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Cytotoxic Activity of Four Mexican Medicinal Plants

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ABSTRACT

Ibervillea sonorae Greene, *Cucurbita ficifolia* Bouché, *Tagetes lucida* Cav and *Justicia spicigera* Scheltdd are Mexican native plants used in the treatment of different illnesses. The ethanolic extract of *J. spicigera* and *T. lucida* as well as aqueous extracts from *I. sonorae*, *C. ficifolia*, *T. lucida* and *J. spicigera* were investigated using sulforhodamine B assay. These extracts were assessed using two cell line: T47D (Human Breast cancer) and HeLa (Human cervix cancer). Colchicine was used as the positive control. Data are presented as the dose that inhibited 50% control growth (ED_{50}). All of the assessed extracts were cytotoxic (ED_{50} < 20µg/ml) against T47D cell line, meanwhile only the aqueous extract from *T. lucida* and the ethanolic extract from *J. spicigera* were cytotoxic to HeLa cell line. Ethanolic extract from *J. spicigera* presented the best cytotoxic effect. The cytotoxic activity of *J.* spicigera correlated with one of the popular uses, the treatment of cancer.

INTRODUCTION

Plant based treatments continue to play an essential role in primary health care, and it has been estimated by the World Health Organization that almost 65% of the world's inhabitants rely mainly of traditional medicines for their primary health care [1]. In Mexico, about 25% of the population still depends on the use of medicinal plants [2]. Mexico has a great heritage from pre-Hispanic Indian cultures and proof of this is the Aztec manuscript "Códice De la Cruz- Badiano" written in 1552 which contains 251 plants descriptions and their medical uses [3].

A renewed interest in the systematic study of indigenous medicines and associated medicinal plant arose in the 1970s, and in 1975 the first medicinal herbarium of Mexico was created (IMSSM herbarium), which preserves 11,000 vouchers of medicinal plants collected around the country. The taxonomical studies of this collection showed that Mexican medicinal plants are known by 10,000 synonyms in 52 Indian languages and Spanish. These studies allowed investigators to conclude the existence of a "basic group" of 1,000 plants used in Mexican traditional medicine throughout almost all the country for almost 400 years [4].

According to a recent inventory carried out by Instituto Nacional Indigenista (INI), there are more than 3,015 medicinal plants species commonly used for treatment of common diseases in Mexico [5]. However, the most of these species have not been subjected to chemical, toxicological, pharmaceutical or chemical investigation.

For the present study, we selected four Mexican medicinal plants. *T. lucida* is an herbaceous, perennial and endemic plant from Mexico to Central America. It has been used since Aztec times for religious and medicinal purposes. Today, it is a popular herb in the Southern States of the United States since it can be a substitute for the well known European tarragon [6].

C. ficifolia is an annual climbing plant native to the Americas, although the exact center of domestication until now is unclear. Linguistic evidence suggests Mexico, because of the wide use of Nahuatl- derived name as far south as Argentina, while archaeological evidence suggests Peru because the earliest remains have been found there [7]. In Mexico it is consumed as food as well as used as a traditional remedy [8].

J. spicigera is a shrub which is native to Mexico [9], and it is used since prehispanic time to treat scabies as documented by Francisco Hernández. [10]. This plant is still used to treat various illnesses [11].

I. sonorae is a perennial dioecious plant native of Northwestern Mexico. For centuries, its roots have been used as a medicinal plant [12]. Today it is still used as such.

We selected these plants to evaluate their cytotoxic activity because they share some common medicinal uses, and two of them are used to treat cancer. A chart of these medicinal plants (Table 1) has been prepared based on a bibliographic review.

MATERIALS AND METHODS

Plant material. *J. spicigera,* and *T. lucida* were acquired from the "Mercado de Sonora" in Mexico City (a public market), and they were identified in the herbarium at the Universidad Autónoma Metropolitana-Iztapalapa by Dr. Adolfo Espejo

Serna. The vouchers are kept with the following numbers: UAMIZ-52718 (*T. lucida*) and UAMIZ 65465 (*J.spicigera*).

