVLC8, and FVLC10 was > 1000 μ g/mL. FVLC1 exhibited a toxic effect similar to the positive control but with 7-fold higher concentration (LC₅₀ = $123.5 \pm 0.2 \mu$ g/mL). Interestingly, FVLC7 (LC₅₀ = $16.0 \pm 0.0 \mu$ g/mL) had the same toxic effect than the control positive (Figure 1). The negative control had no toxic effect.

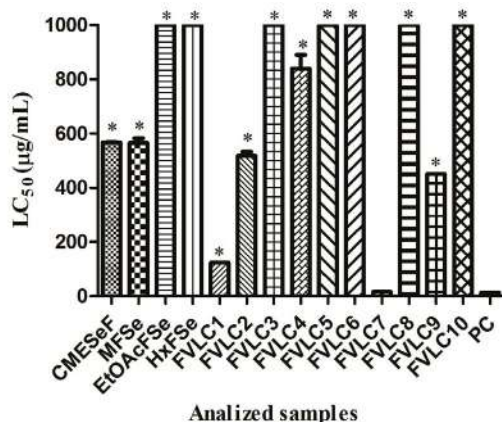


Figure 1. Median lethal concentration in *A. salina* of CMESeF and its isolated fractions. All comparison were performed against positive control (PC). *, $p \leq 0.05$.

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 $\text{CHCl}_3:\text{MeOH}:\text{AcOH}$ (47.5:47.5:5) three fractions with different R_f 's ($R_{f1} = 0.68$, $R_{f2} = 0.80$ and $R_{f3} = 0.90$) were separated. With $\text{EtOAc}:\text{MeOH}:\text{H}_2\text{O}$ (100:13.5:10) four fractions with various R_f 's ($R_{f1} = 0.03$, $R_{f2} = 0.05$, $R_{f3} = 0.13$ and $R_{f4} = 0.23$) were obtained. With $\text{EtOAc}:\text{MeOH}$ (60:20) and $\text{CHCl}_3:\text{MeOH}$ eluent (9:1), a fraction ($R_{f1} = 0.08$) and three fractions ($R_{f1} = 0.08$, $R_{f2} = 0.13$ and $R_{f3} = 0.50$) 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 $\mu\text{g}/\text{mL}$) reduced the viability of all cell lines at 39 ± 1.67 , 15.05 ± 0.09 and $66.10 \pm 4.44\%$ 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

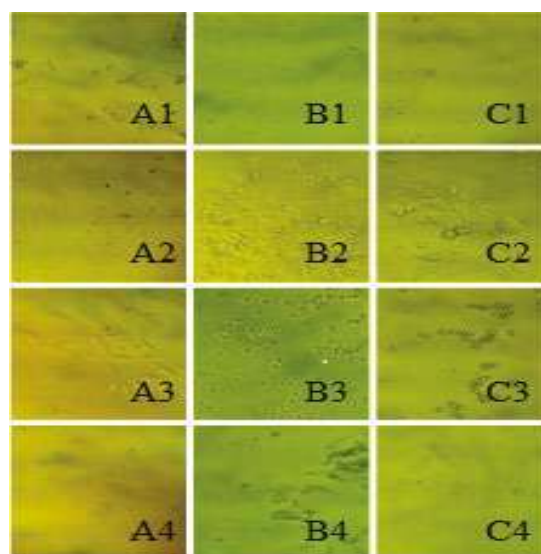


Figure 2. Microscopic observations of Vero, HeLa, and MCF-7 cell lines against FVLC7 (100 $\mu\text{g}/\text{mL}$) after 48 h of exposure. The images include treatments described below: A1= cell line Vero + positive control (Triton 1X), A2= cell line Vero + negative control with culture medium, A3= cell line Vero + negative control 10% ethanol and 3.8% DMSO, A4= cell line Vero + 100 $\mu\text{g}/\text{mL}$ 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 $\mu\text{g}/\text{mL}$ 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 $\mu\text{g}/\text{mL}$ FVLC7.

Evaluation from antitumor effect

FVLC7 (10 $\mu\text{g}/\text{mL}$) decreased $26.23 \pm 9.6\%$ 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 $[\text{M}-\text{H}]^{-1}$), chlorogenic acid (2) (ESI m/z 353 $[\text{M}-\text{H}]^{-1}$), dicaffeoylquinic acid (3) (ESI m/z 515 $[\text{M}-\text{H}]^{-1}$). The signal (ESI m/z 395 $[\text{M}-\text{H}]^{-1}$) might correspond to an alkaloid (ESI m/z 395 $[\text{M}-\text{H}]^{-1}$) (Figure 3).

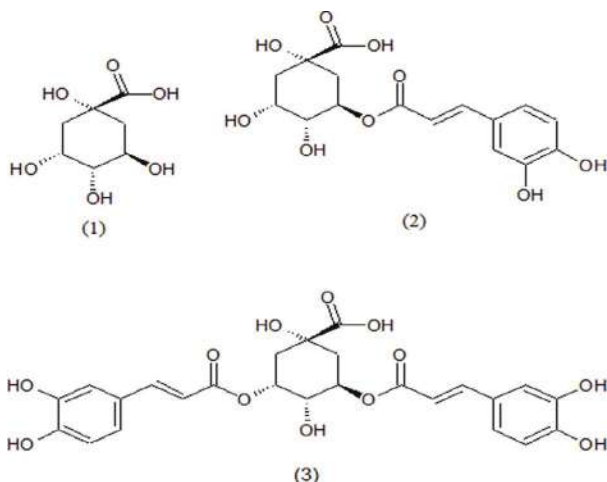


Figure 3. Quinic acid (1), chlorogenic acid (2) and dicaffeoylquinic acid (3) identified on FVLC7.

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 $\text{CHCl}_3:\text{MeOH}:\text{AcOH}$ (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 to those reported for crude methanolic extracts from other solanaceae in ranges of 107.2 to $\geq 1000 \mu\text{g}/\text{mL}$ (33, 34). The positive control had a $\text{LC}_{50} = 13.90 \pm 0.29 \mu\text{g}/\text{mL}$ 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 $\mu\text{g}/\text{mL}$, moderate lethality = 100-300 $\mu\text{g}/\text{mL}$, low lethality = 300 - 640 $\mu\text{g}/\text{mL}$ and minimum lethality = $\geq 640 \mu\text{g}/\text{mL}$ (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 raised during the separation process, whereby a larger amount was obtained with $\text{CHCl}_3:\text{MeOH}$ (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 $\mu\text{g}/\text{mL}$) 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 $\mu\text{g}/\text{mL}$) 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, dicaffeoylquinic 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 which 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 FVLC7 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.

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