BOLETIN LATINOAMERICANO Y DEL CARIBE DE PLANTAS MEDICINALES Y AROMÁTICAS
© / ISSN 0717 7917 / www.blacpma.ms-editions.cl

Articulo Original / Original Article

Cytotoxic activity of ethanolic extracts of *Lippia graveolens* HBK leaves and stem against lung cancer cell line SK-LU-1

[Actividad citotóxica de extractos etanólicos de hojas y tallos de *Lippia graveolens* HBK contra la línea celular de cáncer de pulmón SK-LU-1]

María Estela Frías-Zepeda[1], Jorge Ibarra-Berumen[1], Cynthia Ordaz-Pichardo[2] & Martha Rosales-Castro[1]

[1]Laboratorio de Fitoquímica, Instituto Politécnico Nacional, (CIIDIR) Unidad Durango, Durango, México
[2]Laboratorio de Biología Celular y Productos Naturales, Instituto Politécnico Nacional, Escuela Nacional de Medicina y Homeopatía, Ciudad de México, México.

Reviewed by:
Amina Unis
Ras El Khaimah University
EAU

Ahmed Salah Naser
University of Mosul
Iraq

Correspondence:
Martha **ROSALES-CASTRO**:
mrciidirdgo@yahoo.com

Section Biological Activity

Received: 20 March 2021
Accepted: 10 June 2021
Accepted corrected: 19 September 2021
Published: 30 September 2022

Citation:
Frías-Zepeda ME, Ibarra-Berumen J, Ordaz-Pichardo C, Rosales-Castro M Cytotoxic activity of ethanolic extracts of Lippia graveolens HBK leaves and stem against lung cancer cell line SK-LU-1
Bol Latinoam Caribe Plant Med Aromat 21 (5): 646 - 653 (2022).
https://doi.org/10.37360/blacpma.22.21.5.40

Abstract: *Lippia graveolens* HBK (Verbenaceae) is an aromatic herb of economic importance in Mexico, known as oregano. The leaves are used as condiments, and people use this species for respiratory and digestive disorders. The aim of this work was to evaluate the cytotoxic effect of ethanolic extracts obtained from free-oil leaves (L9) and stem (S15), against lung cancer cell line SK-LU-1, through tetrazolium salt (MTT) assay. Extracts concentrations of 0.3 to 300 µg/mL were used and HFF-1 as normal control cells. Both L9 and S15 extracts, showed cytotoxic effect, although stem was stronger than leaves and without damage to normal cell control. The phenolic compounds caffeic acid and acacetin were in higher concentration in L9, whereas naringenin, taxifolin, eriodictyol, luteolin, and apigenin had higher concentrations in S15. The ethanolic extracts of *L. graveolens* have excellent cytotoxic activity, and have a wide possibility of use in lung cancer treatment.

Keywords: *Lippia graveolens* HBK; Cytotoxic activity; Ethanolic extract; Lung cancer; SK-LU-1.

Resumen: *Lippia graveolens* HBK. (Verbenaceae) es una hierba aromática de importancia económica en México, conocida como orégano. Las hojas se usan como condimento y en medicina tradicional se utiliza para aliviar malestares respiratorios y digestivos. El objetivo de este trabajo fue evaluar el efecto citotóxico de extractos etanólicos obtenidos de hojas sin aceite (L9) y tallo (S15), sobre la línea celular de cáncer de pulmón SK-LU-1, mediante el ensayo de la sal de tetrazolium (MTT). Los extractos se aplicaron a concentraciones de 0,3 a 300 µg/mL y se utilizaron células HFF-1 como control normal. Tanto los extractos L9 como S15 mostraron efecto citotóxico, aunque el efecto del tallo fue mayor al de las hojas y sin daño al control celular normal. Los compuestos fenólicos ácido cafeico y acacetina se encontraron en mayor concentración en L9, mientras que naringenina, taxifolina, eriodictyol, luteolina y apigenina tuvieron mayor concentracion en S15. Los extractos etanólicos de *L. graveolens* tienen una excelente actividad citotóxica, con amplia posibilidad de utilizar en el tratamiento de cáncer de pulmón.

Palabras clave: *Lippia graveolens* HBK; Actividad citotóxica; Extracto etanólico; Cáncer de pulmón; SK-LU-1.

