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Article

Antiproliferative activity of methanolic macerate from medicinal plants *Kalanchoe laetivirens* and *Tidestromia lanuginosa* against cancer lines HeLa (cervical cancer) and MCF-7 (breast cancer).

Actividad antiproliferativa del macerado metanólico de las plantas medicinales *Kalanchoe laetivirens* y *Tidestromia lanuginosa* contra las líneas cancerosas HeLa (cáncer cervicouterino) y MCF-7 (cáncer de mama).

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Abstract: Medicinal plants are those used in herbalism due to their healing properties. These plants contain secondary metabolites that can be used in the development of medicines; the phytochemicals present in these plants are commonly extracted by a maceration process, where the plant material gets in touch with the solvent. *Kalanchoe laetivirens* and *Tidestromia lanuginosa* are plants with medicinal properties found in Sonora, Mexico. In the present study, maceration with a polar solvent was carried out to extract bioactive compounds. The plants extracts were used to evaluate its antiproliferative activity over HeLa (cervical cancer) and MCF-7 (breast cancer) cancer lines. Results indicate that *K. laetivirens* has better antiproliferative properties against both lines compared to *T. lanuginose*; future research is suggested to know the active compounds of *K. laetivirens*.

Keywords: Plant extracts; biological properties; *Kalanchoe laetivirens*; *Tidestromia lanuginosa*.

Resumen: Las plantas medicinales son aquellas usadas en herbolaria debido a sus propiedades curativas. Estas plantas contienen metabolitos secundarios que pueden ser usados en el desarrollo de medicamentos; los fitoquímicos presentes en las plantan suelen extraerse comúnmente por un proceso de maceración al poner en contacto el material vegetal con el solvente. *Kalanchoe laetivirens* y *Tidestromia lanuginosa* son plantas con propiedades medicinales encontradas en la región de Sonora, México. En el presente estudio se realizó una maceración con solvente polar para extraer compuestos bioactivos. Este extracto vegetal se empleó para evaluar su actividad antiproliferativa en líneas cancerosas HeLa (cáncer cervicouterino) y MCF-7 (cáncer de mama). Se encontró que *K. laetivirens* posee mejores propiedades antiproliferativas ante ambas líneas en comparación con *T. lanuginosa*; se sugieren futuras investigaciones para conocer los compuestos activos de *K. laetivirens*.

Palabras Clave: Extractos vegetales; propiedades biológicas; *Kalanchoe laetivirens*; *Tidestromia lanuginosa.*

1. Introduction

Natural medicine has been present worldwide for several generations. Many current medicines are based on natural products and imitate bioactive components from plants [1].

Cancer is a disease that causes formation of tumors due to mutations at the molecular level. When uncontrolled cell division occurs, cancer cells can spread to all organs and tissues of the body. Worldwide, it is estimated that one in eight deaths is caused by cancer [2]. This disease develops slowly, so is often detected in advanced stages. Cervical cancer is the second most common cancer among women in the world, reporting about 288,000 deaths annually [3]. Breast cancer is even more worrisome since it causes about 684,996 deaths annually [4]. Treatments for this disease include surgery, chemotherapy, and radiotherapy; unfortunately, they hurt adjacent normal cells. To combat these secondary effects, new drugs are needed; they could be found in medicinal plants, for this propose, isolation and clinical tests are needed from plants [5].

Kalanchoe is a genus characterized by being herbs, suffrutices or succulent shrubs. They are widely used in gardening thanks to their easy cultivation, remarkable adaptation under water-restricted conditions, and vigorous clonal growth [6]. The most notable substances biosynthesized by the different species of this genus belong to flavonoid glycosides, a group of plant pigments, and bufadienolides, a group of cardioactive steroids with potential application in anticancer drugs [7].

Tidestromia belongs to *Amaranthaceae* family characterized by its chemical diversity, which includes betalains, flavonoids, phenolic acids, essential oils, sesquiterpenes, diterpenes, and triterpenes [8]. It is native from desert regions of the western and southwestern of United States and Mexico [9]. *Tidestromia lanuginosa* is the most abundant specie of the genus, according to Felger & Moser [10]. According to Seri pharmacopoeia, it is used by the Seri Indians of the Sonora region, Mexico, to treat headaches and foot pain [10].

The objective of this research was to investigate the antiproliferative activity of methanol extracts of *Kalanchoe laetivirens* and *Tidestromia lanuginosa* against HeLa (cervical cancer) and MCF-7 (breast cancer) cell lines.

2. Materials and Methods

2.1. Plant collection

The aerial parts of *Tidestromia lanuginosa* and *Kalanchoe laetivirens* were collected in September 2022 on Hermosillo, Sonora, at the coordinates 29°02'30"N 111°04'22"W and 29°03'28"N 110°58'17"W, respectively.

2.2. Preparation of extracts

Both plants were dry at room temperature, once dry they were liquefied to form a powder. 50 g of each specimen were placed in methanol in a 1:10 (w/v) ratio, left under constant agitation for 10 days at room temperature. At the end of the maceration period, the samples were filtered, subjected to a rotary evaporation process, and the remainder was dried at room temperature. The extract obtained was stored, refrigerated, and protected from light until use.

