3rd International Electronic Conference on Medicinal Chemistry
1-30 November 2017
chaired by Dr. Jean Jacques Vanden Eynde
sponsored by pharmaceuticals

Searching for bioactive molecules in prostate cancer from Mayan traditional medicinal plants.

Juan Trinidad[1,2], Rafael Fort[2], Juliette Dourron[1], Francisco J. Aguirre[3], María Ana Duhagon[2]* and Guzmán Álvarez[1]*.

[1] Laboratorio de Moléculas Bioactivas, CENUR Litoral Norte, Universidad de la República, Ruta 3 (km 363), Paysandú, C.P. 60000, Uruguay;
[2] Laboratorio de Interacciones Moleculares, Facultad de Ciencias, Universidad de la República;
[3] Cuerpo Académico en Ciencias de la Salud y Biotecnología, Facultad de Ciencias Químico Biológicas, Universidad Autónoma de Campeche, México.
* Corresponding author: GA guzmanalvarezlqo@gmail.com, MAD mduhagon@fcien.edu.uy

CENUR Litoral Norte Paysandú
FACULTAD DE CIENCIAS
UNIVERSIDAD DE LA REPÚBLICA URUGUAY

Searching for bioactive molecules in prostate cancer from Mayan traditional medicinal plants.

Graphical Abstract

Etnobotanic Exploration

Plants Extract Library

Phenotypic Screening

Bioguided Fractionation

Nature Inspired Design

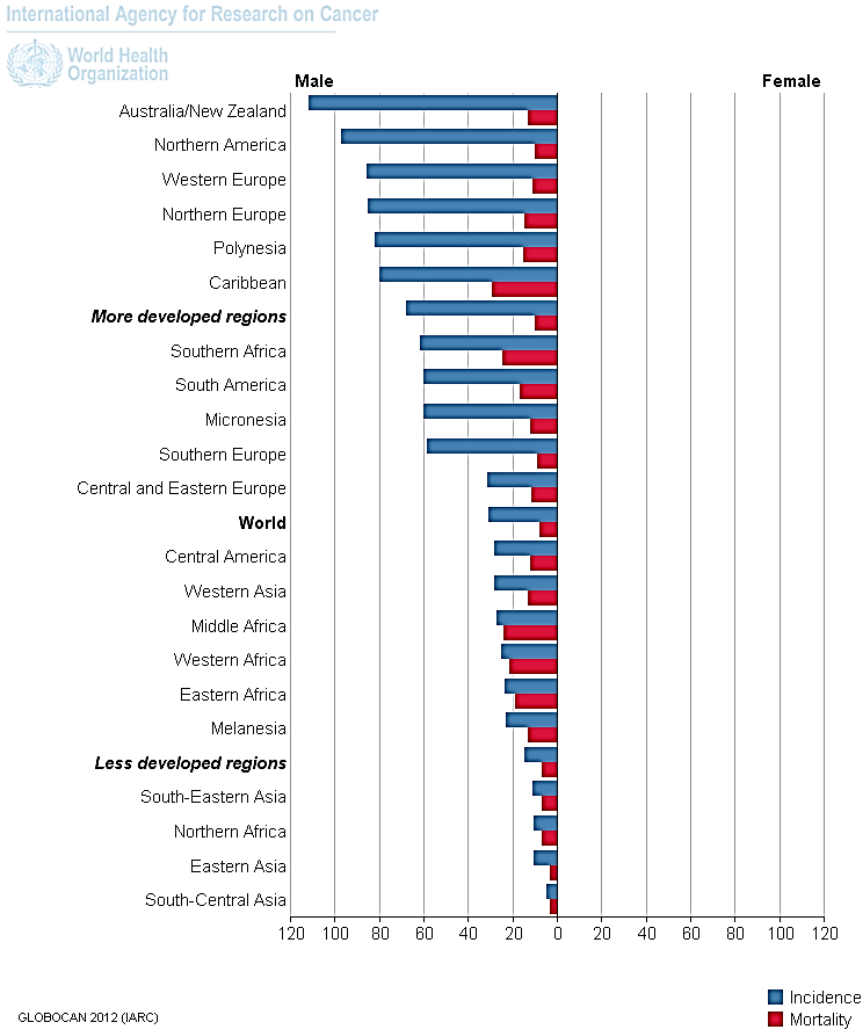
NEW HIT

ABSTRACT

Prostate cancer (PC) is the most common cancer in men around the world. It is a complex and heterogeneous disease in which androgens and their receptor play a crucial role in the progression and development. The current treatment for PC is a combination of surgery, radiation and chemotherapy. Therapeutic agents commonly used in the clinic include steroidal and non- steroidal anti-androgens, such as cyproterone acetate. These few agents have multiple adverse effects and are not 100% effective. Several plant compounds and mixtures, have been shown to be effective against PC cell growth. Some insolated compounds were reported with *in vivo* activity on PC murine model like capsaicin and curcumin. We prepared a library of plant extracts from traditional Mayan medicine. These plants were selected for their use in the contemporaneous Maya communities with application in different types of diseases and treatments. These extracts were used in a phenotypic screening in LNCaP (androgen sensitive) prostate cancer cells in a fixed dose (25 µg / mL). Ten plants out of 11 were identified with cytotoxic activity in these cells. With the active extracts, a bioguided fractionation method was performed until the elucidation of the mayor components. We identified 3 compounds with activity and design one hybrid molecule with the natural product structure and steroid analog to enhance the antiproliferative activity.