INTRODUCTION

Lung cancer is the leading cause of cancer death among men and the second cause of cancer death among women worldwide (Sitarek *et al*., 2020), and historical smoking patterns among the population caused differences in trends and rates related to various factors like age, race, sex, socioeconomic status and geography (Torre *et al*., 2016). Approximately 80% to 85% of the newly diagnosed cases of lung cancer will be Non Small-Cell Lung Cancer (NSCLC) and 15% to 20% will be small cell lung cancer (SCLC) (Khanna *et al*., 2017).

Through innovative research on cancer treatment, new drugs and treatment technologies are currently being developed (Leri *et al*., 2020). Chemotherapeutic agents damage normal cells, thus causing adverse side effects like chest pain, constipation, diarrhea, dyspnea, fatigue, mucositis, pain, rash and vomiting among others (Pearce *et al*., 2017). Therefore, traditional and complementary medicine has been explored in order to propose new alternatives that complement chemotherapy (Cheng *et al.*, 2018). An important source of novel effective agents with medicinal potential is the plant kingdom.

The use of natural phytochemical products, in single or combinatorial therapy, is an emerging strategy for prevention and cure of cancer, because of the various remarkable anticancer properties of these compounds (Sitarek *et al*., 2020). Cell death, which primarily occurs via apoptosis and nonapoptotic mechanisms (necrosis, autophagy, and cellular senescence), is one of the antineoplastic effects of natural compounds (Wattanathamsan *et al*., 2019). In lung cancer cell lines, the anti-cancer mechanisms include cell cycle arrest, over-expression of pro-apoptotic protein Bax, over-expression of P21 and P53 genes, increase of caspases-3 and 6, among others (Zhai *et al*., 2011; Wang *et al*., 2016; Wang *et al*., 2019).

Phytotherapy applies the traditional knowledge of native communities around the world in order to treat ailments, and various plant derived natural products have demonstrated promising effects against several diseases (Colalto, 2018).

Lippia graveolens HBK (Verbenaceae) is an aromatic herb of economic importance that grows in arid and semi-arid regions of Mexico, were known as "oregano". The species has been used for centuries because of its multiple culinary, cosmetic and medicinal properties (Lin *et al.*, 2007). Currently the leaves are marketed mainly for use a food seasoning, as well as for obtaining essential oil. The stem does not have an established use, although represents a high percentage of the plant (50% - 70%) (González-Güereca *et al*., 2007).

Mexican native communities have used various plants like oregano to treat respiratory diseases (Leyva-López *et al*., 2016). Many of the studies are focused on oil extracted from its leaves (Leyva-López *et al*., 2017). The beneficial properties of oregano, such as its antioxidant and anti-inflammatory activities, have been related to the presence of metabolites, such as terpenes, phenolic acids and flavonoids, both in leaves and stem (González-Güereca *et al*., 2007; Gutiérrez-Grijalva *et al*., 2018), that could be used to treat and prevent cell damage related to some illnesses.

Therefore, the objective of this study was to evaluate the cytotoxic effect of ethanolic extracts obtained from free-oil leaves (L9) and stem (S15) against non-small cell lung cancer cell line SK-LU-1.

MATERIALS AND METHODS

Plant materials

Oregano samples were collected in Cuencamé Durango, México, (coordinates N 24° 52' 12"; W 103° 41' 45"), on August 2018 and taxonomically identified as *Lippia graveolens* by Instituto Politécnico Nacional (CIIDIR-IPN) Durango's herbarium, with voucher number 40073.

In this investigation, oregano waste was used, which correspond to residual leaves, after essential oil extracted through a supercritical process with CO_2, and the remaining stems from the collection of plant material.

Both materials were dried under controlled temperature of 23°C, and powdered to 60 mesh screens.

Reagents

Ethanol was purchased from Merck. Fetal Bovine Serum (FBS) were from Bio West, antibiotic-antimycotic (streptomycin sulfate - amphotericin B), phosphate buffered saline (PBS) and 0.25% trypsin-EDTA were from Gibco, MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), DMSO, Roswell Park Memorial Institute, (RPMI), cis-diammine dichloride platinum (II) (Cis-platin), were from Sigma Aldrich. Standards for identification were used and purchased from Sigma-Aldrich Co. The rest of chemicals and reagents used for the experimentation all were of analytical grade.

