Article

Antiproliferative activity of Cucurbitaceae species extracts from southeast of Mexico

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Abstract

There are many species of endemic plants from Mexico, without food or commercial use, but with different applications in traditional medicine and valuable for their content of secondary metabolites. In this sense, we found two species of Cucurbitacea family plants natives of southeast and gulf of México, with traditionally use how soap and laundry agent, control of some pests, and it has also been used how infusion for the treatment of different types of dermatitis and stomachache. In the present work, we evaluate the antiproliferative activity in vitro, of six crude organic extracts, tested against six human tumor cell lines, A549 (lung), HBL-100 (breast), HeLa (cervix), SW1573 (lung), T-47D (breast) and WiDr (colon), the results indicated that at least three extracts from both species presents an interesting antiproliferative activity on five tumor cell lines.

Key words: Cucurbitaceae, cucurbitacines, triterpenic saponines, cell cancer lines, antiproliferative activities.

1. Introduction

 doi:10.20944/preprints201908.0127.v1

Plants have been since the beginning of the civilization source of almost all the therapeutic principles known today. Nowadays, plants are still being used empirically to mitigate and cure various disease conditions in many developing countries. In Mexico, the prehispanic knowledge of medicinal properties from several plants species has been verbally transmitted from generation to generation and this information is useful for the monitoring and prospecting of several species with potentially bioactive principles, such as some compounds currently used in cancer chemotherapy [1]. In this sense, a wide biodiversity of plants exists in the southeast of Mexico and many uses of them are reported in the ancestral pharmacopoeia[2-3].

The Cucurbitaceae family of plants, have 120 genera and approximately 825 species, which are widely distributed in tropical and temperate regions [4]. Many species of the Cucurbitaceae family are used as human food [5-6]. The majority of the species in this family are annual lianas or shrubs and their most representative genera are Cucurbita, Luffa, Citrullus, and Cucumis. Additionally, about 130 wild no commercial species of Cucurbitacea family plants are present in Mexico [4,7-8]. However, few of these have chemical or biological studies. In this context, we documented in the cloudy forest ecosystem from Veracruz, two species of Cucurbitacea, Microsechium hellerii (Pyer) Cong, and Cucurbita okeechobeensis martinezii Bailey, which are wild plants, without comestible uses. These plants are found also in rural roads and are able to colonize successfully disturbed sites.

Natives living nearby sites of cloudy forests used M. helleri roots (amolli or chichicamolli in Nahuatl) as a soap substitute [4], and more recently use aqueous root infusion as food detractor on seed pests after planting since their roots are very bitter.

Chemical studies on methanolic crude extract from M. helleri roots established two saponins, named amoles F and G, as oleanane-type triterpene with five to seven monosaccharide moieties [9-10]. In the same region, fruits from C. okeechobeensis martinezii (morchete or calabacilla loca) are traditionally used for some dermatitis and control of hematophage pests in animals such as fleas [11]. Furthermore, decoction from fruit leaves and stem have been used for some stomach upsets and diarrhea [6, 12]. At present, no phytochemical studies had been carried out with this species.

At respect, saponins like cucurbitane [13], dammarane and oleanane type glycosides [9]) are tetracyclic triterpenes compounds more abundant in Cucurbitaceae family.

In Cucurbita genus, more specifically are cucurbitane type skeleton named cucurbitacines, characterized by a 19-(10-9-b) abeo-10-α-lanost-5-ene. There are 12 main categories to group cucurbitacins based on their differently side-chain (A to T), joined to one or more monosacharid moieties, commonly four to seven units of rhamnose, arabinose, xylose or glucose [14]. The cucurbitacines are of interest because of the wide range of biological and pharmacological properties such as anti-inflammatory, anti-ulcerogenic, analgesic [15], antiallergic [13], antitumor [16], antioxidant [17], hepatoprotective and fungicide effects too [18], also as attractors o repelents for some herbivores [14,19-20]. To respect some cucurbitacins showed inhibitory effects on cancer pathways signaling, such as JAK2/STAT3 pathway [21-22], Cdc2 cyclins, COX-2, Wnt, PI3K/Akt, and others MAP-kinases signaling pathways, and likewise actin cytoskeleton appears to be an early target [23-27].