Keywords: Prostate cancer, *in vitro* LNCaP cell, natural product.

INTRODUCTION

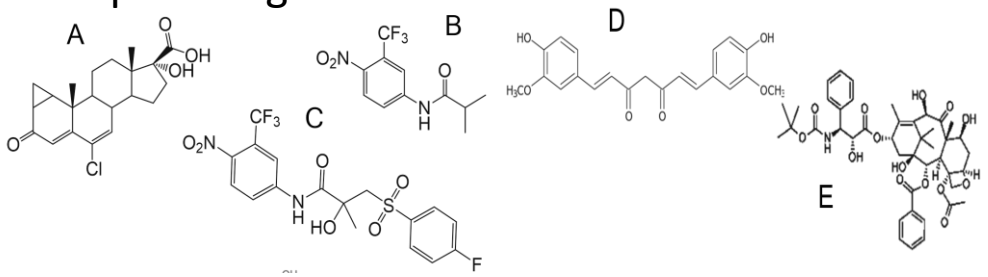


Prostate cancer is the most common cancer in men around the world.

The best option to handle it is the prevention and an early diagnostic.[1]

There are currently four types of treatment for prostate cancer: Surgery, radiation therapy, hormone therapy and chemotherapy.[2] Usually any of them are combined depending on the progression of the disease.

Therapeutic agents:



Drugs used in the treatment of prostate cancer. Cyproterone acetate (A), Flutamide (B) and Bicalutamide (C). Curcumin (D) and docetaxel (E).[3-8]

INTRODUCTION

Folk medicine is used around the world in different cultures such as African, Indian and South American. It is based in natural products and a hundred of years of experience. Actually there are contemporary indigenous communities like the Mayan, They treat sick people with some success using this medicine.

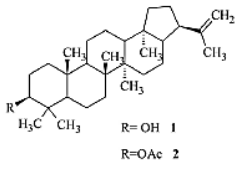
Natural products from a variety of organisms serve as an inspiration to successfully drug design and drug discovery such as Penicillin or Paclitaxel (Taxol)[9].

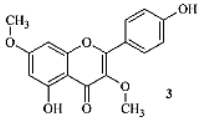
We used this knowledge to select 30 plants with therapeutic potential, from a large diversity of tropical plants. Mayans have been using them for a long time to treat a large variety of diseases.

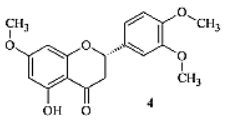
Active Plants' Profiles

Cnidoscolus chayamansa

Plant with high nutritional Value. Antimycobacterial and antiprotozoal activities. Low acute oral toxicity in mice. Some isolated compound has been previously described [10]

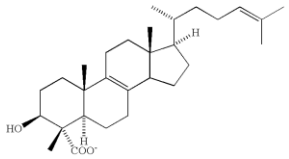






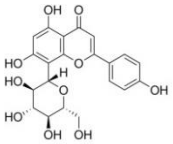
Leucaena leucocephala

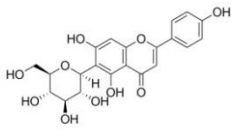
No effect at 80µg/mL SCC9 and SAS cells.[11] Anticancer activity and hair growth inhibition. Some components are significant cancer chemo-preventive and antiproliferative activities. [12]



Terminalia catappa

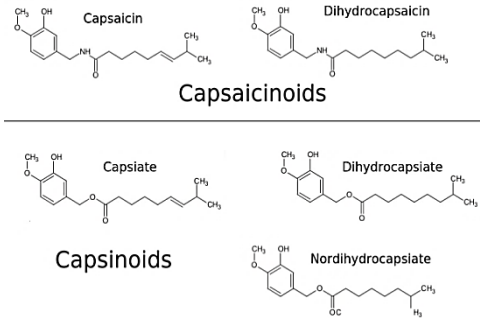
Some antibacterial activity. In vitro activity in Lewis lung carcinoma cells. No effects in SCC-4 and A549 cells viability.[13]





Capsicum chinense

Widely used in Mexican food as a spicy sauce. In vitro and in vivo[14-16] activity in different types of cancer



METHODOLOGY

Searching for bioactive molecules from Mayan traditional medicinal plants for prostate cancer treatment