Preparation of oregano extracts

Pulverized material of leaves and stem with 8.0% and 8.5% humidity, respectively, were extracted independently, with a mixture ethanol/water 50/50 v/v, and a mass/volume ratio of 1/30, according to Soto-Garcia & Rosales-Castro (2016). Preparation consisted of macerating the samples under controlled temperature of 23°C and stirring for 24 h, then filtered. Fresh solvent was added and the procedure was repeated. The extracts obtained in the first and second maceration were combined and concentrated under vacuum in a rotary evaporator at 40°C, after frozen at -20°C and then lyophilized. Yielding after lyophilization were 30.5% of dry extractfor leaves **(L9)** and 19.9% for stem **(S15)**.

Phenolic profile determination by Liquid chromatography- coupled to mass spectrometer (UPLC-MS)

Phenolic profile of oregano extracts was analyzed by UPLC-MS, according to Villegas-Novoa *et al*., (2019), using an Acquity UPLC system (Waters Corp. Milford, MA, USA), C_{18} 100 mm x

2.1 mm x 1.7 µm column (Water Corp., Milford, MA, USA), operated at 40°C. UPLC system coupled with a tandem Xevo TQ-S triple quadrupole mass spectrometer (Waters Corp., Milford,MA, USA).

The elution profile included two solvents: acidified water with 7.5 mM formic acid (A) and acetonitrile LC/MS-grade (B); initial 97% A; 1.2 min, 91% A; 3.8 min, 84% A; 11.4 min, 50% A; 13.2 min, 97% A and 15.0 min, 97% A. MRM data were collected from 0 to 16 min. The flow rate was 210 µL/ min and ionization was carried out using as co-solvent methanol with 0.1% formic acid (v/v) at a flow of 5 µL/min with the use of an isocratic solvent manager. The compounds were identified through the interpretation of their mass spectra, the MS/MS arrangement and using standards (Sigma-Aldrich) of different phenolic compounds (phenolic acids and flavonoids). The negative ionization mode was used. They were quantified from the standard curve of each of the compounds (standards). The UPLC and tandem Xevo TQ-S triple quadrupole mass spectrometer control and data processing were performed using MassLinx software (Waters Corp., Milford, MA, USA) (Villegas-Novoa *et al*., 2019).

Cell lines

SK-LU-1 and HFF-1 cell lines were purchased from American Type Culture Collection. SK-LU-1 cell line was maintained with Roswell Park Memorial Institute (RPMI) medium and HFF-1 with Dulbecco´s Eagle Modified Medium (DMEM) (Sigma Aldrich). Both mediums were supplemented with 10% FBS (Biowest), and 1% antibiotic-antimycotic (Gibco, CA, USA). All cell cultures were grown at 37°C in a 5% CO_2.

Cell treatment and tetrazolium salt (MTT) viability assay

The MTT viability assay was performed according to Mosmann (1983). This assay is based on the reduction of yellow MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) to formazan by mitochondrial succinate dehydrogenase, which is present only in viable cells. The resulting absorbance value is directly proportional to the number of viable cells. Cells were harvested with 0.25% trypsin and resuspended in RPMI and DMEM medium. Cell lines were deposited in 96-well microplates at 8,000 cells/well. The microplates were incubated at 37°C in 5% CO_2 for 24 h before adding treatments.

The leaves and stem extracts were dissolved in dimethyl sulfoxide (DMSO; Sigma-Aldrich, MO, USA) and after with DMEM and RPMI to reach the concentrations tested (0.3, 1, 3, 10, 30, 100 y 300 µg/mL). DMSO was used as vehicle control (0.1%) and cisplatin as positive control (5 µg/mL). Cells were exposed to treatments for 24 or 48 h. After time exposure, every treatment was replaced with 100 µL of MTT (0.5 mg/mL) and then cells were incubated for an additional 4 h. MTT solution was removed and formazan crystals were dissolved in 100 µL DMSO. Finally, the plates were read at 570 nm in a micro plate spectrophotometer (Epoch; BioTek, VT, USA). The assays were performed in triplicate in three independent studies. The percentage of viability was calculated according to the formula:

% viability = [mean Optical Density (O.D.) treated cells×100]/(mean O.D. control cells)

(Espinosa-Paredes *et al*., 2020).

