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ORIGINAL ARTICLE

Gas Chromatography Coupled With Mass Analysis Phytochemical Profiling, Antiproliferative and Antimigratory Effect of *Tagetes lucida* Leaves Extracts on Cervical Cancer Cell Lines

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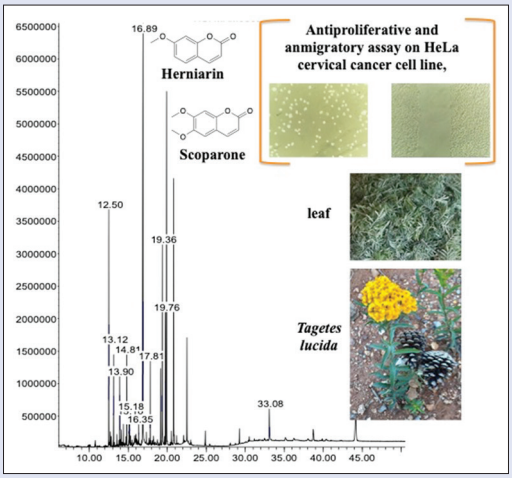
ABSTRACT

Background: *Tagetes* species have been extensively employed in folk medicine and have been demonstrated to exert various biological activities due to its phytochemical constituents, including those related to cancer. **Objectives:** The aim of this study was to identify, in *Tagetes lucida* organic leaf extracts, the bioactive compounds with antiproliferative and antimigratory activities on the human cervical cancer (CCa) cell lines human cervical cancer cell line (HPV-16) (SiHa) and human cervical cancer cell line (HPV-18) (HeLa), and on the non-cancer cell line human immortalized keratinocyte cell line (HaCaT). **Materials and Methods:** The phytochemical profile of all *Tagetes* leaves extracts was determined by Gas chromatography mass spectroscopy analysis and tested on SiHa, HeLa, and HaCaT cell lines using (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) and wound-healing assays. **Results:** The Gas chromatography coupled with mass analysis analysis revealed two main constituents identified in all extracts, the coumarins herniarin (17.152%–56.904% content) and scoparone (2.778%–34.817% content), the rest of identified constituents were terpenes where the geranyl acetate was the major constituent on hexane extract. On the anti-proliferative potential of *T. lucida* extracts, our present investigation revealed that all extracts decreased HeLa cell viability (half-maximal inhibitory concentration value of <190.26 mg/mL at 24 h and <220.41 mg/mL at 48 h) dose dependently; the cell viability of SiHa and HaCaT was not affected. Furthermore, the migration capacity of SiHa and HeLa cells decreased after 24 and 48 h with acetonic and methanolic extracts treatment, while on HeLa cells a more evident effect was observed with a <80% decrease in wound closure. **Conclusion:** Our findings revealed the coumarins presented in *T. lucida* as potential candidates of inhibiting cell proliferation and migration preferably toward CCa (HeLa cell line).

Key words: Antiproliferative, cervical cancer, human cervical cancer cell line (HPV-18) cells, migration, natural products, *Tagetes lucida*

SUMMARY

- Two main constituents were identified on *Tagetes lucida* leaves extracts, the cumarins herniarin (17.152%–56.904% content) and scoparone (2.778%–34.817% content). All extracts showed anti-proliferative activity by inhibiting human cervical cancer cell line (HPV-18) (HeLa) cell viability with a half-maximal inhibitory concentration value of <190.26 µg/mL at 24 h and <220.41 µg/mL at 48 h in a dose-dependent manner, while human cervical cancer cell line (HPV-16) (SiHa) and human immortalized keratinocyte cell line cell viability was not affected. Also, the migration capacity of SiHa and HeLa cells decreased after 24 and 48 h with acetonic and methanolic extracts treatment, where a more evident effect was observed with a <80% decrease in wound closure on HeLa cells. The coumarins presented in *T. lucida* are suggested potential candidates of inhibiting cell proliferation and migration preferably towards cervical cancer (CCa) (HeLa cell line).



Abbreviations used: H: *T. lucida* hexane extract; D: *T. lucida* dichloromethane extract; A: *T. lucida* acetone extract; M: *T. lucida* methanol extract; GC-MS: Gas chromatography coupled with mass analysis; HPV: Human papillomavirus; SiHa: Human cervical cancer cell line (HPV-16); HeLa: Human cervical cancer cell line (HPV-18); HaCaT: Human immortalized keratinocyte cell line; DMEM/F-12: A 1:1 mixture of Dulbecco's Modified Essential Medium (DMEM) and Ham's F-12 Medium; FBS: Fetal bovine serum; DMSO: Dimethyl sulfoxide; AraC: Cytarabine, cytosine β-D-arabinofuranoside; PBS: Phosphate-Buffered Saline; OD: Optical density; IC_{50}: Half-maximal inhibitory concentration; SD: Standard deviation; ANOVA: One-way analysis of variance; RT: Retention time; MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.