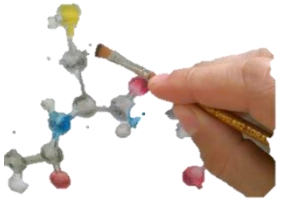
Etnobotanic Exploration

Plants Extract Library

Phenotypic Screening

Bioguided Fractionation

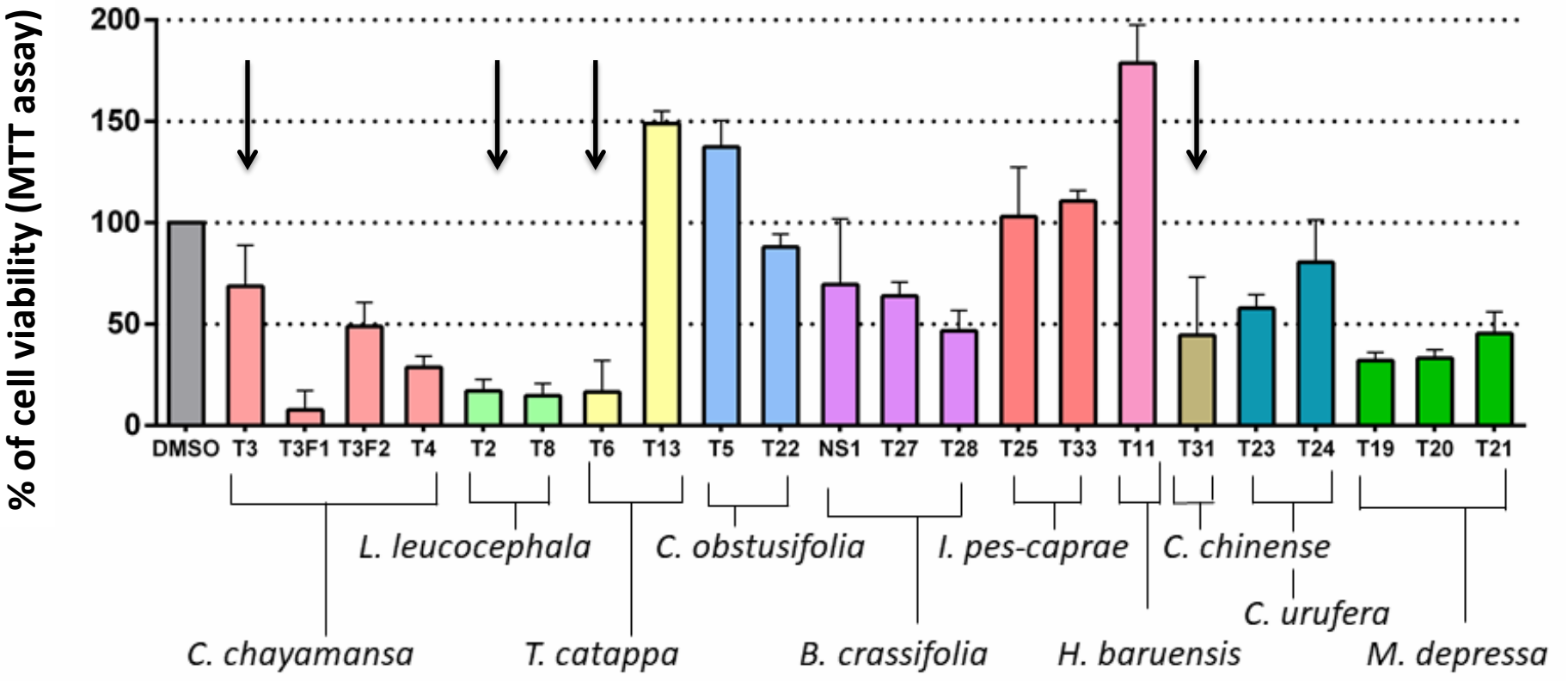
Nature Inspiration Design



NEW HIT

RESULTS

Phenotypic screening: Cytotoxic activity of the extracts (25 µg/mL) in LNCaP cells



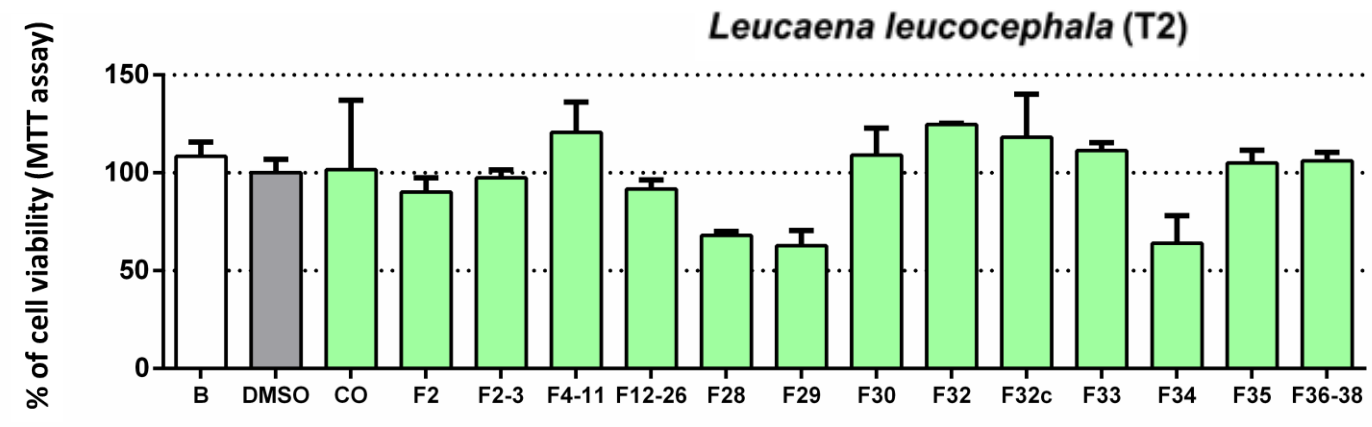
90% of the Selected Plants had anti-proliferative effect in LNCaP cells.