C. ficifolia was collected in Acolman, State of Mexico, and *I. Sonorae* was obtained from the "Mercado de Sonora". They were identified in the herbarium of Instituto Mexicano del Seguro Social by Dr. Santiago Xolalpa Molina. Their voucher specimen references are IMSSM-11119 (*C. ficifolia*) and IMSSM-14184 (*I. sonorae*).

Cancer Cell Lines. Breast cancer T47D and cervix cancer HeLa were obtained from the American Type Culture Collection (ATCC, Rockville, Maryland, USA).

Preparation of Extracts. The aerial parts from *J. spicigera* and *T. lucida* were dried at room temperature, protected from dust and sunlight. They were ground using a mixer, and then 500 g from each plant were macerated separately at room temperature using hexane, dichloromethane, ethyl acetate, ethanol and water for 3 days. The extracts were filtered and the organic solvents were evaporated to dryness under reduced pressure in a Savant speed Vac Plus SC210A (Farmigdale USA). The water from aqueous extracts was eliminated by evaporation using a bath water.

Decoctions from *J. spicigera* and *T. lucida* were obtained by refluxing the ground dried plant in water for 30 minutes. Each liquid was filtrated and the water was eliminated. Mature fruits of *C. ficifolia* were cut in halves. Fiber and the seeds were removed and the pulp was tried in the same way as the two previous plants. To obtain organic and aqueous extracts from *I. sonorae*, the ground dried roots were processed in the same way as *J. spicigera.*

Evaluation of Cytotoxic Activity. T47D (human breast cancer) and HeLa (human cervix cancer) were grown in RMPI 1640 medium supplemented with 2 mM L-glutamine (In Vitro, Mexico), 10% heat inactivated fetal calf serum (GIBCO) and antibiotics; mixture of streptomycin (50 µg/ml) and penicillin (50UI/ml) (In Vitro, Mexico). Both cancer cells lines were cultured at 37°C in an atmosphere of 5% CO_2/95% air (100 % humidity). At 70-80% confluence cells were detached from the cultured flask by treatment with 0.05% trypsin-EDTA (GIBCO). The cells were counted and their viability determined using trypan-blue (Sigma) exclusion dye on a hemocytometer (Boecco, Germany). After counting, dilutions were made to obtain the required cell density. In each case, 96 well tissue culture plates were used.

One hundred µL of cells suspension (T47D = 1.0 x 10^5 cel/mL; HeLa = 5.0 x 10^4 cel/mL) was added to 96-well plate and preincubated for 24 hours at 37°C under an atmosphere of 5% CO_2/95% air (100% humidity) incubator to allow stabilization prior the addition of the samples. After this, the cells were treated with 100 µL of four different concentrations (for triplicate) of extract dissolved in dimethyl sulfoxide (Sigma, Mexico) (DMSO) to final concentration of 0.5% in the culture medium and incubated for 48 hours and fixed by addition 50 µL/well of cold 50% (w/v) trichloroacetic acid –TCA- final concentration of 10% in the culture medium and incubated for 1 hr at 4ºC. The supernatant was discarded, and the plates were washed five times with tap water and air dried. The cells were stained with 50 µL/well of sulforhodamine B –SRB- (Sigma, USA) solution (0.4%, w/v, in acetic acid) for 30 min at room temperature. Unbound SRB was removed by washing five times with 1 % acetic acid and the plates were air-dried; bound SRB stain was solubilized with 200 µL of 10mM unbuffered Tris base (Sigma, Mexico), pH 10, and the absorbance was read on a spectrophotometric plate reader at 490 nm (BIO-RAD model 450,USA). A measure was made of the cell population density at time zero (the time at which the sample was added) from reference plate of cells fixed with TCA just priori to sample addition and processed as described above [18]. The absorption values obtained with each of the treatment procedures were averaged, and the average value obtained with zero-day control subtracted. Cell survival was obtained as (T/C) x 100 where T and C represent the mean absorbance cell treated with sample and vehicle control (DMSO), respectively. Results are expressed as the dose that inhibits 50% control growth after the incubation period (ED_{50}).The ED_{50} values were calculated by regression analysis (percent survival versus log concentration). Colchicine (Sigma) was used as positive control. According to the standards of the National Cancer Institute (NCI), ED_{50} values of ≤20 µg/mL for extracts and ≤4 µg/ml for pure compounds are considered active [19].

