

Efficacy Assessment for Several Natural Products with Potential Cytotoxic Activity Against Breast and Cervix Cancers

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Abstract: Over more than five decades, cancer remains a national and international health problem regardless of the discovery of several dozens of novel anticancer drugs (natural and synthetic). Accordingly, screening of natural products for promising anti cancer activity is very initiative field of studies in several countries with diversity of gardenia. Breast and cervix cancers are of the most common gynecological female solid tumors that represent major health problem. Herein, we have assessed the cytotoxic characteristics of several molecules of natural origin (cerulinin, chrysin, honikiol, limonin, mevinolin, resveratrol, salicin, retinol, ascorbic acid and calciferol) against two different gynecological breast (MCF-7) and Cervix (HeLa) solid tumor cell lines. After exposure to serial concentrations of the test compounds, SRB-u assay was undertaken and viability assessment was performed via fitting to Emax model to identify the cytotoxicity parameters such as, IC50 and resistant fraction (R-value). Tested compounds showed cytotoxic efficacy against both gynecological solid tumor cell lines (MCF-7 and HeLa) with IC50's ranged from 0.61 to 131.1 µg/ml. In MCF-7 breast cancer cell line; cerulinin, honikiol, mevinolin and calciferol showed the highest potency with IC50 less than 5 µg/ml. Chrysin showed moderate potency with IC50 of 7.27 µg/ml. Ascorbic acid and resveratrol showed the weakest cytotoxic activity with IC50 more than 10 µg/ml. In HeLa cervix cancer cell line; cerulinin and mevinolin showed the highest potency with IC50 less than 5 µg/ml. Chrysin, honikiol, resveratrol and calciferol showed moderate potency with IC50 of ranging from 5-10 µg/ml. Ascorbic acid was the weakest cytotoxic molecule with IC50 more than 10 µg/ml. Limonine, salicin and retinol failed to exert any cytotoxic effect against MCF-7 or HeLa cancer cell lines in-vitro. RT-PCR analysis revealed that the cytotoxicity of these products is multi-factorial and not solely dependent on p53 expression. Impressively, molecules of potent and moderate potency (except chrysin) showed low resistant fraction in both gynecological solid tumor cancer cell lines. In conclusion, our data showed wide range of variable efficacy of several molecules of natural origin against two hormone dependent solid tumor cell lines.

Key Words: Anti-cancer screening, Breast cancer, Cervix cancer, Cytotoxicity, Natural products

1. Introduction

Far East, Middle East Sahara, and tropical regions are considered of the richest terrains of natural products in the world (El-Shemy *et al.*, 2007). Currently, isolation and purification of the active fraction or active ingredient amongst potentially active natural product is getting great scientific and industrial interests (Shanab *et al.*, 2010).

New compounds for the treatment of neoplasm might be *de novo* identified in terms of structure and function (investigational new drug, IND). Also there might be several well known compounds that recently showed promising activity against cancer (investigational new action drugs, INA) (Van Den Berghe *et al.*, 1978). Therefore, the aim of this study is to determine the effect of some natural compounds as anticancer agent.

2. Materials and Methods

2.1 Chemicals and drugs.

Pure natural compounds were purchased from Sigma Chemical Co. (St. Louis, MO). RPMI-164 media, fetal bovine serum and other cell culture materials were purchased from AATC (Houston, TX). Other reagents were of the highest analytical grade.

2.2. Cell culture

Breast cancer cell line (MCF-7) and Cervix cancer cell line (HeLa) were obtained from the Vaccera (Giza, Egypt). Cells were maintained in RPMI-1640 supplemented with 100 µg/mL streptomycin, 100 units/mL penicillin and 10% heat-inactivated fetal bovine serum in a humidified, 5% (v/v) CO₂ atmosphere at 37°C (**Fig. 1**).

2.3. Cytotoxicity assays

The cytotoxicity of crude extract and the fractionated compounds were tested against MCF-7 and HeLa cells by

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Table 1. Effect of natural compounds on the cytotoxicity parameters of HeLa cell line.