Also, cucurbitacin B isolated from Luffa cylindrica (smooth luffa), showed apoptosis in several human cancer cell lines through caspase-3 and caspase-9 activity [28-29], observing proliferation inhibition against breast cancer cells in a dose-dependent form [30].

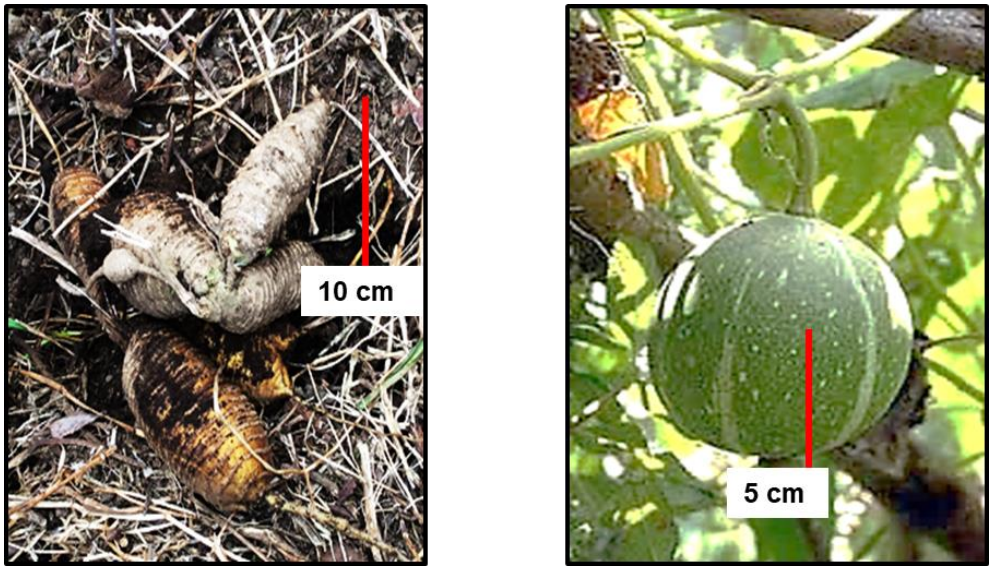
About this disease condition, several kinds of cancer, have gone from number 9 to 2 as cause of worldwide mortality from 2012 to 2017; and the same way, number 6 to 3 place in Mexico [31-32], and other developing countries, which means that there is an annual increase in the rates of this condition as well as the need to treat it and prevent it.

In Mexico, there is a list of species that do not present food, or commercial use, however they are valuable for their secondary metabolites content, likewise, there are many with traditional use. Therefore, we decided to test the antiproliferative activity of extracts on some cancer cell lines, of extracts obtained from two species of the Cucurbitaceae founded in middle Veracruz state.

2. Results.

In the field surveys, near to Cofre de Perote mountain, traditional knowledge shared with us the use of two species of wild cucurbitaceae without food use and with use in folkloric medicine, both herbaceous and crawling or climbing species, and were found in some wicked places, or on slopes in a cloudy forest habitat. The species were identified and a complete specimen was herborized and deposited, at Biology Herbarium from Universidad Veracruzana (N° 24100), and INECOL-MX herbarium

(XAL0147669) respectively. **the extracts obtained from aerial part and fruits yielded a 0.5 to 0.8 % from dry vegetal tissues.**



(a) **(b)**

Figure 1. a) Tubercle root of Microsechium helleri Pyer y -cognd, collected near Cofre de Perote mountain b) Fruit of Cucurbita okeechobeensis martinezii Bailey collected near Coatepec municipality.

The *in vitro* antiproliferative activity against six representative human solid tumor cell lines, was evaluated for crude extracts. As shown in table 1, ethyl acetate extracts from root of *M. helleri*, and fruit of *C. okeechobeensis martinezii*, revealed a remarkable activity, $GI_{50} \leq 2.5$ μg-mL against five of six cell tumor lines.The methanolic and ethyl acetate extracts of the leaves form m helleri, not show antiproliferative activity (≥30 μg-mL) and methanolic extract form fruit of c ok was active against three lines (11-16 μg-mL).