2.3. Cell line growth

HeLa and MCF-7 lines were grown in Dulbecco's Medium Eagle Modified (DMEM) supplemented with 5% heat-inactivated Fetal Bovine Serum (FBS). Cells were placed in an incubator with atmospheric conditions of 5% CO_2 and 37°C [11].

2.4. Antiproliferative activity evaluation

The standardized MTT assay [3-(4,5 dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] was used. Cells were placed in 96-well plate (100,000 cells per well). After 24 hours of incubation, aliquots of 50 μL of medium (DMEM 5% FBS) with concentrations of 200, 100, 50, 25, 12.5 and 6.25 μg/mL in DMSO were added. Cells with DMSO were used as control. Cells were incubated for 48 hours.

After 44 hours, wells were washed with phosphate buffered saline (PBS) and 10 μL of MTT solution (5 mg/mL) was added in a 1:10 dilution with DMEM 5% FBS. At the end of 48 hours, the formazan crystals were dissolved with 100 μL of acidic isopropanol. The absorbance was measured in a microplate reader (Thermo Fisher Scientific Inc. Multiskan GO, Waltham, MA, USA) at the wavelengths of 570 and 630 nm. The experiments were performed in triplicate. The results are expressed in IC50 values. Analysis was performed using GraphPad Prism 5 software, GraphPad Software, Inc., CA, USA [11].

3. Results

3.1. Extract yield

The extracts were obtained by maceration giving 2.9% and 6.26% yield of *Kalanchoe laetivirens* and *Tidestromia lanuginose*, respectively.

3.2. Antiproliferative activity evaluation

Antiproliferative activity of methanol extracts was evaluated *in vitro* using HeLa and MCF-7 lines. Table 1 shows IC_{50} values for evaluated extracts.

Table 1. Antiproliferative activity (IC_{50}) of methanol extracts

Methanol extracts	IC_{50} (μg/mL)	
	MCF-7	HeLa
Kalanchoe laetivirens	40.84 ± 1.83	8.394 ± 1.97
Tidestromia lanuginosa	>400	>400

K. laetivirens methanolic extract IC_{50} demonstrated antiproliferative activity on HeLa (cervical cancer) and MCF-7 (breast adenocarcinoma) cell lines. *T. lanuginosa* did not demonstrate antiproliferative activity since IC_{50} values are greater than 400 μg/mL. According to "National Cancer Institute of the United States of America" an extract is considered to have high activity if IC_{50} is ≤ 30 μg/mL, medium if it is between 31 to 60 μg/mL, and low if it is 61 to 99 μg/mL [12]. *K. leativirens* is considered to have high activity against HeLa cells and medium activity against MCF-7 cells. *T. lanuginosa* is not useful for this property.

4. Discussion

4.1. Extract yield

This yield was reached because plant material was pulverized in order to reduce the particle size and increase contact area between plant sample and solvent [13]. Maceration is intended to soften and break the cell wall of plant to release its phytochemicals. The process took place in 3 steps. The first is penetration of solvent into plant matrix to reach the phytochemicals; the second is dissolution of phytochemicals from the matrix into the organic phase; and the third step is diffusion of solvent with the phytochemical out of the matrix [14–15].

4.2. Antiproliferative activity

Tidestromia lanuginosa belongs to *Amaranthus* family capable of producing alkaloids, flavonoids, saponins, tannins. In addition, genus *Tidestromia* is capable of producing sesquiterpenoids [6,13].

However, since antiproliferative activity is absent at the concentrations analyzed, is inferred that this plant is not capable of producing metabolites with this biological property.

Kalanchoe is a genus capable of producing alkaloids, sterols, terpenes, flavonoids, tannins, phenolic acids, bufadienolides, stigmasterol, some of them with antiproliferative activity [14]. Phytochemicals present in *Kalanchoe* cause cell death by inducing necrosis by TRAIL (TNFSF10) gene expression. TRAIL triggers RIPK1-dependent (receptor-interacting serine/threonine kinase 1) necrosis [15]. In addition, reactive oxygen species (ROS) are generated. Excess production of superoxide radicals and oxidative stress occur due to an inefficient cellular antioxidant system, causing mitochondrial membrane destruction and damage to lipids, proteins, and nucleic acids in cells. ROS at high concentration prevent cancer cell proliferation and arrest cell cycle in S and G2/M phases [16–17].

Another possible mechanism is inhibition of Na^+/K^+-ATPase, responsible for creating and maintaining Na^+ and K^+ gradients in cell membrane and activating kinases responsible for survival, proliferation, adhesion, and migration cellular by decreasing the expression of subunits $\alpha 1$ and $\alpha 3$ [18–19].

K. laetivirens contains phytochemicals capable of preventing cell proliferation as verified by the IC_{50} values.

5. Conclusions

Medicinal plants are an alternative for obtaining phytochemicals with therapeutic properties. The extraction procedure of bioactive agents is simple as they can be extracted by a solid-liquid extraction such as maceration. *Tidestromia lanuginosa* did not show antiproliferative activity against cell lines studied. *Kalanchoe laetivirens* was able to inhibit cell proliferation of HeLa and MCF-7 lines, so is necessary to carry out more studies to identify the phytochemicals present on it.

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Conflicts of Interest: The authors declare no conflict of interest.

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