Compounds Parameter	Resveratrol	Cerulinine	chrysin	chrysin	Honikiol	Limonene	Mevinolin	Salicin	VIT A	Vit-C	Vit D
IC ₅₀	8.0±0.25 µg/ml	3.9±0.9 µg/ml	6.5 µg/ml	6.5 µg/ml	5.2±0.71 µg/ml	--	0.61±0.02 µg/ml	--	> 300 µg/ml	33.10±2.75 µg/ml	5.7±1.95 µg/ml
Resistance Fraction	3.03±0.4%	0.7±0.4%	45.40%	45.40%	2.47±0.17%	>50%	0.33±0.09%	>60%	>100%	0.39±0.1%	0.20±0.10%

Table 2. Effect of natural compounds on the cytotoxicity parameters of MCF7 cell line.

Compounds Parameter	Resveratrol	Cerulinine	chrysin	Honikiol	Mevinolin	VIT A	Vit-C	Vit D -
IC ₅₀	19.13±5.9 µg/ml	3.13±0.15 µg/ml	7.27±2.56 µg/ml	4.23±1.4 µg/ml	0.67±0.2 µg/ml	---	30.3±3.2 µg/ml	2.62±0.61 µg/ml
Resistance Fraction	0.00±0.00	2.57±2.57%	0.93±0.45%	1.5±0.34%	1.4±1.4%	>100%	0.0±0.0%	0.00±0.00%



(a) MCF-7 monolayer culture at confluent state



(b) HeLa monolayer culture at confluent state

Fig. 1. Cell Line Image (a)MCF-7 monolayer culture at confluent state, (b)HeLa monolayer culture at confluent state.

SRB assay as previously described (Skehan *et al.*, 1990). Exponentially growing cells were collected using 0.25% Trypsin-EDTA and plated in 96-well plates at 1000-2000 cells/well. Cells were exposed to each test compound for 72 h and subsequently fixed with TCA (10%) for 1 h at 4°C. After several washings, cells were exposed to 0.4% SRB solution for 10 min in dark place and subsequently washed with 1% glacial acetic acid. After drying overnight, Tris-HCl was used to dissolve the SRB-stained cells and color intensity was measured at 540 nm (Skehan *et al.*, 1990).

2.4. Cytotoxicity assays

The cytotoxicity of crude extract and the fractionated

compounds were tested against MCF-7 and HeLa cells by SRB assay as previously described (Skehan *et al.*, 1990). Exponentially growing cells were collected using 0.25% Trypsin-EDTA and plated in 96-well plates at 1000-2000 cells/well. Cells were exposed to each test compound for 72 h and subsequently fixed with TCA (10%) for 1 h at 4°C. After several washings, cells were exposed to 0.4% SRB solution for 10 min in dark place and subsequently washed with 1% glacial acetic acid. After drying overnight, Tris-HCl was used to dissolve the SRB-stained cells and color intensity was measured at 540 nm (Skehan *et al.*, 1990).

2.5. Data analysis

The dose response curve of compounds was analyzed using E_{max} model (Eq. 1).

$$\% \text{ CR} = (100 - R) \times \left(1 - \frac{[D]^m}{K_d^m + [D]^m} \right) + R \dots\dots\dots \text{(Eq. 1)}$$

Where R is the residual unaffected fraction (the resistance fraction), [D] is the drug concentration used, K_d is the drug concentration that produces a 50% reduction of the maximum inhibition rate and m is a Hill-type coefficient.

IC₅₀ was defined as the drug concentration required to reduce fluorescence to 50% of that of the control (i.e., K_d = IC₅₀ when R=0 and E_{max} =100-R) (Al-Abd *et al.*, 2008).

3. Results and Discussion

Cerulinine, vitamin D and Honkiol were showed moderately active as single agent for the Breast and cervix cancer. In the other hand, vitamin A and C both showed weak activity according to IC₅₀ in **Tables 1 and 2**.

Regarding Limonene and Salicin, no data's were generated from both compounds' (Table 1).

Mevanoline is fairly active as single agent for both cancer cell lines (MCF7 and Hela; Tables 1 and 2; **Fig. 2**).