The concentration leading to 50% inhibition of cell viability (IC_{50}) was calculated by non-linear regression analysis with GraphPad Prism 7.0 software (GraphPad, CA, USA). The results were expressed as an average ± Standard Deviation. IC_{50} were expressed as an average ± S.E.M. Selectivity Index of each extract was calculated dividing IC_{50} of normal cells control between IC_{50} of cancer cell line.

RESULTS

The phenolic profile in leaves L9 and stem S15 extracts are shown in Table No. 1, which were identified and evaluated according to the reference standards. Some differences between the percentages of the compounds evaluated were found, the more important are caffeic acid and acacetin were in higher concentration in leaves (14.67%, 10.44%) compared to the stem (7.34%, 0.94%), whereas naringenin, taxifolin, eriodictyol, luteolin, and apigenin had higher concentrations in the stem.

Table No. 1
Phenolic compounds identified in *L. graveolens* by UPLC-MS, leaves (L9) and stem (S15)

Rt (min)	$[M-H]^-$	L9 (%)	S15 (%)	Identification
4.32	179	14.67	7.34	Caffeic acid
5.80	463	3.59	2.82	Quercetin-3-glucoside
6.35	609	0.32	0.35	Rutin
6.41	303	17.61	21.31	Taxifolin
7.56	137	2.01	2.86	2-hydroxybenzoic acid
7.79	435	1.00	0.90	Phloridzin
8.67	287	15.13	20.59	Eriodictyol
8.92	285	4.43	6.12	Luteolin
8.96	301	0.80	0.96	Quercetin
9.73	271	25.95	30.11	Naringenin
9.84	269	1.53	2.18	Apigenin
12.51	283	10.44	0.94	Acacetin

Expressed as relative percentage (%) dry extract

The cytotoxic effect of *L. graveolens* leaves L9 and stem S15 extracts against SK-LU-1 cells, are shown in Figure No. 1, as average of three independent experiments. Both extracts decreased cell viability effect against SK-LU-1 cell line. The IC_{50} values for L9 resulted in 14.57 ± 1.33 µg/mL and 41.69 ± 1.15 µg/mL at 24 h and 48 h respectively. The extract S15 showed a strong effect against non-small cell lung carcinoma compared to L9. In the case of the stem extract S15 the IC_{50} values resulted in 7.80 ± 1.32 µg/mL and 0.81 ± 0.28 µg/mL, at 24 h and 48 h respectively. Vehicle control (DMSO 0.1%) did not decreased cell viability compared to negative control (116.49 ± 7.88%). In contrast, chemotherapeutic agent cisplatin (5 µg/mL) decreased cell viability to 60.02 ± 4.10% and 24.80 ± 1.77% at 24 h and 48 h, respectively.

The results of normal control cell line HFF-1, are shown in Figure No. 2. The IC_{50} values for L9 resulted in 59.86 ± 1.22 µg/mL and > 300 µg/mL, at 24 h and 48 h, respectively and S15 the IC_{50} values resulted in 50.77 ± 1.37 µg/mL and >300 µg/mL, at 24 h and 48 h respectively. Regarding Selectivity Index, the extract S15 was more selective than L9, with 6.5 and 4.1, respectively.

DISCUSSION

Smoking is a risk factor that increases the number of cases of Lung cancer which constitutes a worldwide public health problem (Tindle *et al*., 2018). Due to the high incidence of this type of cancer, it is necessary to conduct investigations that propose new therapies, such as traditional and complementary medicine (Liao *et al*., 2017). There is great interest in the search for secondary metabolites obtained from vegetal species for the treatment of these pathologies that affect humans (Ye *et al*., 2017; Yu *et al*., 2018). In this context, polyphenolic compounds as naringenin, taxifolin, eriodictyol, caffeic acid, luteolin, quercetin-3-glucoside, 2-hydroxybenzoic acid, apigenin, quercetin, phlorizin, acacetin and rutin were found in free-oil leaves L9 and stem S15 of *L. graveolens*. Some of these

compounds, in addition to quercetin hexoside, kaempferol, pylosin, cirsimaritin, have been previously reported in leaves and stems of *L. graveolens* (González-Güereca *et al*., 2007; Gutiérrez-Grijalva *et al*., 2018). Another compounds detected in an hydroethanolic extract of *L. graveolens* leaves are Hispidulin, Lancilactone B, Camaric acid and Salviol among others (Cortés-Chitala *et al*., 2021).