Table 1. GI_{50} values of antiproliferative activity (GI_{50} in $\mu g.mL^{-1}$) against human solid tumor cell lines organic extracts from two plant species.

Specie	Sample (Solvent)	Cell line					
		A549 (lung)	HBL-100 (breast)	HeLa (cervix)	SW157 3 (lung)	T-47D (breast)	WiDr (colon)
Microsechium hellerii root	AR (Ethyl Acetate)	<2.5	7.7	<2.5	<2.5	<2.5	<2.5
Microsechium hellerii root	MR (Methanol)	3.9	9.8	14	5.5	3.7	4.2
Microsechium hellerii leaves	AA (Ethyl Acetate)	55	59	88	31	125	125
Microsechium hellerii leaves	MA (Methanol)	55	64	66	47	80	96
Curcubita okechobeensis martinezii fruit	CM (Methanol)	16	221	11	12	54	44
Curcubita okechobeensis martinezii fruit	CA (Ethyl Acetate)	<2.5	7.9	<2.5	<2.5	<2.5	<2.5

3. Discussion

M. hellerii and *C.okeechobeensis martinezii* extracts showed antiproliferative activity against six cell tumoral lines, but in different extents of inhibition. GI_{50} values less than 2.5 µg.mL-1 were ethyl acetate extracts from root of *M. hellerii* for five cell lines. Breast cancer (HBL-100) line cell does result less sensitive. *M. hellerii* root methanolic extract exhibit antiproliferative effect too, the leaves of this specie do not show cell proliferation inhibition. Therefore, the content of bioactive compounds against tumor cells is higher in the acetate extract of root of *M. hellerii* than in its leaves. In other work, two saponins named amole F and G were isolated and identified from root methanolic extract of this specie as well as nine oleanane-type saponins, and were evaluated for their antifeedant, nematicidal and phytotoxic activities [10]; the structures of these compounds were established as bayogenin and polygalacic glycosides (D-glucopyranosyl, L-rhamnopyranosyl, D-xylopyranosyl, L-arabinopyranosides) [9-10]. Regarding the effect of the solvent, can say that of ethyl

acetate polarity is more efficient extracting bioactive compounds, for both cucurbitaceae species, since the extract obtained with ethyl acetate from the fruit of *C. okeechobensis martinezii*, also presented less than 2.5 µg.mL-1 in GI_{50} in five of the six cell lines evaluated, and methanolic doesn't have good antiproliferative activities, but this specie is promising candidate for chemical and pharmacological studies.

Even though there are many wild species of Cucurbitaceae in Mexico and some of them has been recognized traditionally for centuries as diverse medicinal uses, antiparasitic, soap substitute or insecticide [6], most do not have chemical or pharmacological studies. Others Cucurbitaceae species over the world, they cover as laxant, emetic, antipyretic, antidiabetic, antioxidant, anticarcinogenic, anti-inflammatory, and for treatment of malaria and dysenteries among others, and have been documented, and mostly verbally transmitted nowadays, especially in countries where access to medical services is expensive. To respect many herbal preparations contains compounds which can act of synergistic mode [33]. In many cases, different illness is treated with two or more species to obtain benefic results. Synergy may act to protection of the bioactive substance from degradation by enzymes, or facilitate transport across membrane barriers and organelle walls, it may be overcome drug resistance mechanisms, providing signals to the hosts cells, resulting in higher efficacy of the herbal preparation when compared it with its components alone [34].

About pharmacological evidence over major compounds isolated from some species of Cucurbitaceae family, such cucurbitacines they have demostrated their anti-ulcerogenic, analgesic, antiinflamatory, antiallergic and antitumor activities [20, 14, 35, 27]. Cucurbitacines are concentrated in roots and fruits, in most of cases, and to a lesser extent in stems and leaves; however, they have also been founded in other plant families, in some fungi and even in some marine mollusks. For more than a decade work on the anti-tumor properties of cucurbitacin pure compounds, has been reopened, and also and its differential toxicity to the cell lines of renal, brain, and melanoma tumors, its inhibition of cell adhesion and as already mentioned above, can act in different targets of cancer signaling pathways, which play important roles in the apoptosis and survival of cancer cells [36]. Among these Cucurbitacin B, D, E and I (figure 3), exerts strong anticancer activities meanwhile other type of cucurbitacin have modest anticancer activities [14, 35, 37].

