

## Evaluate the Activity of *Cucumis melo* Ethanol Extract against Skin Cancer Named Melanoma

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### Abstract

Chinese medicine herbs have a large library of bioactive compounds and *Cucumis melo* is one of Chinese herbs has been used for medical treatment and exhibit anticancer activity against colon cancer. In the present study, we evaluate anticancer activity of *Cucumis melo* ethanol extract at first by using high throughput screening HTS for herb fractions screening in concentration of  $100\mu\text{M}$  to identify the effective fraction and then we measured the cytotoxic effects by using morphology changes, and detect apoptosis by AO/EB staining, our results showed that *Cucumis melo* ethanol extract have a strong toxicity against human melanoma A375 cancer cells and requires more investigation to identify the small natural molecule who is responsible for this cancer activity.

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**Keywords:** Chinese medicinal herbs, *Cucumis melo* L, Melanoma.

## Introduction

Melanoma chemotherapy treatment includes Alkylating Agents such as Dacarbazine (DTIC), response rate is 15% to 20% (Lucia BJ *et al.*, 2009; Kyaw MH *et al.*, 2010), Platinum Drugs (such as cisplatin, response of 15% as a single treatment) (Azadeh O *et al.*, 2010; Svetromir NM *et al.*, 2007), and Microtubule-toxin Agents (such as paclitaxel, response rate of 16% as single treatment) (Philip PA *et al.*, 1994; Hill GJ *et al.*, 1979). The side effects are still the main problem of chemotherapy agents (Bajetta E *et al.*, 2002; Bleehen NM *et al.*, 1995). Effectively, High throughput screening and the use of Chinese herbs provides better results of new small and natural molecules of anti-melanoma; this concludes acceleration in the ways by generating many new anti-melanomas (James AD and Edward SA, 1985; Wee YC and Hsuan K, 1992; Xianghui M and Zhiwen W, 2009). *Cucumis melo* is one of Chinese herbs belongs to cucurbitaceous family and it is a species of melon that has been developed into many cultivated varieties (Desai, B.B, 2004) and has been used in common medicine exclusively for curing

disease. The plant fruit is used as vomitive but on few amounts by honey is tonic for stomach also Cucurbitacine glycosides are tetra cycle three terpenic and have anti-tumor effect (M. Asadi *et al.*, 2012; Adekunle, A.A. and O.A. Oluwo, 2008). The herb compositions have studies and isolated several compounds (Lignou S *et al.*, 2013; Albishri HM *et al.*, 2013). Nakamura Y *et al.* in his research they identified a small natural molecule (3-Methylthiopropionic acid ethyl ester) from Japanese *Cucumis melo* and found it is enhanced differentiation in human colon cancer cells (Nakamura Y *et al.*, 2008). Future research will focus on the identification of new anti-melanoma and small anti-tumor molecules by using high throughput screening technology that speeds up the chemists to discover new anticancer agents, which is expected to generate new structures. In our study, we have used high throughput screening of *Cucumis melo* ethanol fraction extract herb to evaluate anticancer toxicity as anti-melanoma by morphology changes, AO/EB staining to detect apoptosis affect and to use the herb extract for further mechanistic studies.

## Materials and Methods

### 1. Chemical and reagents

Fetal bovine serum Hangzhou Sijiqing Biological Engineering Materials Co., Ltd. DMEM culture medium, and Dimethyl sulfoxide (DMSO) were purchased from Sigma. Acridine orange/ethidium bromide apoptosis staining was purchased from Beyotime Institute of Biotechnology Jiangsu China.

### 2. Preparation of *Cucumis melo* ethanol extract

*Cucumis melo* herb was purchased from national institute for food and drug control and jilin xiancao medical herb limited company by the Key Research Laboratory of Cell Biology, Membrane Channels Research and Anti-Cancer Drug Discovery in the School of Life Science, Northeast Normal University, Changchun, Jilin Province China. Specimen was deposited in this Key Laboratory.