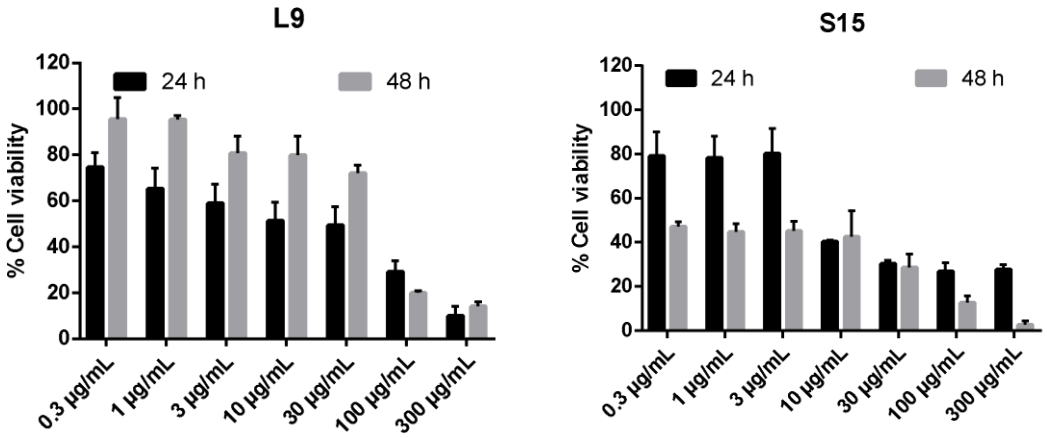

Figure No. 1
Cytotoxic effect of *Lippia graveolens* leaves (L9) and stem (S15) extracts against SK-LU-1 cells

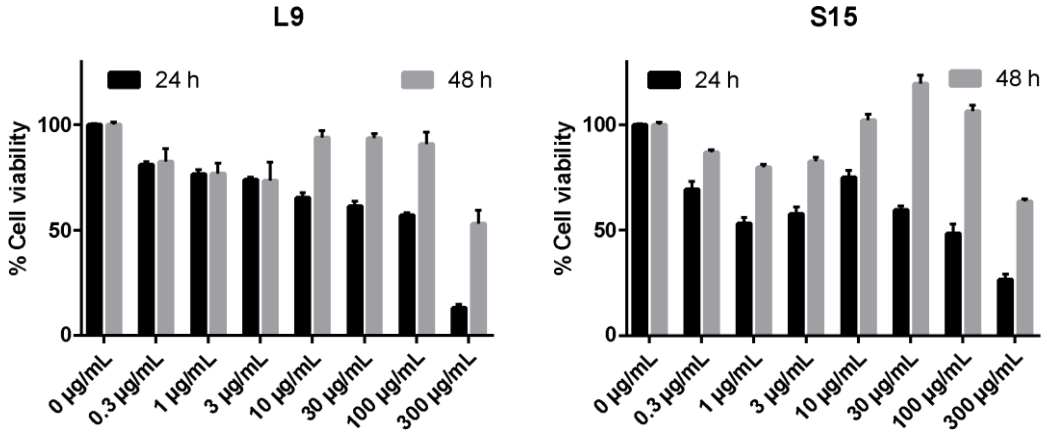

Figure No. 2
Cytotoxic effect of *Lippia graveolens* leaves and stem extracts againstHFF-1 cells

These phenolic compounds are related to reduce inflammation and treatment of respiratory and digestive disorders, headaches, rheumatism and diabetes (Gutiérrez-Grijalva *et al*., 2018; Tungmunnithum *et al*., 2018). In *L. graveolens* leaves extract, the biological effect as antioxidant and anti-inflammatory, is attributed at these compounds (Lin *et al*., 2007; Pérez-Gutiérrez, 2014; Gutiérrez-Grijalva *et al*., 2018).

Results indicated that Mexican oregano leaves and stem extract showed a favorable effect by decreasing the percentage of cell viability against SK-LU-1 cells. The stem extract S15 exerted a higher effect than leaves extract L9. The flavonoid

galagin, isolated from a methanolic extract from leaves and flowers of *L. graveolens*, exhibited high anti-proliferative effect, resulting an IC_{50} of 37.1 ± 1.0 µM against U251 (glioblastoma) (Amador *et al*., 2020).