Black arrows indicates the samples selected for the bioguided fractionation procedure.

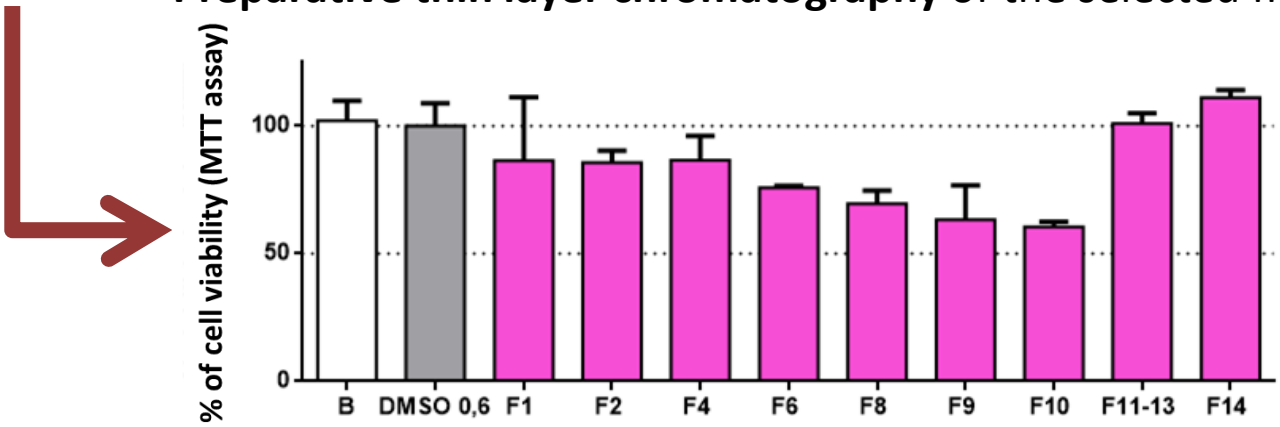
RESULTS

1st Fractionation by **Silica Gel Chromatography** in a petroleum ether/ ethyl acetate gradient.

Cytotoxic activity of the extracts (25µg/mL) in LNCaP cells



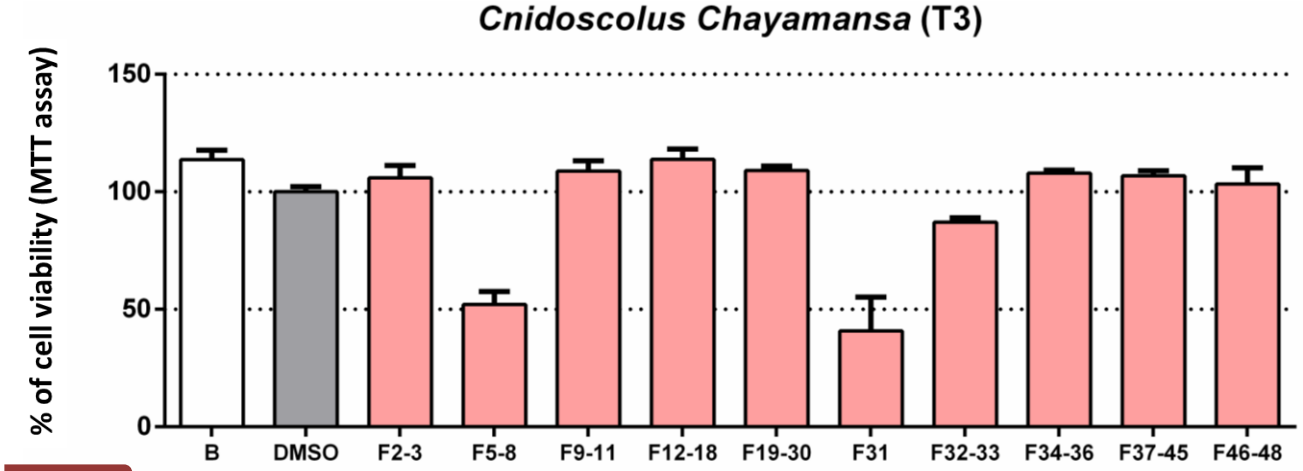
Preparative thin layer chromatography of the selected fraction (F28-29).



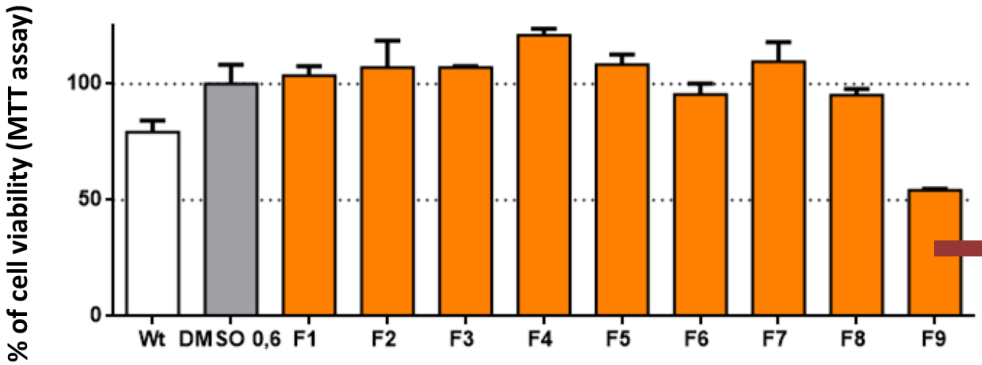
RESULTS

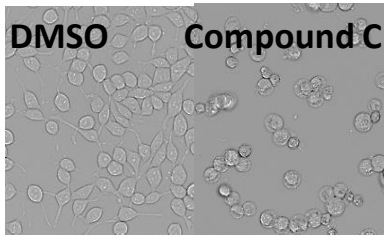
1st Fractionation by **Silica Gel Chromatography** in a petroleum ether/ ethyl acetate gradient.