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 11 August 2019 doi:10.20944/preprints201908.0127.v1

M. helleri extract root´s, or it's compounds have not yet been reported with antiproliferative or cytotoxic activities, in this work the methanolic extract showed antiproliferative activity too, but in more concentration. The compounds isolated and reported from these extracts, was several glycosides of bayogenin and polygalacic acid, see figure 4, [9-10], which differ from the cucurbitacines in the number and type of fused carbon cycles in their skeleton molecule, however it's not ruled out, that these pentacyclic oleanane triterpenes, could have antiproliferative activity in cancer cell lines, or maybe the bioactive compounds will be different sapogenins due to polarity range of dissolvent used for extraction, so we need to continue with the work of molecular elucidation with the bioactive extracts.

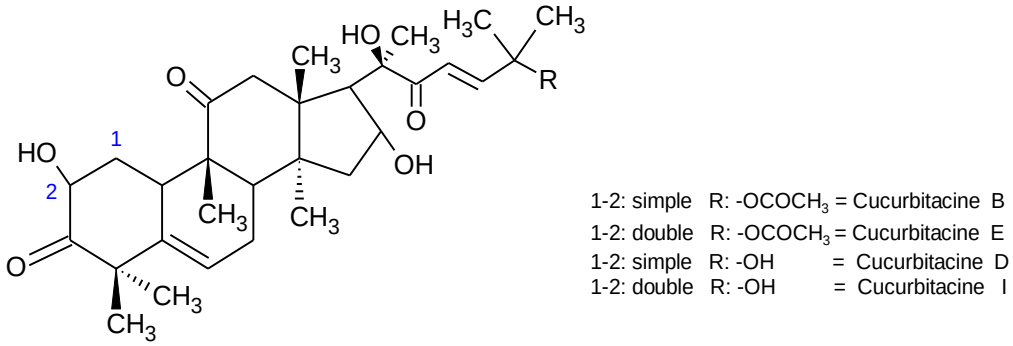


Figure 3. Structure of some bioactive cucurbitacines [35].

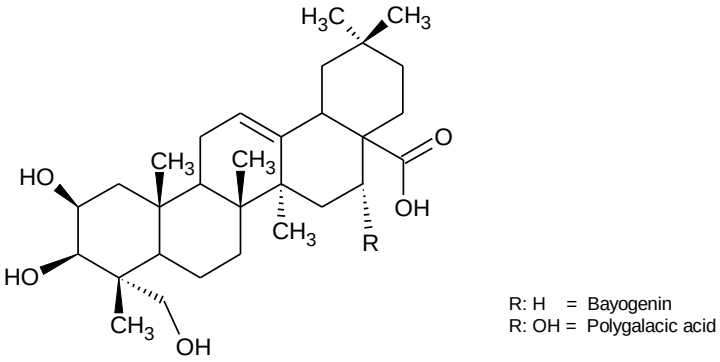


Figure 4. Triterpenes (sapogenins) isolated from *M. helleri* [9-10]

The other Cucurbitaceae specie reported here, there aren't, chemical studies, and it's interesting too, due to its ethnical uses and their antiproliferative bioactivity. To respect, many species of *Cucurbita* genus, have been the subject of chemical studies, showed many biological and pharmacological activities, and we hope, with such background, to find compounds in the extract of *C. okeechobeensis martinezii* that resulted bioactive and specifically antiproliferative active.

 doi:10.20944/preprints201908.0127.v1

193 Therefore we will continue, with the search for compounds with possible
194 antiproliferative activity of the ethyl acetate extracts from M. helleri roots and C.
195 okeechobeensis martinezii fruits, and the sustainable use of unexplored flora like
196 this. Due to the multiple biological and pharmacological properties exhibited by
197 principal secondary metabolites from Cucurbita species, multidisciplinary
198 research is required for seeking and bioprospection of potential molecules that can
199 mitigate some degenerative diseases, and helps us to generate scientifically
200 validated data regarding the effectiveness of endemic plants and their biologically
201 active metabolites contents, also to support the alternative use of different herbal or
202 semi-herbal therapies against this degenerative malignancy.