Briefly, *Cucumis melo* was crushed and then extracted in Soxhlet extractor with ethanol for more than 12 cycles to achieve maximum extraction of its ingredients. The ethanol extract was hemi dried using rotary evaporator and

then dissolved in 80% methanol. After centrifugation at 12000 rpm for 15 minutes, the supernatant was separated and filtered with 0.18  $\mu\text{m}$  filter paper. Starting from the first peak to the end of the last peak, the extracted material was divided into 80 fractions on the basis of time (30 seconds per fraction) using HPLC. The fractions were dried and dissolved in dimethylsulfoxide (DMSO) to obtain a 1 mg/mL stock solution. These fractions were subjected to screening for cytotoxicity against human melanoma A375 cells, and evaluated by morphological changes.

### 3.0 Cell Culture

Human melanoma cell line A375 was purchased from the Cell Bank of Shanghai Institute of Cell Biology, Chinese Academy of Sciences (Shanghai, China). The cells were cultured in DMEM supplemented with 10% FBS and 100 unit of Penicillin at 37°C in a CO<sub>2</sub> incubator with 5% CO<sub>2</sub>, 95% air and 100% humidity. Cells were plated in 10 cm culture dish and allowed to grow to approximately 60-80% confluence before experimentation.

### 3.1 Cells morphology changes

The cells were harvested in  $1 \times 10^4$  cells per well in 96-well plates. The cells were treated with 0, and 100  $\mu\text{M}$  of *Cucumis melo* ethanol extract. 24h later, we observed the morphology changes of melanoma cells, we used untreated cells as a control and take pictures by using an optical microscope.

### 3.2 AO/EB staining to detect apoptosis

Acridine orange/ethidium bromide (AO/EB) was purchased from Bio Basic, INC, China. AO/EB was used to stain untreated and treated melanoma A375 cells. To detect apoptosis, the cells were harvested in  $1 \times 10^4$  cells per well in 96-well plates. The cells were treated with 0, and 100  $\mu\text{M}$  of *Cucumis melo* ethanol extract. 24h later; we started the staining procedure by removing the medium and adding 8 $\mu\text{l}$  of AO/EB (1:1) (The dye mix for the AO/EB staining was 100  $\mu\text{g}/\text{ml}$  acridine orange and 100  $\mu\text{g}/\text{ml}$  ethidium bromide in PBS) in each well. After mixing them gently; the mixture was incubated for 10-20min at 37°C, then we observed the staining cells and taken photos by using an optical microscope (Deborah R *et al.*, 2005).

### Results and Discussion

High throughput screening technology is a technique accelerates screening herbs fraction to identify active fraction and isolate small natural molecules can be used for cancer researches and treatment, and we used it in this study to screen about 80 fraction of *Cucumis melo* ethanol extract against melanoma A375 cells to identify active fractions and as showing in table (1) D2-D11; E2-E11; F2-F11; G2-G11 are exhibit high toxicity against melanoma cells and we can use these fractions to identify the responsible small compound for this anticancer activity and cells were treated with 100  $\mu\text{M}$  of *Cucumis melo* ethanol extract and showed clearly a strong effect on melanoma A375, perhaps due to the natural compounds mixture in *Cucumis melo* ethanol extract that enhances the activity against melanoma cells. Also, the cells were treated with 0 and 100 $\mu\text{M}$  of *Cucumis melo* ethanol extract and showed an effect on morphology changes of melanoma cells, as showing in Figure (1).