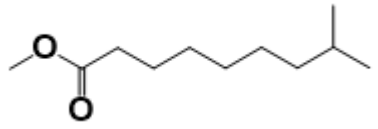
Cytotoxic activity of the extracts (25µg/mL) in LNCaP cells.



Preparative thin layer chromatography of the selected fraction (F5-8).





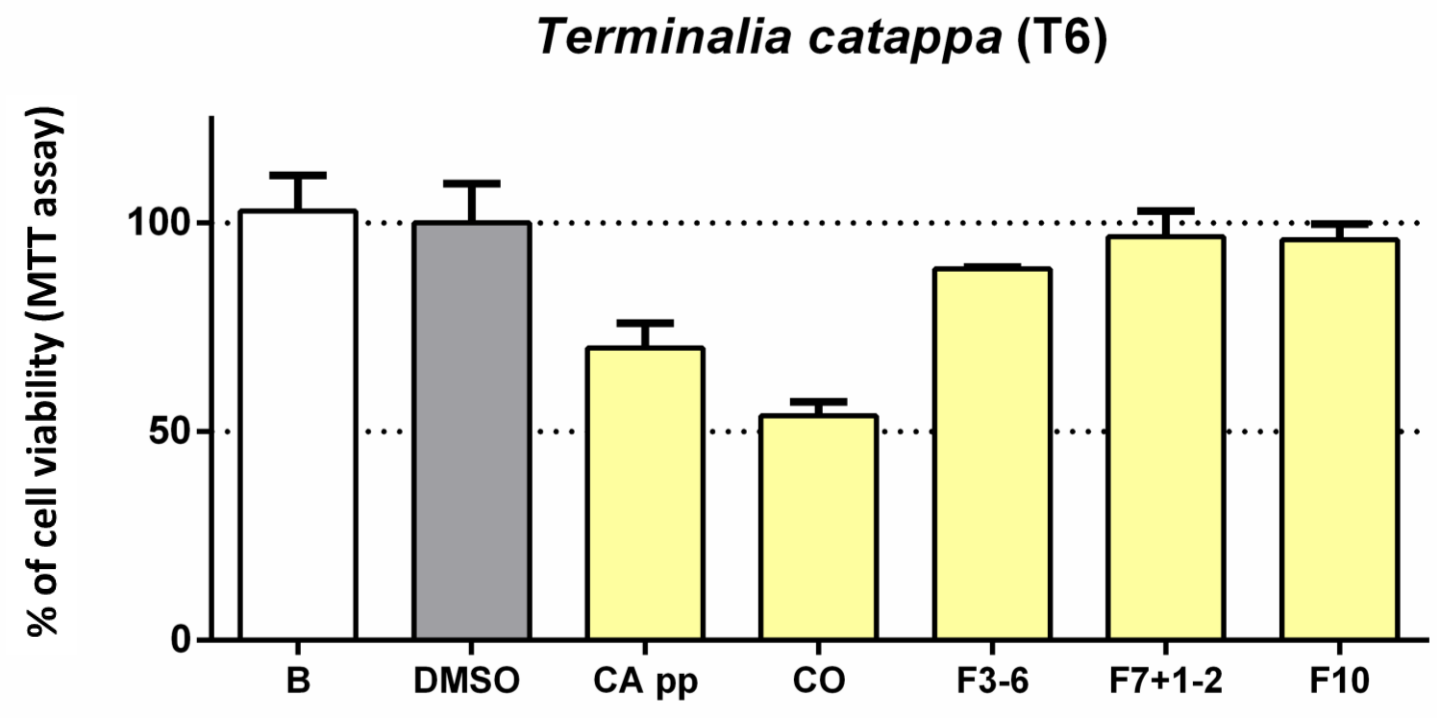


Compound C

RESULTS

1st Fractionation by **Silica Gel Chromatography** in a petroleum ether/ ethyl acetate gradient.