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INTRODUCTION

The fourth most frequently found cancer in women throughout the world is cervical cancer (CCa); the latter ranks in second place in Mexico.[1] Current chemopreventive therapies in cancer patients include surgery, radiotherapy, immunotherapy, targeted molecular therapy and chemotherapy, of which, chemotherapy in combination with radiotherapy (chemoradiotherapy) is one of the most used.[2] However, exist 90% of the deficiency on chemotherapy treatments as a result of drug resistance developed during invasion and metastasis cancer.[3] Despite side effects, nowadays, there is a gamma of antineoplastic drugs that have different cell objectives, among which are those that cause DNA damage, as anthracyclines, antimetabolites, antitumor antibiotics; and nutritional supplements of camptothecins, epipodophyllotoxins, platinum analogs, taxanes, and alkaloids of vinca, whom the last are derived from natural sources.[4]

Recently, the search for bioactive molecules to replace specific drugs in cancer therapy has increased significantly. Plant natural compounds have a fundamental role in defense, protection against ultraviolet radiation, parasites, and predators.[5] The growing interest is due to the presence of infinity of secondary metabolites, such as terpenes, phenolic compounds, and alkaloids with anticancer activity.[6] Phenolic compounds are one of the most studied phytochemical families, such compounds have various biological properties that include, antioxidant, antienzymatic, antiestrogenic, antiproliferative activities, etc.; on the other hand, these compounds halt the cell cycle, promoting apoptosis as well as differentiation, with this wide variety of biological functions makes them one of the most versatile chemopreventive agents in the intervention of each of the stages of carcinogenesis.[7]

Tagetes is a genus of plants that belongs to the Asteraceae family and comprises about 56 species.[8] This genus has been investigated as a possible source of chemical compounds with high pharmaceutical and nutritional values. The natural compounds that have been found are phenyl compounds as coumarins,[9-13] terpenes,[9,10,13] and thiophenes compounds that they have been attributed various properties such as antibacterial, anticoagulant, anti-Alzheimer, anti-HIV, and anticancer activities.[14-18]

Tagetes lucida cav. is an important aromatic ritual plant widespread from the Central to America South. It is commonly known by the name of "pericón," "yauhtli" (náhuatl), "anis," "Santa María," or "San Juan" herb.[12,19] In traditional medicine, *T. lucida* is one of the most widely used medicinal plants of Western Mexico, a decoction of the entire plant o various parts of the plant have been useful in treating digestive tract conditions, spitting blood, anxiety, depression and as an anti-inflammatory and malarial remedy. In addition, pharmacological studies have reported it as antibacterial, insecticidal, cytotoxic, antioxidant, antidepressant-like effects, anxiolytic, sedative, and anti-*Candida albicans* activity.[12,20,21]

Despite the great potential of species of this genus as a source of secondary metabolites with therapeutic interest, there are currently insufficient *in vitro* studies conducted in the area of cancer, the current background on the antiproliferative and anti-migratory activity of this genus remains poorly studied.[12,22] In addition, this species does not have sufficient phytochemical or pharmacological studies that support the properties that have been attributed to it. Thus, our workgroup decided to select, as the objective of our work, the evaluation of the effect of *T. lucida* organic extracts on proliferation and migration in two CCa cell lines, human cervical cancer cell line (HPV-16) (SiHa) and human cervical cancer cell line (HPV-18) (HeLa), and in a nontumor human immortalized keratinocyte cell line (HaCaT) cell line. The results obtained from this research will generate basic information regarding the biological properties and phytochemical profiles of the species *Tagetes lucida* collected, which will contribute to the proper exploitation of a valuable native natural resource of Mexico.

MATERIALS AND METHODS

Plant material

In December 2018, *T. lucida* leaves were gathered in Petaquillas (17°28'26' N, 99°27'43' O), Guerrero State, Mexico. The identification plant was authenticated by Arturo Hernández Abarca, biologist of the Herbarium from the Institute of Scientific Research, Natural Sciences Area (Autonomous University of Guerrero). A voucher specimen (11555) was deposited at the same Institute for future references.

Extracts preparation

The *T. lucida* plant material (1.4 Kg) dried at room temperature (24°C) and grinded, was successively macerated with hexane, dichloromethane, acetone, and methanol (reactive-grade, 500 mL for 72 h, three times). After the macerated volume was filtered, the organic phase was concentrated at 60°C under reduced pressure in a rotating evaporator. Hexane (H), dichloromethane (D), acetone (A), and methanol (M) extracts were stored at-20°C in amber vials until there were used.

Gas Chromatography-Mass Spectrometry analysis

The *T. lucida* extracts phytochemical profile showed the presence of bioactive components. gas chromatography coupled with mass analysis (GC-MS) analysis was carried out using the Agilent Technology 6890 gas chromatograph interfaced to a 5973N mass spectrometer equipped with an HP-5MS capillary nonpolar column (30 m, ID: 0.25 mm, film thickness: 0.25 µm), connected to an ion trap detector operating in electron impact mode at 70 eV; carrier gas was He, with a flow rate of 1 mL/min and there was an injection volume of 20 µL (in HPLC grade Hexane). The oven temperature was programmed within a range of 50°C-230°C with an increase of 2°C/min. The NIST/EPA/NIH Mass Spectral library version 1.7a/ChemStation was employed to compare the results.