Other flavonoids demonstrated an exerted anti-proliferative effect against lung cancer through specific mechanism. Naringin, tangeretin and hesperidin isolated from *Citrus platymamma* fruit exerted a cytotoxic effect against NSCLC cell line A549 showing an IC_{50} value of 364 µg/mL at 24 h (Nagappan *et al*., 2016). Eriodictyol induced cell cycle arrest in G_2/M phase on A549 cells (lung cancer), as well as an inhibition of mTOR/PI3K/Akt (Zhang *et al.*, 2020). Moreover, the combination of flavonoids and chemotherapy drug increases the apoptotic rate. In NSCLC cells, Apigenin-cisplatin combination produced S phase prolongation, more G_2/M cell cycle arrest and increase apoptosis compared with cisplatin or apigenin alone, by inducing p21 and PUMA respectively (Yan *et al*., 2020).

It is advisable to evaluate the effect of extracts against normal cell to determine the selectivity of extracts to produce damage to cancer cells. Both extracts (L9 and S15) were selective to SK-LU-1 cells compared to HFF-1 given the selectivity indexes. Authors recommend selectivity indexes ≥2 (Suffness & Pezzuto, 1990). Thus, both extracts decrease cell viability on malignant cells rather than normal cells.

Considering the effectiveness of *L. graveolens* extracts against SK-LU-1 cells and that stem is valueless waste byproduct, development of an anticancer drug represents an opportunity to use the stem in a valuable way. However, like other agro-industrial by-products represents an opportunity of economic profit that is rarely explored, which could improve the exploitation of easily available vegetal material.

CONCLUSION

The ethanolic extracts from *L. graveolens* oil-free leaves and stem decreased cell viability in lung cancer cell line SK-LU-1, without affecting cell viability in cells HFF-1. The effect may be attributed to its polyphenolic composition. These by-products (residual leaf after the extraction of essential oil and the stem) are an important source of compounds that can be exploited by the pharmaceutical industry and applied as a complement in the treatment of lung cancer.

ACKNOWLEDGEMENTS

The authors are very grateful to CONACYT (Mexican Council of Science & Technology) for the scholarship granted to Maria Estela Frias-Zepeda and Jorge Ibarra-Berumen. Our thanks also go to Socorro González-Elizondo for *L. graveolens* identification. Thanks to Nuria E. Rocha-Guzmán for UPLC/MS use.

REFERENCES

Amador S, Nieto-Camacho A, Ramírez-Apan MT, Martínez M, Maldonado E. 2020. Cytotoxic, anti-inflammatory, and α-glucosidase inhibitory effects of flavonoids from *Lippia graveolens* (Mexican oregano). **Med Chem Res** 29: 1497 - 1506. **https://doi.org/10.1007/s00044-020-02569-6**

Cheng YY, Hsieh CH, Tsai TH. 2018. Concurrent administration of anticancer chemotherapy drug and herbal medicine on the perspective of pharmacokinetics. **J Food Drug Anal** 26: S88 - S95. **https://doi.org/10.1016/j.jfda.2018.01.003**

Colalto C. 2018. What phytotherapy needs: Evidence-based guidelines for better clinical practice. **Phytother Res** 32: 413 - 425. **https://doi.org/10.1002/ptr.5977**

Cortés-Chitala MC, Flores-Martínez H, Orozco-Ávila I, León-Campos C, Suárez-Jacobo Á, Estarrón-Espinosa M, López-Muraira I. 2021. Identification and quantification of phenolic compounds from Mexican oregano (*Lippia graveolens* HBK) hydroethanolic extracts and evaluation of its antioxidant capacity. **Molecules** 26: 702. **https://doi.org/10.3390/molecules26030702**

Espinosa-Paredes DA, Cornejo-Garrido J, Moreno-Eutimio MA, Martínez-Rodríguez OP, Jaramillo-Flores ME, Ordaz-Pichardo C. 2020. *Echinacea Angustifolia* DC extract induces apoptosis and cell cycle arrest and synergizes with paclitaxel in the MDA-MB-231 and MCF-7 human breast cancer cell lines. **Nutr Cancer** 1 - 19. **https://doi.org/10.1080/01635581.2020.1817956**

González-Güereca MC, Soto-Hernández M, Kite G, Martínez-Vázquez M. 2007. Actividad antioxidante de flavonoides del tallo de Orégano Mexicano (*Lippia graveolens* HBK var. berlandieri Schauer). **Rev Fitotec Mex** 30: 43 - 49.