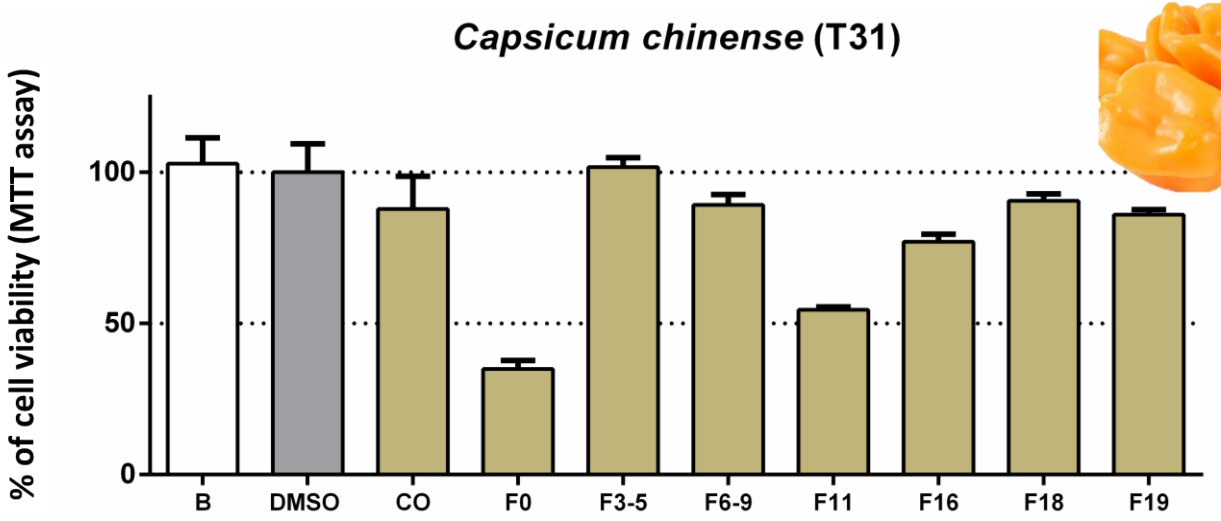
Cytotoxic activity of the extracts (25µg/mL) in LNCaP cells



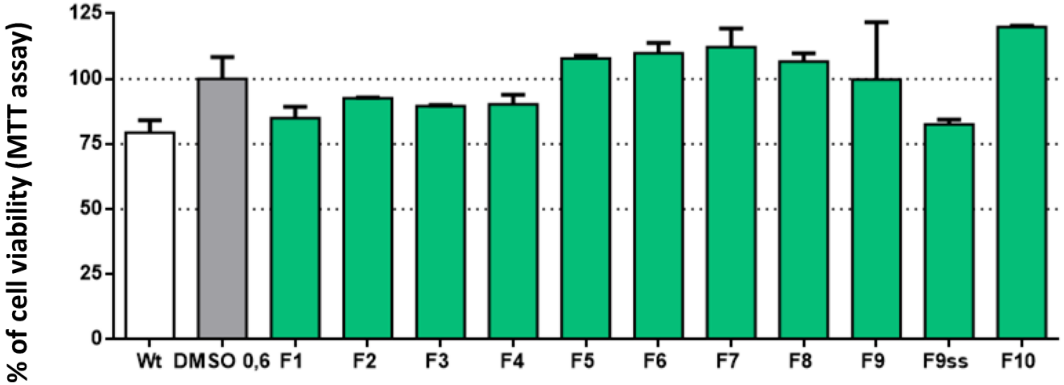
RESULTS

1st Fractionation by **Silica Gel Chromatography** in a petroleum ether/ ethyl acetate gradient.

Cytotoxic activity of the extracts (25µg/mL) in LNCaP cells



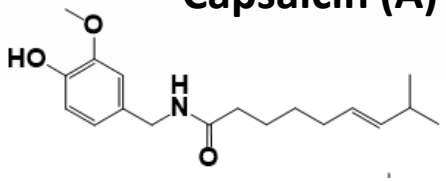
Preparative thin layer chromatography of the selected fraction (F0 and F11).

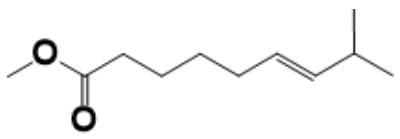


from F11

from F0

Capsaicin (A)

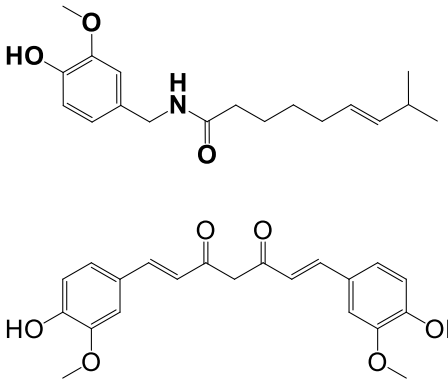


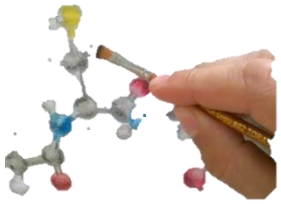


Compound (B)