We used AO/EB staining to observe apoptosis affected by *Cucumis melo* ethanol extract, the previous studies showed that after staining by AO/EB, the

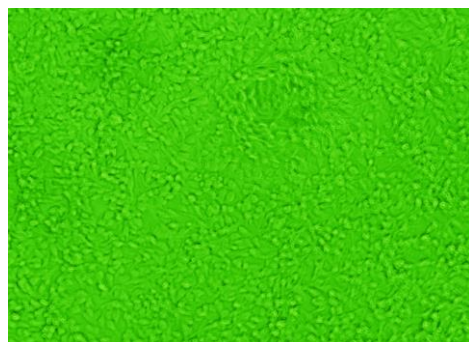
cells will stain with red for necrosis, bright green and DNA fragmentation for apoptosis and green for live cells (Leite M *et al.*, 1999). The cells were treated with *Cucumis melo* ethanol extract and showed clearly the apoptosis compared with the control, and as mentioned in the previous studies to detect apoptosis by using AO/EB staining method as showing in figure (2).

In conclusion, high throughput screening technology speeds up the identification

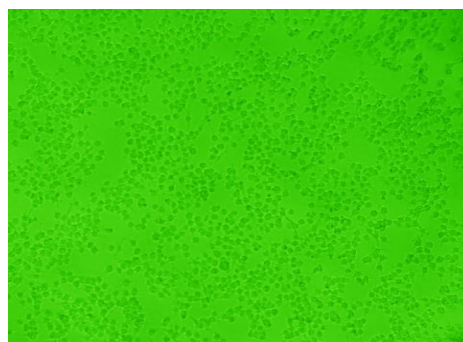
of new natural drugs against cancer cells and paves the way to discover new anti-drugs. In the present study results *in vitro*, we suggested that Chinese herb *Cucumis melo* extract have strong toxicity against human melanoma A375 and require further studies to identify the responsible small natural molecules for the cancer activity and study the mechanistic, in addition will generate new small natural anti-melanoma molecules to discover new natural agents.

**Table 1:** show first high throughput screening HTS procedure to detect different toxicity fractions of *Cucumis melo* ethanol extract (negative control is untreated cells and positive control is cells treated with DMSO) (+ = low toxicity; ++ = medium toxicity; +++ = high toxicity) against melanoma A375 cancer cells

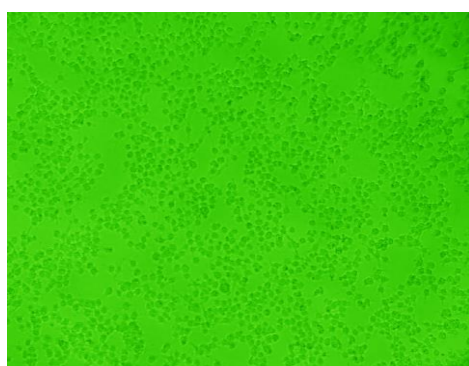
	Cucumis melo ethanol extract fractions												
Negative control	1	2	3	4	5	6	7	8	9	10	11	12	Positive control
<b>A</b>	CS												DMSO+CS
<b>B</b>	CS												DMSO+CS
<b>C</b>	CS				C5 +++	C6 +++		C8 +++	C9 +++	C10 +++	C11 +++		DMSO+CS
<b>D</b>	CS	D2 +++	D3 +++	D4 +++	D5 +++	D6 +++	D7 +++	D8 +++	D9 +++	D10 +++	D11 +++		DMSO+CS
<b>E</b>	CS	E2 +++	E3 +++	E4 +++	E5 +++	E6 +++	E7 +++	E8 +++	E9 +++	E10 +++	E11 +++		DMSO+CS
<b>F</b>	CS	F2 +++	F3 +++	F4 +++	F5 +++	F6 +++	F7 +++	F8 +++	F9 +++	F10 +++	F11 +++		DMSO+CS
<b>G</b>	CS	G2 +++	G3 +++	G4 +++	G5 +++	G6 +++	G7 +++	G8 +++	G9 +++	G10 +++	G11 +++		DMSO+CS
<b>H</b>	CS												DMSO+CS