Cell culture and exposure to extracts

Human SiHa, HeLa, and HaCaT cells were cultured in Dulbecco's Medium Modified Eagle Medium nutrient mixture F-12 (DMEM/F12) supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS), 1% (v/v) antibiotic (Ampicillin/Streptomycin) and incubated under a 5% CO_2 atmosphere and 100% humidity at 37°C.

Cell proliferation assay

The proliferation effect of the extracts on SiHa, HeLa, and HaCaT cells was assessed utilizing the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, according to Mossman (1983). 1×10^4 cells per well were seeded on 96-well flat-bottom culture plate in 100 µL of medium and incubated at 37°C overnight. The *T. lucida* extracts in 100 µL of fresh medium were added to reconstitute the final concentrations (5–320 µg/mL) during 24 and 48 h. Medium-containing vehicle solvent dimethylsulfoxide (DMSO, ≤1% [v/v]) was added as untreated controls (V, vehicle). After treatment, the medium was replaced by fresh medium and the MTT reagent (100 µL) was added to each well and incubated for 4 h at 37°C. The formazan crystals were diluted with isopropanol (100 µL), and supernatant optical density was measured at 545 nm employing Statfax 2100 microplate reader (Awareness Technology, Palm City, FL, USA).[23] Paclitaxel (5 µg/mL) compound was used as a positive control.[24] Each concentration was tested with three replicates in each

experiment and the experiment was performed independently at least thrice. The extract concentration required for 50% inhibition (IC_{50}) was calculated through the linear equation (Y = mX + b) using GraphPad Prism software v6.0 (GraphPad Software, Inc, La Jolla, CA, USA).

Migration: Wound-healing assays

Cells were grown in 12-well plates until reach ~90% confluence and then in a linear wound, the cell monolayer was scratched with a pipette tip (2 mm). After, cells were preincubated with cytosine β-D-arabinofuranoside (AraC) (5 μg/mL) for 2 h to eliminate migration induced by cell proliferation. Then, the cells were washed twice with phosphate-buffered saline. The cells were treated with *T. lucida* extracts (5–20 μg/mL) with FBS (1%) and incubated for 24 h and 48 h at 37°C. Cell migration over the denuded area was visualized and photographed under a phase-contrast inverted microscope (NIKON, Ts2FL, USA) at 0, 24, and 48 h. The width of the wound was measured with the ImageJ 1.44p software and the MRI wound healing tool, compared to zero time. The experiment was repeated at least thrice.[25,26]

Statistical analysis

Data analysis was performed using the GraphPad Prism version 6.0 (GraphPad Software, Inc., La Jolla, CA, EE. UU.) statistical software program. One-way analysis of variance was used with the Dunnett test. Data were shown as mean ± standard deviation. A statistically significant difference was considered when $P < 0.05$. The analysis and adjustment of the images were carried out in ImageJ statistical software. All experiments were carried out in triplicate.

RESULTS AND DISCUSSION

The *T. lucida* hexane, dichloromethane, acetone, and methanol extracts provided yields of 5.5%, 16.26%, 3.45%, and 5.44%, respectively. Table 1 presents GC-MS analyses. The phytochemical study by GC-MS analysis revealed 23 identified constituents where different fatty acids, heterocyclic compounds, and esters among others were presented. Interestingly, the highest percentage (~70%) of the coumarins herniarin (15) and scoparone (17) [Figure 1] were predominant in dichloromethane, acetone, and methanol extracts compared with these compounds. Moreover, the geranyl acetate (4) was the major identified constituent on hexane. These compounds were previously reported in the *Tagetes* genus.[27,28]

Phenolic compounds identified from studied species of the *Tagetes* genus are mainly thiophenes, flavonoids, and coumarins, which they normally are described as antifungal and antibacterial compounds.[29,30] However, this is the first work where a high content of these coumarins is reported both in *T. lucida* and the other *Tagetes* species.[31-34]

Antiproliferative activity

To evaluate the antiproliferative activity of *Tagetes* extracts on SiHa, HeLa, and HaCaT cell lines for 24 and 48 h, MTT assays were conducted. However, extracts after 24 h of treatment did not show an effect on antiproliferative activity (data not shown). In HeLa cells treated during 48 h with 160 and 320 μg/mL of all extracts, cell viability of <50% and 40%, respectively, was observed. Similarly, in SiHa cells treated with 160 and 320 μg/mL, cell viability of < 60% and 50%, respectively, was observed. While in HaCaT cells treated with 160 μg/mL of hexane, dichloromethane, and acetone extract, the cell viability remained above 60% [Figure 2].