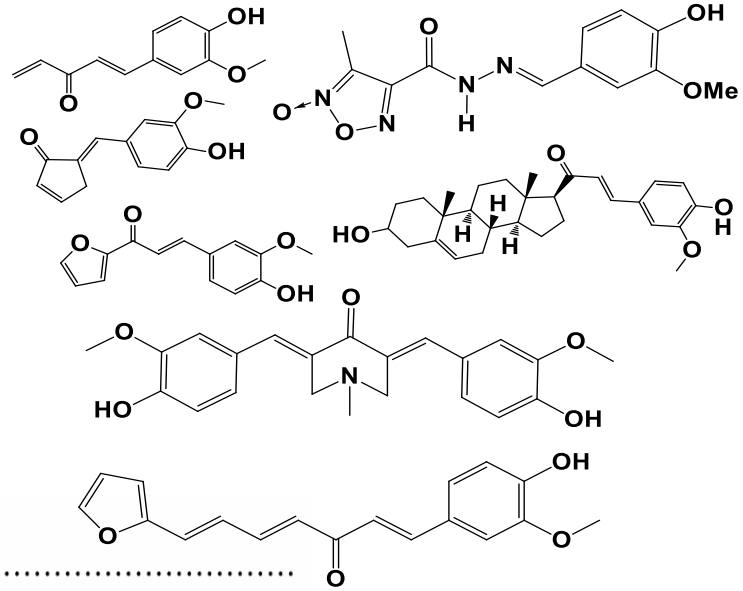
RESULTS

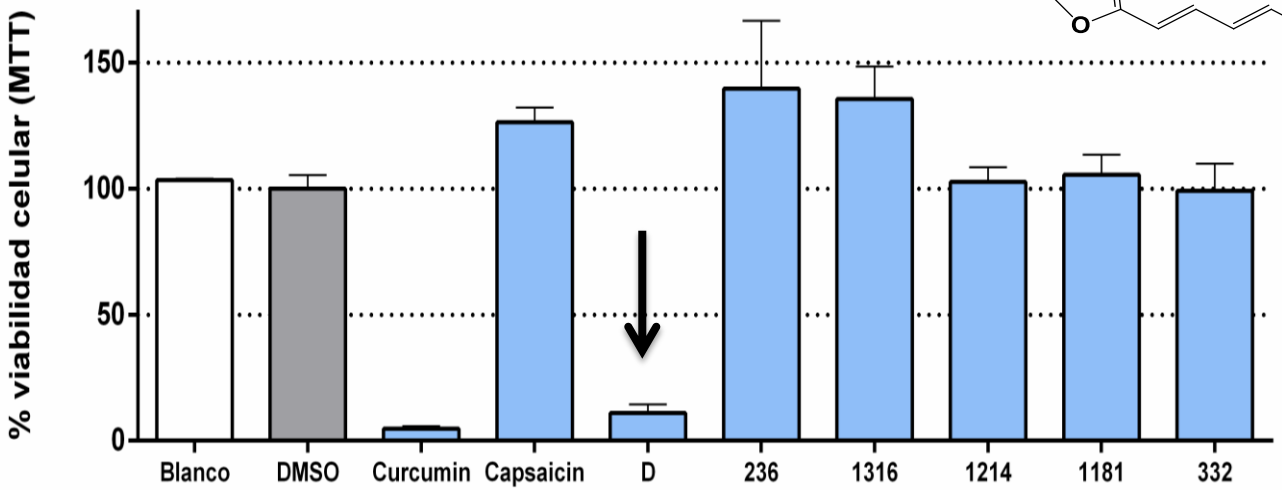
Inspired by Nature





Synthetic capsaicinoid derivatives

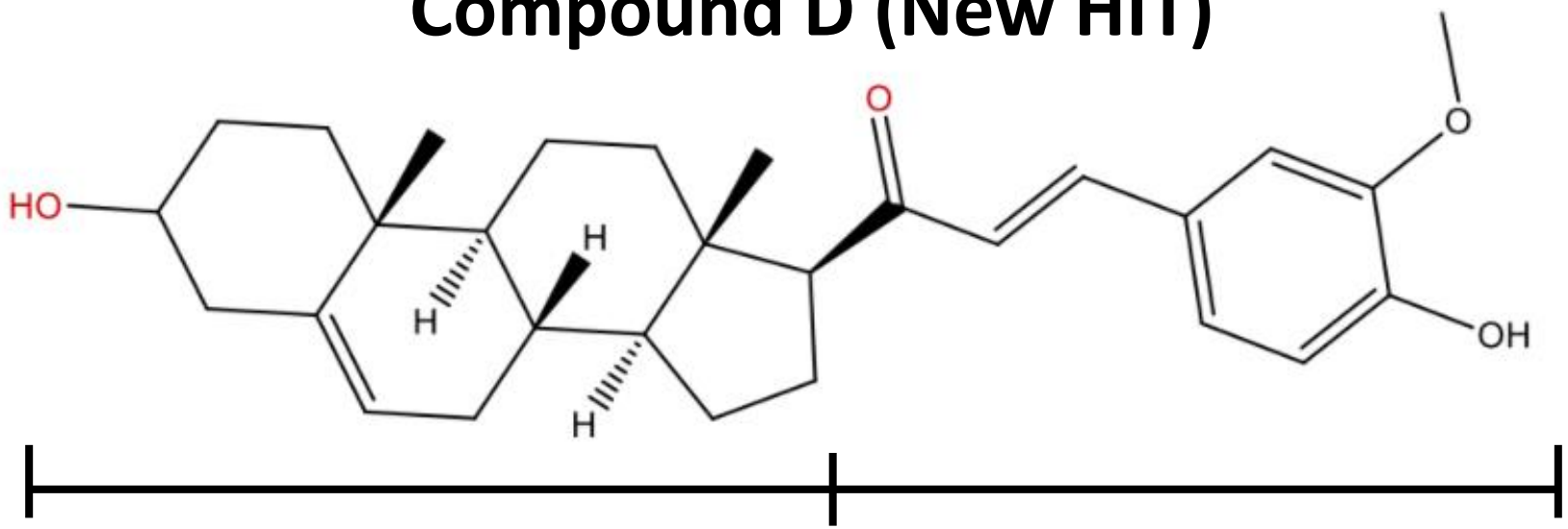




RESULTS

IC50 < 25 µM in LNCaP androgen dependent cell line

Compound D (New HIT)



