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

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Graphical Abstract

Nature Inspired Design

NEW HIT
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 insulated 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.
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
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).

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.
INTRODUCTION

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 chemopreventive 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 vivo¹⁴⁻¹⁶ activity in different types of cancer

![Chemical structures of various compounds](image-url)
Searching for bioactive molecules from Mayan traditional medicinal plants for prostate cancer treatment

**METHODOLOGY**

**Etnobotanic Exploration** → **Plants Extract Library** → **Phenotypic Screening**

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**NEW HIT** → **Bioguided Fractionation**
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.
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).
1st Fractionation by **Silica Gel Chromatography** in a petroleum ether/ethyl acetate gradient.

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

**RESULTS**

![Graph showing % of cell viability (MTT assay) for different fractions](image1)

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

![Graph showing % of cell viability (MTT assay) for different compounds](image2)
RESULTS

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

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

*Terminalia catappa* (T6)

![Graph showing cell viability](image)
RESULTS

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

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

% of cell viability (MTT assay)

**Capsicum chinense (T31)**

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

% of cell viability (MTT assay)

Capsaicin (A)

from F11

Compound (B)

from F0
RESULTS

*Inspired by Nature*

Synthetic capsaicinoid derivatives

![Chemical structures](image1.png)

![Results graph](image2.png)
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$_{50}$ < 25µM (Compound D). Then we identified a new Hit for the drug development process.

**Acknowledgments**