Gutiérrez-Grijalva EP, Picos-Salas MA, Leyva-López N, Criollo-Mendoza MS, Vázquez-Olivo G, Heredia JB.

2018. Flavonoids and phenolic acids from Oregano: Occurrence, biological activity and health benefits. **Plants** 7: 1 - 23. **https://doi.org/10.3390/plants7010002**

Khanna P, Blais N, Gaudreau PO, Corrales-Rodriguez L. 2017. Immunotherapy comes of age in lung cancer. **Clin Lung Cancer** 18: 13 - 22. **https://doi.org/10.1016/j.cllc.2016.06.006**

Leri M, Scuto M, Ontario ML, Calabrese V, Calabrese EJ, Bucciantini M, Stefani M. 2020. Healthy effects of plant polyphenols: molecular mechanisms. **Int J Mol Sci** 21: 1250. **https://doi.org/10.3390/ijms21041250**

Leyva-López N, Nair V, Bang WY, Cisneros-Zevallos L, Heredia JB. 2016. Protective role of terpenes and polyphenols from three species of Oregano (*Lippia graveolens*, *Lippia palmeri* and *Hedeoma patens*) on the suppression of lipopolysaccharide-induced inflammation in RAW 264.7 macrophage cells. **J Ethnopharmacol** 187: 302 - 312. **https://doi.org/10.1016/j.jep.2016.04.051**

Leyva-López N, Gutiérrez-Grijalva EP, Vazquez-Olivo G, Heredia JB. 2017. Essential oils of oregano: Biological activity beyond their antimicrobial properties. **Molecules** 22: 989. **https://doi.org/10.3390/molecules22060989**

Liao YH, Li CI, Lin CC, Lin JG, Chiang JH, Li TC. 2017. Traditional Chinese medicine as adjunctive therapy improves the long-term survival of lung cancer patients. **J Cancer Res Clin Oncol** 143: 2425 - 2435. **https://doi.org/10.1007/s00432-017-2491-6**

Lin LZ, Mukhopadhyay S, Robbins RJ, Harnly JM. 2007. Identification and quantification of flavonoids of Mexican oregano (*Lippia graveolens*) by LC-DAD-ESI/MS analysis. **J Food Compos Anal** 20: 361 - 369. **https://doi.org/10.1016/j.jfca.2006.09.005**

Mosmann T. 1983. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. **J Immunol Methods** 65: 55 - 63.

Nagappan A, Lee HJ, Saralamma VVG, Park HS, Hong GE, Yumnam S, Raha S, Charles SN, Shin SC, Kim EH, Lee WS, Kim GS. 2016. Flavonoids isolated from *Citrus platymamma* induced G2/M cell cycle arrest and apoptosis in A549 human lung cancer cells. **Oncol Lett** 12: 1394 - 1402. **https://doi.org/10.3892/ol.2016.4793**

Pearce A, Haas M, Viney R, Pearson SA, Haywood P, Brown C, Ward R. 2017. Incidence and severity of self-reported chemotherapy side effects in routine care: A prospective cohort study. **Plos One** 12: e0184360. **https://doi.org/10.1371/journal.pone.0184360**

Pérez-Gutiérrez RM. 2014. Effect of Mexican oregano (*Lippia graveolens* Kunth) on streptozotocin induced diabetic mice and its role in regulating carbohydrate metabolic enzymes and their inhibitory effect on the formation of advanced glycation end products. **Ann Res Rev Biol** 4: 3470 - 3491.

Sitarek P, Merecz-Sadowska A, Sliwinski T, Zajdel R, Kowalczyk T. 2020. An *in vitro* evaluation of the molecular mechanisms of action of medical plants from the Lamiaceae family as effective sources of active compounds against human cancer cell lines. **Cancer** 12: 2957. **https://doi.org/10.3390/cancers12102957**

Suffness M, Pezzuto J. 1990. **Assays related to cancer drug discovery**. In: Hostettmann K. (Ed.), Methods in plant biochemistry: Assay for bioactivity. Academic Press, London, UK.