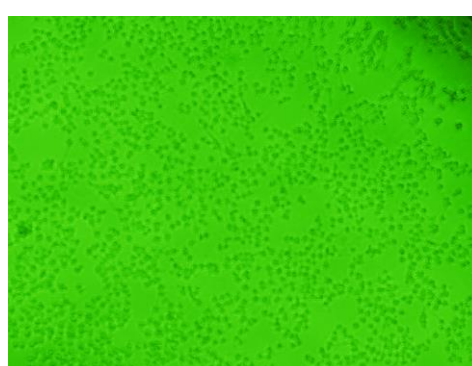
Melanoma untreated cells, the control



Cells treated with Cucumis melo ethanol extract- C8 fraction



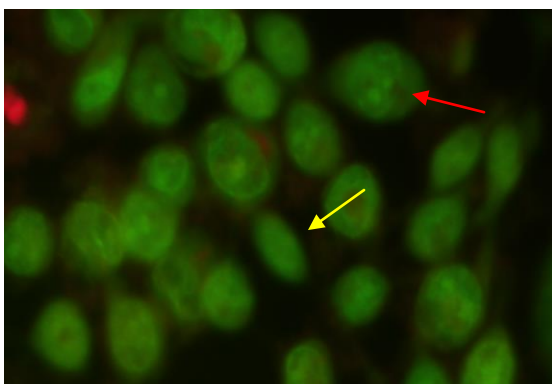
Cells treated with Cucumis melo ethanol extract- C10 fraction



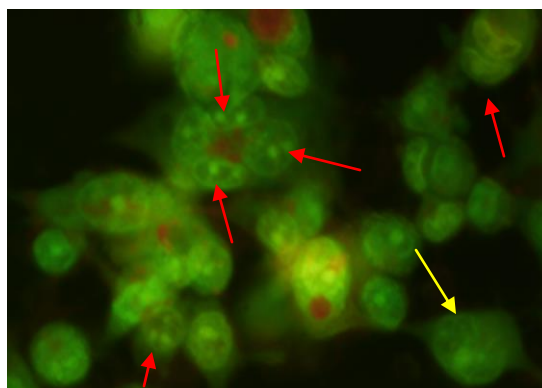
Cells treated with Cucumis melo ethanol extract- C9 fraction

**Figure 1:** Morphology changes of melanoma A375; Cucumis melo ethanol extract fractions (0 and 100 $\mu$ M) showed strong toxicity compared with the control

Untreated A375 cells- control



A375 cells treated with Cucumis melo ethanol extract



**Figure 2.** AO/EB staining to detect apoptosis of human melanoma A375 cells after 24h of Cucumis melo ethanol extract treated with 0 and 100 $\mu$ M. (Yellow arrows refer to live cells and red arrows refer to DNA fragmentation (dead cells))

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## تقييم فعالية المستخلص الايثانولي لكوكيوس ميلو ضد سرطان الجلد المسمى الميلانوما

علي عبد الواحد عبد الحسين الشاوي

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### الخلاصة :

تمتلك النباتات الطبية الصينية مكتبة كبيرة من المركبات الفعالة بايولوجيا" ويعد كوكيوس ميلو احد النباتات الصينية والذي يستعمل في العلاج الطبي والذي له فعالية مضادة لسرطان القولون. في الدراسة الحالية قيمت الفعالية المضادة للسرطان لمستخلص الايثانول لنبات كوكيوس ميلو باستعمال تقنية الحقن الانتاجي العالي الجريان بتركيز 100 مايكرومولار لتحديد الفعالية السرطانية كما قيست الفعالية السمية باستخدام تغير شكل وطبيعة الخلايا السرطانية وكشف برنامج الخلايا المميت باستخدام صبغة خاصة لخلايا سرطان الجلد المسمى ميلانوما ، دلت النتائج التي تم الحصول عليها ان المستخلص يمتلك فعالية قوية مضادة لسرطان الميلانوما ويحتاج الى دراسة اعمق من اجل عزل وتشخيص المركب المسؤول عن هذه الفعالية المضادة للسرطان مستقبلا" ودراسة الميكانيكية الفعالة للبروتينات المنثرة.