RESULTS AND DISCUSSION

For the analysis of cytotoxic activity, cells were treated for 48 hr with aqueous and ethanol extracts or decoctions from *J. spicigera*, *T. lucida*, *I. sonorae* and *C. ficifolia* in a concentration range of (50 – 0.4 µg/mL). Half-maximal inhibitory concentrations (ED_{50} values) are summarized in Table 2.

The cytotoxic activities of the extracts were different in the two cancer cell lines employed and all the extracts assessed shown cytotoxic activity on T47D cells (Table 2). The best cytotoxic activity was observed with ethanol extract from *J. spicigera* (ED_{50} = 0.43 µg/mL) and *T. lucida* (ED_{50}= 1.82 µg/mL). Both extracts were more cytotoxic than colchine (ED_{50}= 2.89 µg/mL), compound pure with antitumor activity [1] that was used as cytotoxic reference.

On the other hand, HeLa cell line was less sensitive than T47D cells and the best cytotoxic activity on the first cancer cell line (ED_{50} = 0.341 µg/mL) was presented by colchicine. Both the ethanol extract from *J. spicigera* (ED_{50} = 5.59 µg/mL) and aqueous extracts from *T. lucida* (ED_{50} = 13.2 µg/mL) were cytotoxic.

The aqueous and decoction extracts from *J. spicigera* showed similar activity in both cancer cell lines because both extracts have been obtained with the same solvent and both preparations have related secondary metabolites and similar biological activity.

Table 1. Ethnomedical information of uses of medicinal plants collected for cytotoxic screening.

Family & Botanical Name	Synonym	Common Names (Spanish/ English)	Part Used	Mode of Prep.	Ethno-medicinal Uses	Ref.
Acanthaceae *Justicia Spicigera* Schult.	*Jacobina spicigera* Schult.	Muitle, muicle Mexican honeysuckle	Aerial part	Decoction	Kidney infection Stimulant Dysenteric Menstruation Uterine cancer Diabetes	[9,10] [11,13] [14,15]
Asteraceae *Tagetes lucida* Cav.	*T. florida* Sweet *T. schiedeana* Less.	Pericón, Marigold	Aerial part	Decoction	Diarrhea Dysentery Rheumatism Vomiting Asthma Varicose veins Colds	[6,11] [13] [14]
Cucurbit-aceae *Cucurbita ficifolia* Bouché		Chilacayote, Figleaf gourd	Mature fruit	Juice	Wounds Hemorrhoids Fever Diabetes	[7,8] [13]
Cucurb-iataceae *Ibervillea sonorae* Greene	*Maxim-owiczia sonorae* S. Wats	Wareke	Root	Decoction	Skin ailment Wound treatment Diabetes mellitus Rheumatism Antiinflammatory Analgesic Hypoglycemic Cancer healing	[12] [13] [16] [17]

Table 2. Cytotoxicity activity of four Mexican medicinal plants in human cancer cell lines T 47D[1] and HeLa[2].

Preparation	Plant	TD_{50} [1]T47D (µg/ml)	TD_{50} [2]Hela (µg/ml)
Ethanol	*J. spicigera*	0.43	5.59
Aqueous	*J spicigera*	14.79	>50
Decoction	*J. spicigera*	13.54	>50
Ethanol	*T. lucida*	1.82	>50
Aqueous	*T. lucida*	18.94	13.2
Aqueous	*I. sonorae*	4.23	>50
Aqueous	*C. ficifolia*	8.85	>50
Colchicine[3]	C. autumnale	2.89	0.341

[1]*Human breast cancer,* [2] *Human cervix cancer,* [3]*Positive control.*

Other studies performed with aqueous extract from *J. spicigera* were shown to have cytotoxic effects on leukaemic cells and have no proliferative activity on normal haematopoietic progenitor's cells. Research found that this plant extract induced apoptosis in the human leukemia cell line TF-1 but not in the bcl-2 transfectant cell line TB-1. Additional experiments with this extract had cytotoxic effects on mouse fibroblasts (3T3) and two cervical carcinoma cells line CALO and INBL. The cell presented apoptotic bodies which indicate that the main cause of death was apoptosis [22]. These studies support our experimental evidence that *J. spicigera* has cytotoxic activity and the death cell could be by apoptosis.