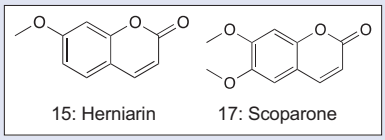

Figure 1: Chemical structure of coumarins with a high content in *Tagetes lucida* extracts

In addition, all of the extracts gave rise to morphologic changes in the cancer cell lines, including a decrease in cell size, a rounded shape, and the formation of intracellular vacuoles, suggestive of apoptosis (data not shown). It is reported that growth regulation in SiHa and HeLa is due to variations in p53 activation due to the low p53 levels, which induce cell cycle arrest, while high p53 levels induce apoptosis.[35,36] Therefore, the *T. lucida* antiproliferative effect could be due to that p53 is activated to a greater degree in HeLa compared with SiHa in response to treatments.

The better antiproliferative effect in a dose-dependent manner compared to untreated control cells was induced by all *T. lucida* extracts for HeLa at 24 h (CI_{50} < 190.26 μg/mL) and 48 h (CI_{50} < 220.41 μg/mL), while for SiHa there was no effect [Table 2].

Positive control: Paclitaxel (5 μg/mL). na: Non-active (half-maximal inhibitory concentration >250 μg/mL)

Since CCa is the second major cause of death from cancer worldwide, the HPV-16 (SiHa) and HPV-18 (HeLa) genotypes are attributed as the major risk factors, accounting for nearly 70% of cancers. In addition, SiHa contains around 1–2 integrated copies of the HPV 16 genome, while HeLa possesses 10–50 integrated copies of HPV 18, resulting in a higher replication rate in HeLa cells, thus becoming a more aggressive cancer.[35,36] Our results are interesting because the evaluated extracts were only active against the HeLa cell line [Table 2].

Based on high coumarin content in the GC-MS analysis and on previous investigations results, we can infer that the coumarins are directly related to the antiproliferative effect. The coumarins, depending on their structure, can act on various tumor cells by different mechanisms.[37-39] Hence, a selective difference of the cytotoxic effect on the tumor cells (SiHa and HeLa) could be more noticeable by isolated coumarins from *T. lucida*. Thereby the results were consistent with other studies where is reported to exhibit negligible or mild side effects in humans using doses up to 7 g daily, after 2 weeks of continued treatment; even they were excellent agents for treating side effects caused by radiotherapy.[36-41]

Although coumarins are considered as one of the most important phytochemicals from *Tagetes* genus, cancer studies are focused on flavonoids and thiophenes of mainly *T. minuta* and *T. erecta* species,[9-10,13,42,43] within included studies where is attributed to these compounds activity against HeLa cells,[11,14,43] this could be due to low coumarin quantities found into the studied species. The coumarins antitumor activity is extensively explored by many researchers at present due to is considered promising anticancer compounds, however, the compounds used in their research were not isolated from natural species.[44] This is the first study, which recognizes antitumor activity to identify coumarins from *T. lucida* extracts and the first, where two CCa and one non-cancer cell line were employed. While our results would appear to propose *T. lucida* extracts as an alternative or complementary therapy against cancer, cytotoxicity, and molecular mechanisms must be analyzed in more study models.

In addition, the GC-MS analysis revealed that several terpenes presented on *Tagetes* extracts have been reported with attractive anticancer properties. Considering the monoterpenes, the linalool exhibited an antiproliferative effect on human melanoma cells (RPMI 7932) at

Table 1: Compounds detected by gas chromatography coupled with mass analysis in *T. lucida* extract

Component	Content (%) RT (min)			
	Hexane	Dichloromethane	Acetone	Methanol extracts
Linalool	0.127			
	8.463			
2,6-Dimethyl-3,5,7-octatriene-2-ol, E	0.810			
	10.112			
Geraniol	1.358			
	10.762			
Geranyl acetate	21.230	5.918	9.300	7.206
	12.542	12.509	12.503	12.246
Nerolidol acetate	0.076			
	12.614			
β-Elemene	2.493			
	12.719			
Isohomogenol	0.557			
	12.818			
Caryophyllene	9.887	3.744	3.515	1.340
	13.146	13.127	13.120	13.114
α-Humulene	1.074			
	13.560			
β-Cubebene	7.931	2.173	1.494	1.035
	13.915	13.903	13.120	13.895
α-Cadinene	1.735			0.541
	14.381			14.105
E-Nerolidol	6.410	2.916	2.673	2.777
	14.821	14.808	14.808	14.802
(-)-Spathulenol	1.692	0.469	0.729	0.370
	15.117	15.110	15.104	15.097
Caryophyllene oxide	0.943	0.844	0.843	0.447
	15.189	15.183	15.176	15.176
Herniarin	17.152	38.815	56.904	53.639
	16.897	16.956	16.891	16.838
Phytol	7.000	3.396	3.575	4.190 0.708
	17.830	17.817	17.817	17.810 20.516
Scoparone	2.778	34.817	12.638	21.855
	19.367	19.498	19.360	19.347
Geranyl linalool	5.811	3.833	4.332	3.444
	19.781	19.774	19.761	19.754
Squalene	3.736			
	29.259			
α-Tocopherol	7.199	3.075	3.603	
	33.122	33.389	33.082	
Tricyclo[5.2.2.0 (1,6)]undecano-3-ol, 2-methylene-6,8,8,-trimethyl-			0.394	
			16.352	
α-Muurolene				1.156
				14.375
Ethyl α-linolenate				1.290
				20.398