There are national and indigenous rights over plant derived

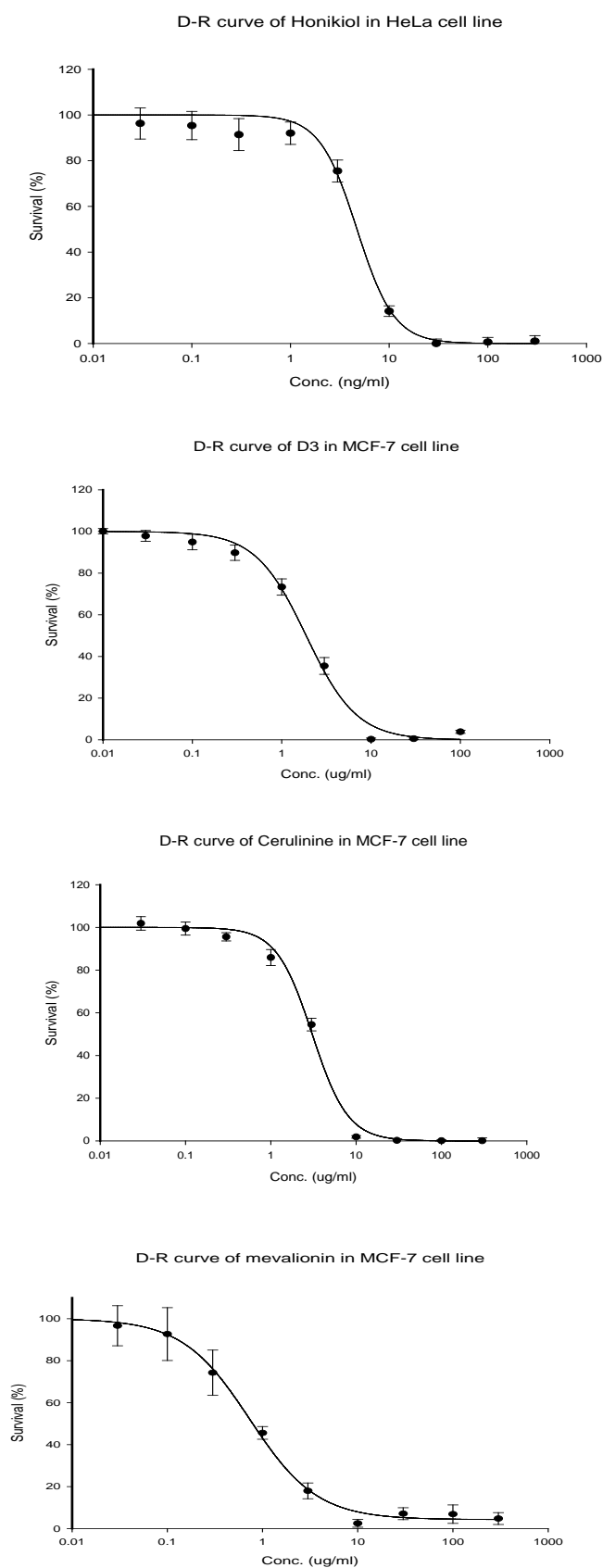


Fig. 2. The effect of natural compounds on the dose response curve (D-R) of MCF-7 and HeLa cell lines.

resources. Basic scientific investigations based on medicinal plants and indigenous medical systems have increased. A

screening program was initiated more than three decades ago (Ieven *et al.*, 1979), that identified many antibacterial antifungal, antiviral, antiparasitic, and other pharmacologically active substance activities in higher plants (Jang *et al.*, 1997). For example, Resveratrol has shown as being anti-oxidant, anti-inflammatory and neuro-protective (Li *et al.*, 2009).

To the major health and economic impact of cancer and leukemia, considerable attention has been drawn to the discovery of cytotoxic agents (Elgendi *et al.*, 2010). The dramatic side effects of the vast majority of cytotoxic agents urged scientific research in the direction of modifying treatment options or treatment modalities (Shaked *et al.*, 2005). The re-assessment of INA drugs in the field of neoplasia worth intensive investigation by research teams and us (O'Quigley *et al.*, 2010). Particularly, natural compounds of mild to moderate adverse effect would represent very rich soil for INA anti-cancer drug fishing.

Resveratrol (RES) has shown a diversity of beneficial health effects such as, anti-oxidant, anti-aging, cardio-protective, anti-inflammatory and neuro-protective properties (Li *et al.*, 2009). Due to its anti-oxidant properties, cancer preventive activities of RES have been studied and proved (Luther *et al.*, 2009). However, the first report mentioning potential anti-cancer activity of RES was as late as 1997 (Signorelli *et al.*, 2005; Jang *et al.*, 1997). Besides its anti-leukemic properties (Puissant *et al.*, 2010).

4. Conclusion

In conclusion, the effect of some natural compounds as anticancer was promising for further clinical studies.

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