Soto-García M, Rosales-Castro M. 2016. Efecto del solvente y de la relación masa/solvente, sobre la extracción de compuestos fenólicos y la capacidad antioxidante de extractos de corteza de *Pinus durangensis* y *Quercus sideroxyla*. **Maderas Ciencia y Tecnología** 18: 701 - 714. **https://doi.org/10.4067/S0718-221X2016005000061**

Tindle HA, Stevenson Duncan M, Greevy RA, Vasan RS, Kundu S, Massion PP, Freiberg MS. 2018. Lifetime smoking history and risk of lung cancer: Results from the framingham heart study. **J Natl Cancer Inst** 110: 1201 - 1207. **https://doi.org/10.1093/jnci/djy041**

Torre LA, Siegel RL, Jemal A. 2016. **Lung cancer statistics**. In: Lung cancer and personalized medicine. **https://doi.org/10.1007/978-3-319-24223-1**

Tungmunnithum D, Thongboonyou A, Pholboon A, Yangsabai A. 2018. Flavonoids and other phenolic compounds from medicinal plants for pharmaceutical and medical aspects: Anoverview. **Medicines** 5: 93. **https://doi.org/10.3390/medicines5030093**

Villegas-Novoa C, Gallegos-Infante JA, González-Laredo RF, García-Carrancá AM, Herrera-Rocha KM, Jacobo-Karam JS, Moreno-Jiménez MR, Rocha-Guzmán NE. 2019. Acetone effects on *Buddleja scordioides* polyphenol extraction process and assessment of their cellular antioxidant capacity and anti-inflammatory

activity. **Med Chem Res** 28: 2218 - 2231. **https://doi.org/10.1007/s00044-019-02448-9**

Wang F, Wang YH, Wang JJ, Xu HL, Wang CM. 2016. Eriodictyol-induced anti-cancer and apoptotic effects in human hepatocellular carcinoma cells are associated with cell cycle arrest and modulation of apoptosis-related proteins. **Bangladesh J Pharmacol** 11: 285 - 291. **https://doi.org/10.3329/bjp.v11i2.25549**

Wang R, Wang J, Dong T, Shen J, Gao X, Zhou J. 2019. Naringenin has a chemoprotective effect in MDA-MB-231 breast cancer cells via inhibition of caspase-3 and-9 activities. **Oncol Lett** 17: 1217 - 1222. **https://doi.org/10.3892/ol.2018.9704**

Wattanathamsan O, Hayakawa Y, Pongrakhananon P. 2019. Molecular mechanisms of natural compounds in cell death induction and sensitization to chemotherapeutic drugs in lung cancer: Review. **Phytother Res** 33: 2531 -2547. **https://doi.org/10.1002/ptr.6422**

Yan W, Wu THY, Leung SSY, To KKW. 2020. Flavonoids potentiated anticancer activity of cisplatin in non-small cell lung cancer cells *in vitro* by inhibiting histone deacetylases. **Life Sci** 258: 118211. **https://doi.org/10.1016/j.lfs.2020.118211**

Ye YT, Zhong W, Sun P, Wang D, Wang C, Hu LM, Qian JQ. 2017. Apoptosis induced by the methanol extract of *Salvia miltiorrhiza* Bunge in non-small cell lung cancer through PTEN-mediated inhibition of PI3K/Akt pathway. **J Ethnopharmacol** 200: 107 - 116. **https://doi.org/10.1016/j.jep.2016.12.051**

Yu X, Lin H, Wang Y, Lv W, Zhang S, Qian Y, Deng X, Feng N, Yu H, Qian B. 2018. D-limonene exhibits antitumor activity by inducing autophagy and apoptosis in lung cancer. **Oncol Targets Ther** 11: 1833 - 1847. **https://doi.org/10.2147/OTT.S155716**

Zhai YJ, Cheng F, Wang TM, Chu ZY, Li N. 2011. *In vitro* anticancer activity of taxifolin on human cervical cancer Hela cells and its mechanism. **Chinese Trad Patent Med** 12

Zhang Y, Zhang R, Ni H. 2020. Eriodictyol exerts potent anticancer activity against A549 human lung cancer cell line by inducing mitochondrial-mediated apoptosis, G2/M cell cycle arrest and inhibition of m-TOR/PI3K/Akt signalling pathway. **Arch Med Sci** 16: 446. **https://doi.org/10.5114/aoms**