Steroid group

Capsaicinoid pharmacophore group

CONCLUSION

- We tested 10 plant species from the Mayan Folk Medicine and found that 9 of them have cytotoxic activity in prostate cancer cells.
- We performed a bioguided fractionation to isolate the active compound, validating this process by the isolation of capsaicin (A) from *Capsicum chinense* fruits.
- We also described 2 more compounds: one from *Capsicum chinense* fruits (Compound B) and another from *Cnidoscolus chayamansa* (Compound C).
- Also we designed 7 new compounds inspired by nature, one of them has $IC_{50} < 25\mu M$ (Compound D). Then we identified a new Hit for the drug development process.

Acknowledgments

CSIC

SRE
SECRETARÍA DE RELACIONES EXTERIORES

REFERENCES

1-Yan D, Lockman D, Brabbins D, Tyburski L, Martinez A. An off-line strategy for constructing a patient-specific planning target volume in adaptive treatment process for prostate cancer. Int J Radiat Oncol Biol Phys. 2000;48(1):289-302.

2-Jemal, A.; Samuels, A.; A., G.; Ward, E.; Thun, M. Cancer statistics, 2003. CA Cancer J. Clin. 2003,53,5-26.

3-Goldenberg, S. L.; Bruchovsky, N. Use of cyproterone acetate in prostate cancer. Urol. Clin. North. Am. 1991,18, 111-112.

5- de Voogt, H. J. The position of cyproterone acetate (CPA), a steroid anti-androgen, in the treatment of prostate cancer. Prostate 1992 (Suppl. 4),91-95.

6- de Voogt, H. J.; Smith, P. H.; Pavone-Macaluso, M.; de Pauw, M.; Suciu, S. Cardiovascular side effects of diethylstilbestrol, cyproterone acetate, medroxyprogesterone acetate and estraumustine phosphate used for the treatment of advanced prostate cancer: Results from European Organization for Research on Treatment of Cancer Trials 30761 and 30762. J. Urol. 1986, 135, 303-307

7-Kelly, W. K.; Scher, H. I. Prostate specific antigen decline after antiandrogen withdrawal: The flutamide withdrawal syndrome. J. Urol. 1993, 149, 607-609.

8-Liu S, Wang Z, Hu Z, et al. Anti-tumor activity of curcumin against androgen-independent prostate cancer cells via inhibition of NF-kb-954;B and AP-1 pathway in vitro. J Huazhong Univ Sci Technolog Med Sci. 2011;31(4):530-534.

9-Henry JY, Lu L, Adams M, et al. Lenalidomide enhances the anti-prostate cancer activity of docetaxel in vitro and in vivo. Prostate. 2012;72(8):856-867.

10- Pérez-González MZ, Gutiérrez-Rebolledo GA, Yépez-Mulia L, Rojas-Tomé IS, Luna-Herrera J, Jiménez-Arellanes MA. Antiprotozoal, antimycobacterial, and anti-inflammatory evaluation of *Cnidoscolus chayamansa* (Mc Vaugh) extract and the isolated compounds. *Biomed Pharmacother.* 2017, 89:89-97.

11-Chung HH, Chen MK, Chang YC, Yang SF, Lin CC, Lin CW, *Environ Toxicol.* Inhibitory effects of *Leucaena leucocephala* on the metastasis and invasion of human oral cancer cells. 2017, 32(6):1765-1774.

12-Liang-Chien S, Chi-Ming L, Ching-Tong C, Hsing-Tan L, Wei-Jen L, Chung-Yi C, The anti-cancer and anti-metastasis effects of phytochemical constituents from Leucaena leucocephala, *Biomedical Research*, 2017; 28 (7): 2893-2897.

13-ChuaShun-FaYang, Shang-Jung, Liuc Wu-Hsien, KuodeYan-ZinChang, Yih-Shou, In vitro and in vivo antimetastatic effects of Terminalia catappa L. leaves on lung cancer cells, Food and Chemical Toxicology, 2007, 45:1194-1201.

14-Aza-González C, Núñez-Palenius HG, Ochoa-Alejo N Molecular biology of capsaicinoid biosynthesis in chili pepper (Capsicum spp.). Plant Cell Rep. 2011, 30:695–706.

15- Amruthraj NJ, Raj P, Saravanan S, Lebel In vitro studies on anticancer activity of capsaicinoids from capsicum chinense against human hepatocellular carcinoma cells. Int. J. Pharm. Pharm. Sci. 2014 6:254-558.

16-1. Mori A, Lehmann S, O'Kelly J, et al. Capsaicin, a component of red peppers, inhibits the growth of androgen-independent, p53 mutant prostate cancer cells. Cancer Res 2006, 66:3222–3229.