RT: Retention time

CI_{50} = 5.60 μM and on prostate cancer cells (DU145) at CI_{50} = 28.3 and 10.5 μM at 12 and 24 h, respectively;[45,46] and the geraniol and geranyl acetate were reported due to their ability to trigger apoptosis, DNA damage and cell cycle arrest on colon cancer cells (Colo-205) at IC_{50} values of 20 and 30 μM, respectively.[47] Similarly, the sesquiterpenes as spathulenol were disclosed effective against the ovarian cancer cell line (OVCAR-3) at CI_{50} = 49.30 μg/mL;[48] the nerolidol was noted with anticancer properties against skin melanoma cells (B16-F10, IC_{50} > 25 μM), hepatocellular carcinoma (HepG2, IC_{50} > 25 μM), human promyelocytic leukemia cells (HL-60, IC_{50} = 21.99 μM) and human erythroleukemic cells (K562, IC_{50} = 17.58 μM);[49] and in combination with their isomers cis-and trans-nerolidol exhibited anticancer effect against HeLa cells (IC_{50} = 1.5 μM) and breast carcinoma cells (BT-20, IC_{50} = 1.5 μM);[50] further, the *in vitro* studies showed a reduction of incidence of intestinal neoplasia (50%) and a reduction of several tumors/rat (about 53%) in rats fed with nerolidol.[51]

Moreover, although the anticancer properties of the β-caryophyllene and β-caryophyllene oxide are poorly recognized, quite a lot of evidence has demonstrated that both sesquiterpenes possessed cytotoxic activities against several types of cancer cells.[52] For