Previous phytochemical determination in ethanol, aqueous and decoction extracts from *J. spicigera* done in our laboratory showed flavones (not published data) compounds. Kaempherol-3,7- bisrhamnoside (kaempferitrin) is a flavone that has been previously isolated from ethanol extract obtained of *J. spicigera* leaves [9]. Kaempferitrin (II) is other flavone isolated from soluble methanol fraction of hexane-ether-methanol (1:1:1) *J. spicigera* extract [21].

Other *Justicia* species are used in various countries as medicinal plants. One of them is *J. procumbes* L., known as "Chu Wei Hung" or "Jué Chuáng" in Chinese folklore that is used for the treatment of fever, pain due pharyngo-latyngeal swelling and cancer. From this plant have been isolated two 2, 3-naphtalide lignans identified as justicidin A, diphellins with potent cytotoxicity on KB cells (human nasopharyngeal cancer) with ED_{50} < 1.0 µg/mL [22].

Isolation of a 1-aryl-2,3-napthalide lignans (justicidin B) from ethanol extract of J. pectoralis showed cytotoxicity on 9PS (murine leukemia) cancer cell line (ED_{50} = 3.3 µg/ml), 9KB (ED_{50}= 7.3 x 10^{-2} µg/mL) but was inactive (ED_{50} = 28 µg/mL)on NSCLCN6 (bronchial epidermoid carcinoma cell line of human origen) [23].

The methanol extract of the aerial parts of *J. gracifolia* (Standt.) D Gibson showed potato disc inhibition (54.6%), and a significant DNA peak reduction (30.7%) [24]. These reports support the cytotoxic role of some species plants belonging to *Justicia* genus [22-24] and the chemical analysis suggests a lignans compounds to exhibit the cytotoxic activity [22, 23].

In Mexico, *T. lucida* is used medicinally as an infusion, and also as an insecticide [25]. In Guatemala extracts of this plant are sold as infusion, tincture and elixir. These products are used for stomach and menstrual pains, gastritis to treat infections and diarrhoea [26]. From methanol extract of the whole plant of *T. lucida* were isolated seven coumarins and three flavonoids, where two dihydroxylated coumarinas isolated presented antibacterial activity [6]. In the scientific literature there are not previous cytotoxic reports of *T. lucida* and the

present work shows the first scientific report about this important biological activity.

The bitter aqueous infusion of the aerial tuber (also referred to a root or caudex) of *I. sonorae* is used in Mexican traditional medicine. The aqueous extract from the root was cytotoxic on T47D (ED_{50}= 4.25 µg/mL). From the polar fraction of the hexane:diisopropyl eter:methanol (1:1:1) extract of the air dried tubers of *I. sonoraea* were isolated three cucurbitane type glycosides that represented about 12% of the total extract. The enzymatic cleavage of these compounds generated aglycones know as Kinoin A, B and C [27]. Kinoin C is a member of the 22,23,24,25,26,27-hexanocucurbitacin. The natural cucurbitacins constitute a group of triterpenoid substances which are well known for their bitterness and toxicity [28]. Cucurbitacins are noted for their cytotoxic behavior [29] and were a hot topic within the medicinal chemistry and drug discovery community from an anti-cancer drug development perspective, particularly in the 1960's. Besides cytotoxicity and anti-cancer activity, cucurbitacins also exhibited further wide-ranging *in vitro* or even *in vivo* pharmacological effects, such as purgative, anti-inflammatory and anti-fertility activities [28]. This is the first report on *I. sonorae* cytotoxic activity and it is possible that cucurbitacin compounds in the plant could possibly be responsible and explain cytotoxic properties of the plant and explain one of the ethnopharmacological uses, to be cancer-healing [17].

The aqueous extract from *C. ficifolia* was cytotoxic on TD47 cancer cell line (ED_{50}=8.85µg/ml). In previous reports it showed the toxicity effects (LD_{50}=625.0 mg/Kg) of the freeze-dried juice of *C. ficifolia* on mice [8]. The chemical investigation of the Cucurbitacea family has shown to cucubitanes compounds as active agents of toxicity and could possibly explain the cytotoxic and toxic effects of *C. ficifolia*.

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