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 doi:10.20944/preprints201908.0127.v1

4. Materials and Methods

4.1 Plant material

The roots and fruits of *M. helleri* and *C. okeechobeensis martinezii*, were identified and collected in region of Coxmatla and Teocelo localities, in the center of Veracruz State, Mexico. A voucher specimen of each, were deposit at Biology Herbarium from Universidad Veracruzana (N° 24100), and INECOL-MX herbarium (XAL0147669) respectively.

4.2 Obtaining extracts

The roots and aerial part (leaves and stem) of M. helleri, and fruits of C. okeechobeensis martinezii were cleaned, grounded, and dried to 40°C for 96 hours (separately) in a laboratory oven, the samples were ground to obtain a smaller particle size, weighted and extracted by 120 hours on maceration, with ethyl acetate (Sigma reactive grade), to room temperature, in amber glass container. The dissolvent were eliminated by reduced pressure and extracts obtained were weight. Other dry samples of roots, leaf and fruits were oven dried, and extracted at same way and time, now using methanol reactive grade (Sigma-México). The crude methanolic and ethyl acetate extracts were kept covered in dryness, and protected from sunlight until their use in bioassays. In total, four extracts from *Microsechium helleri*, roots and leaf, with methanolic and ethyl acetate, and two from fruits of *Cucurbita okeechobeensis*, were used to assays using six tumoral cell lines.

4.3 Cell lines and culture

The human solid tumor cell lines A549, HBL-100, HeLa, SW1573, T-47D and WiDr, donated by Prof. G. J. Peters (VU Medical Center, Amsterdam, The Netherlands), were used in this study. The cells were maintained in 25 cm2 culture flasks in RPMI 1640 supplemented with 5% heat-inactivated fetal calf serum and 2 mM L-glutamine in an incubator at 37°C, 5 % CO2 and 95 % air humidity. Exponentially growing cells were trypsinized and re-suspended in an antibiotic-containing medium100 units penicillin G and 0.1 mg of streptomycin per mL). Single cell suspensions were counted using an Orflow's MoxiZ automated cell counter (Ketchum, ID) and dilutions were made to give the appropriate cell densities for the inoculation onto 96-well microtiter plates. Based on their doubling times, cells were inoculated in 100 µL per well at 10 000 (A-549, HBL-100, HeLa and SW1573), 15 000 (T-47D), and 20 000 (WiDr) cells per well.

4.4. Antiproliferative tests

Dry extracts were initially dissolved in DMSO at 400 times the desired final maximum test concentration, i.e. 10 mg.mL-1 and diluted in the culture media until they reached an assay concentration of 250 µg.mL-1. Control cells were exposed to

an equivalent concentration of DMSO (0.25% v/v) without extracts or negative control. After 24 hours the extracts were incubated for 48 h and that cells were precipitated with 25 µL ice-cold TCA (50 % w/v) and fixed for 60 min at 4 ºC. The sulforhodamine B (SRB) assay in Cell Culture was performed [38], measuring the protein content of adherent and suspension cells in 96-well microtiter plates. Cultures fixed with trichloroacetic acid were stained for 30 minutes with 0.4% (wt/vol.) The optical density (OD) of each well, was measured at 492 nm using BioTek's Power Wave XS Absorbance Microplate Reader (Winooski, VT). The percentage growth was calculated as the OD difference between the start and end of each treatment level, corrected for background OD of the control wells and compared with untreated control cells. The results were expressed as the concentration of extract causing 50 % reduction in the proliferation of cancer cells (GI50).

Author Contributions. Field data collection, recollect of plant material & investigation KMV, FCPS, OMF; Antiproliferative tests JMP. Writing—original draft preparation, OMF; writing—review and editing, OMF, JMP.

Funding: J.M.P. thanks the Spanish Government for financial support through project PGC2018-094503-B-C22 (MCIU/AEI/FEDER, UE)

Acknowledgments: O.M.F. thanks the CIMA Universidad Veracruzana, for some materials donated for experiments.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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