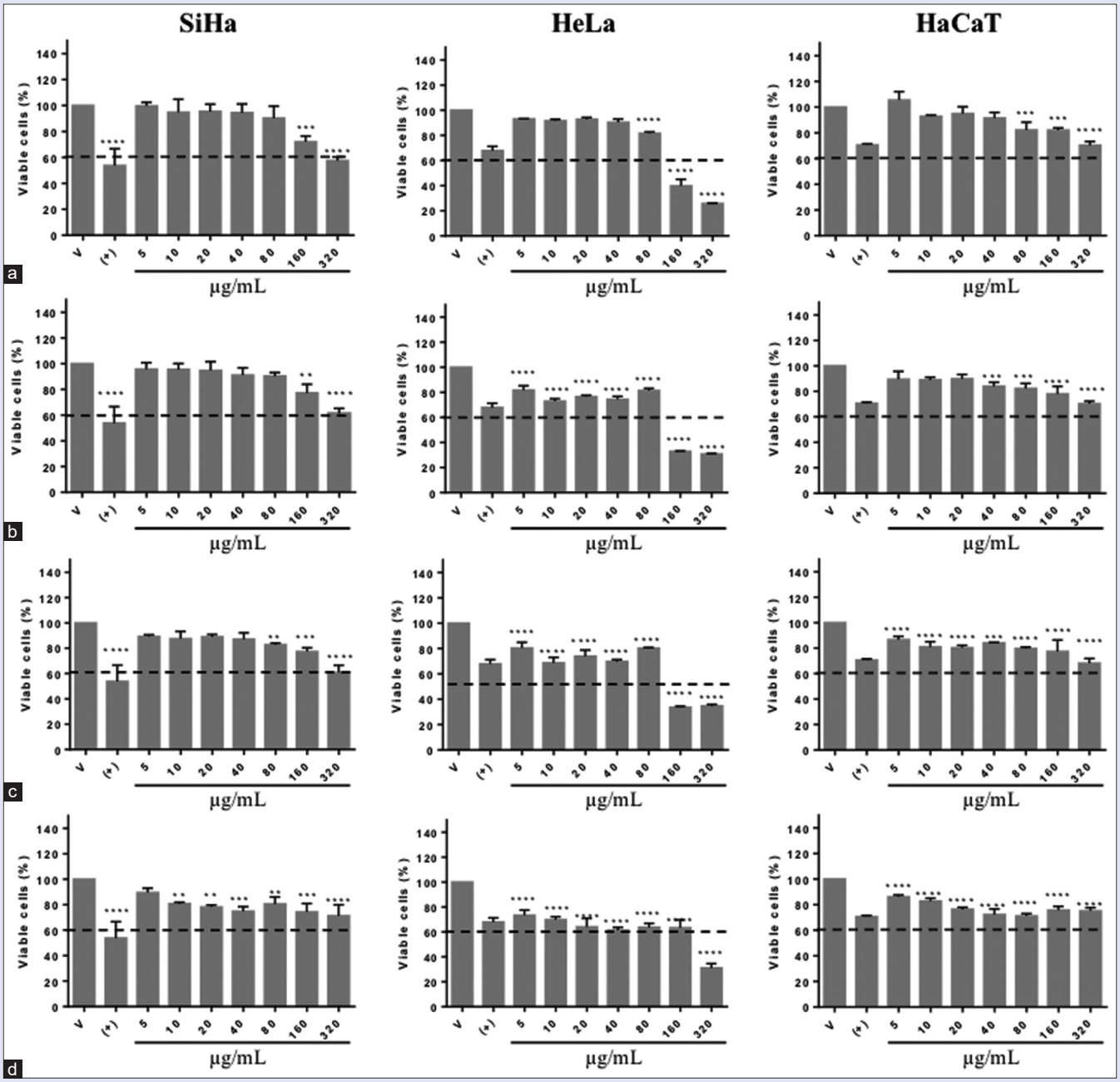

Figure 2: Antiproliferative effect of *Tagetes lucida* extracts on human cervical cancer cell line (HPV-18), human cervical cancer cell line (HPV-16), and human immortalized keratinocyte cell line cells. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay; cells treated with *Tagetes lucida* extracts during 48 h. Hexane (a), Dichloromethane (b), Acetone (c), and Methanol (d) extracts. V: Vehicle, (dimethyl sulfoxide), +: Positive control, Paclitaxel (5 μg/mL). The mean ± standard deviation of three independent experiments expressed the values. We employed one-way analysis of variance and the Dunnett multiple comparison test; *$P < 0.05$; **$P < 0.01$, and ***$P < 0.001$, and ****$P < 0.0001$. *Differences with respect to the control treatment (v)

example, β-caryophyllene was reported led to strong growth inhibition in two colon cancer cell lines, HCT-116 (IC_{50} = 19 μM) and HT-29 (IC_{50} = 63 μM), as well as in pancreatic cancer cells, PANC-1 (IC_{50} = 27 μM) and with combination with their isomers a-humulene and iso-caryophyllene were more effective in the reduction of MCF-7 human breast cancer cell line proliferation (90% at 10 μg/mL) than when used separately.[53,54] Besides, the β-caryophyllene oxide was reported as cytotoxic effect on various cancer cell lines, such as HeLa (IC_{50} = 13.55 μM), HepG2 (human liver cancer cells, IC_{50} = 3.95 μM), AGS (human gastric cancer cells, IC_{50} = 12.6 μM), SNU-1 (human gastric cancer cells, IC_{50} = 16.79 μM), SNU-16 (human stomach cancer cells, IC_{50} = 27.39 μM), and A-2780 (human ovarian cancer cells, IC_{50} = 40.6 μM).[55,56] On human brain cancer studies, β-elemene displayed antiproliferative effect against human brain tumor cells: A172 (80.8 μg/mL), CCF-STTG1 (82.8 μg/mL) and U-87MG (88.6 μg/mL);[57] while on human hepatocellular carcinoma studies, α-humulene, showed antiproliferative effect on Huh7 (IC_{50} = 15.09 μg/mL), SMMC-7721 (IC_{50} = 17.31 μg/mL), HepG2 (IC_{50} = 11.22 μg/mL) and Hep3B (IC_{50} = 13.78 μg/mL).[58] Finally, the diterpene phytol and triterpene squalene showed potent anti-proliferative activity against human lung carcinoma cells (A549, IC_{50} = 70.81 and 60.7 μM at 24 and 48 h, respectively);[59] and

against breast cancer cells (MDA-MB-231 and MCF-7, CI_{50} = 4.35 and 6.05 mg/mL, respectively).[60]

Antimigratory activity

The effect of all extracts of *T. lucida* on the migration capacity of SiHa and HeLa cells was tested to concentrations of 5, 10, and 20 µg/mL for 24–48 h. However, the hexane and dichloromethane extracts did not show an effect on migration capacity cells (data not shown). No significant decrease was observed in the migration capacity of the SiHa cells treated with the acetone extract at 24 h, nonetheless compared to the control (39% decrease in wound closure), the treatment decreased the migration capacity at 48 h, observing a 26% and 23% decrease in wound closure with concentrations of 5 and 20 µg/mL, respectively [Figure 3a and b]. Besides, treatment with the methanolic extract (20 µg/mL) decreased the migration capacity of SiHa cells from 24 h (14% less wound closure, compared to the control); this latter effect continued at 48 h, while at a lower proportion (around 9% less wound closure compared with the control) [Figure 3c and d]. In regard to HeLa cell line, compared to the control (46% decrease in wound closure), the acetonic extract treatment decreased the cell migration capacity at 24 h, from the 5–10 µg/mL (29 and 27% of the wound closure, respectively) to the 20 µg/mL (4% of the wound closure), where a better effect was observed (42% less wound closure, compared to control). The effect of 5 and 10 MG/ML (55 and 38% of the wound closure, respectively) was maintained at 48 h, however, no significant decrease was observed at 20 µg/mL compared to control (82% decrease in wound closure) [Figure 4a and b]. Similarly, treatment with the methanolic extract decreased wound closure by 28% and 8% at 24 h compared to the control (46% decrease in wound closure) at concentrations of 10 and 20 µg/mL, respectively. This effect continued to be observed at 48 h; however, there was a better significant decrease at a concentration of

Table 2: Antiproliferative effect of organic extracts using the MTT assay

Extracts	IC_{50}±SD (µg/mL)					
	24 h			48 h		
	SiHa	HeLa	HaCaT	SiHa	HeLa	HaCaT
Hexane	NA	190.26±2.35	NA	NA	220.41±3.21	NA
Dichloromethane		174.21±3.02			217.41±2.95	
Acetone		178.28±3.12			176.55±3.31	
Methanol extracts		189.70±1.84			203.116±2.26	

Positive control: Paclitaxel (5 µg/mL). NA: Non-active (IC_{50} >250 µg/mL). SiHa: Human cervical cancer cell line (human papillomavirus-16); HeLa: Human cervical cancer cell line (human papillomavirus-18); HaCaT: Human immortalized keratinocyte cell line; IC_{50}: Half-maximal inhibitory concentration; SD: Standard deviation; MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay

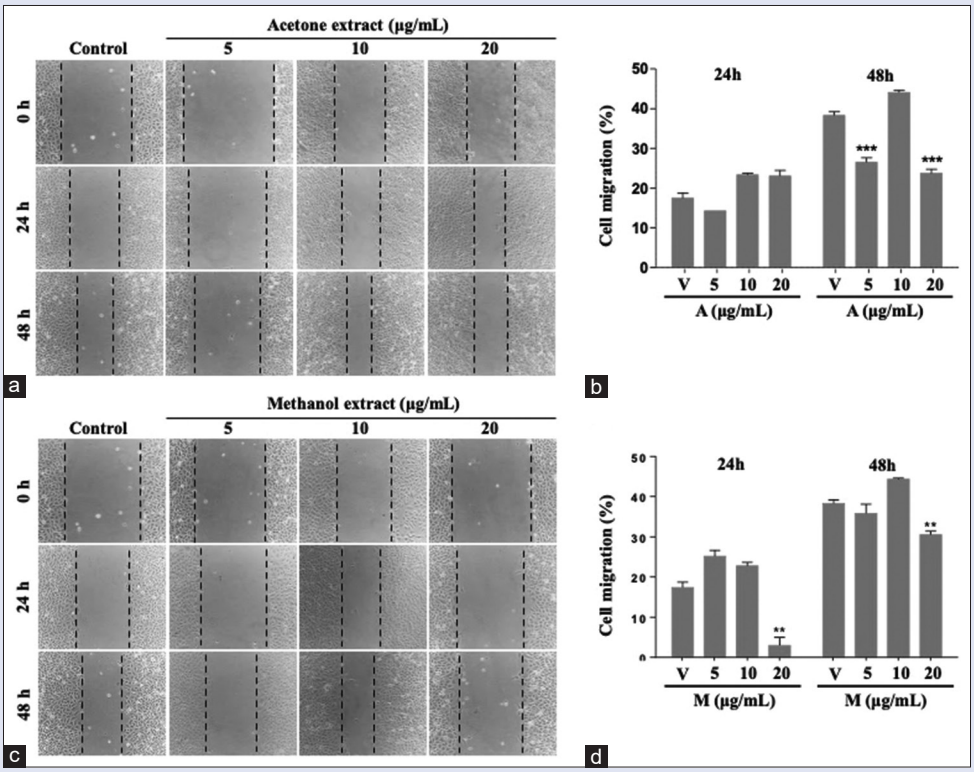

Figure 3: Effect of *Tagetes lucida* extracts on the migration capacity of human cervical cancer cell line (HPV-16) cells. Wound closure test. Negative control (fetal bovine serum 1%). Microscopic images of the effect of acetonic (a) and methanolic (c) extracts at × 10 magnification. Graphic representation of the migration percentage of cells treated with the acetonic (b) and methanolic (d) extracts. The mean ± standard deviation of three independent experiments expressed the values. We employed one-way analysis of variance and the Dunnett multiple comparison test; $^{**}P < 0.01$ and $^{***}P < 0.001$. *Differences with respect to the control treatment

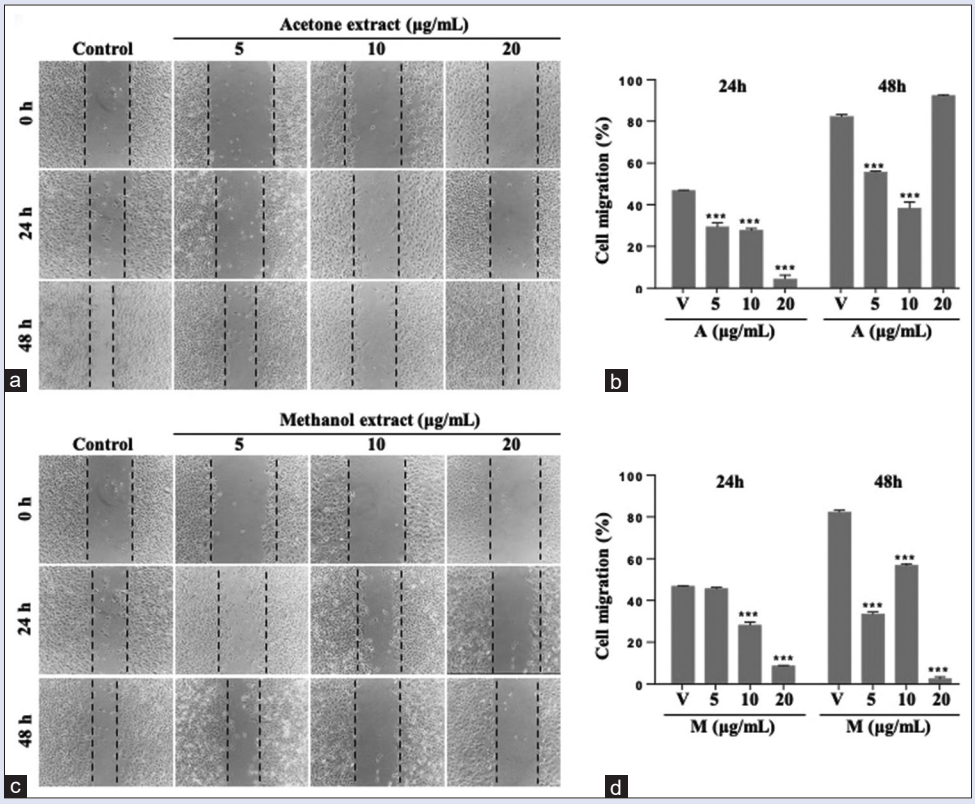

Figure 4: Effect of *Tagetes lucida* extracts on the migration capacity of human cervical cancer cell line (HPV-18) cells. Wound closure test. Negative control (fetal bovine serum 1%). Microscopic images of the effect of acetonic (a) and methanolic (c) extracts at × 10 magnification. Graphic representation of the migration percentage of cells treated with acetonic (b) and methanolic (d) extracts. The mean ± standard deviation of three independent experiments expressed the values. We employed one-way analysis of variance and the Dunnett multiple comparison test; ***$P < 0.001$. *Differences with respect to the control treatment

20 µg/mL (2% of the wound closure), compared to the control (82% decrease in wound closure). At this time, a decrease in cell migration capacity was observed at of 5 µg/mL (49% less wound closure) compared to the control [Figure 4c and d].The results suggest that the metabolites present in the acetonic and methanolic extracts decrease the migration capacity of SiHa and HeLa cells, showing a more evident effect on HeLa cells after 24 h and 48 h of treatment (<80% decrease in wound closure) even to low concentrations, while in SiHa cells, they decreased the cell migration capacity only after 24 and 48 h of treatment (<16% decrease in wound closure) only at the major concentration tested. The possible anti-migratory effect of the acetonic and methanolic extracts could be due to the presence of the coumarins herniarin and scoparone, the most abundant compounds identified in the acetonic and methanolic extracts, since it has been reported that treatment with these compounds in laryngeal cancer cells (RK33), decrease cell migration in a dose-dependent manner.[61]

In that metabolites derived from plants may alter the abnormal cellular signaling pathways that present in cancer, we need to understand the molecular mechanisms involved to identify new targets and to develop novel pharmacological intervention strategies that are effective. Thus, numerous studies have reported new findings of the role of metabolites in the elucidation of signaling pathways.[62-65] Therefore, this study contributes new knowledge for the exploration of the role of *T. lucida* leaf extracts and/or of the coumarins identified as key components in the elucidation of the mechanism of cellular signaling pathways on CCa.

CONCLUSION

The phytochemical study revealed the presence of high quantities of compounds 15 y 17 in all bioactive extracts of *T. lucida* leaves, the principal coumarins suspected of inhibiting cell proliferation and migration preferably towards CCa (HeLa cell line). This study contributes to the phytochemical and biological knowledge of *T. lucida* leaves and the research of native species of Mexico. Therefore, our findings provide a strong basis for the further exploration of *T. lucida*, justifying its potential use as an alternative or complementary therapy against CCa. Notwithstanding this, more studies are required to determine the components responsible for this biological activity, as well as the molecular and cellular mechanisms involved.

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Conflicts of interest

There are no conflicts of interest.

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