



Cytotoxicity of *Euphorbia macroclada* on MDA-MB-468 Breast Cancer Cell Line

Hojjat Sadeghi-Aliabadi^a, Seyed Ebrahim Sajjadi^b, Marziyeh Khodamoradi^c

^aDepartment of Pharmaceutical Chemistry, ^bDepartment of Pharmacognosy,

^cIsfahan Pharmaceutical Sciences Research Center, Faculty of Pharmacy, Isfahan University of Medical Sciences, Isfahan, Iran

Abstract

It has been reported that different species of *Euphorbia* (Euphorbiaceae) have antitumor activity. Some reports also show that these plants have potential cytotoxic effect against different cell lines. In a program to screen the cytotoxicity of Iranian native plants, *Euphorbia macroclada* Boiss. was collected, identified, and the cytotoxic activity of dichloromethane, ethylacetate, methanol extracts, and the plant latex were determined against MDA-MB-468 cell line. Different concentration of extracts and latex were added to 24 h cultured cells and then incubated for 72 h under specific condition (37 °C, 5% CO₂). Cell survival was evaluated using MTT assay. The results of this study indicated that, dichloromethane and ethylacetate extracts had cytotoxic effects on cell line, while the methanol extract and latex were not cytotoxic at the tested concentrations. The data from this investigation suggest that the nonpolar extracts of *E. macroclada* possess higher cytotoxic activity.

Keywords: Brest cancer line; Cytotoxicity; Euphorbiaceae; *Euphorbia macroclada*; MDA-MB-468; MTT assay.

Received: January 20, 2008; **Accepted:** December 2, 2008

1. Introduction

Euphorbiaceae is a large family of flowering plants including 300 genera and over 5000 species [1]. *Euphorbia* is the largest genus in this plant family with about 2000 known species [2]. In the flora of Iran, the genus is represented by 70 species, of which 17 species are endemic [3].

Species of *Euphorbia* have been used in the treatment of cancer, asthma, leukemia, and some warts [4-6]. The plant is also used as a

laxative and diuretic in different parts of the world [7]. Some of the *Euphorbia* species have cytotoxic, antiviral, antibacterial, and antifungal activities [8-10]. Latex of some species of *Euphorbia* (Farfion in Persian) has traditionally been used in the treatment of skin diseases, gonorrhea, migraines, intestinal parasites and warts [11]. In several studies different extracts and isolated compounds from *Euphorbia* showed cytotoxic activity against cell lines such as KB, K₅₆₂, CNE₂, ANA-1, B₁₆, CHO, Hep⁻², and Hela [12-15].

In recent decades a number of species have been tested for their antitumor effects, partly

*Corresponding author: Hojjat Sadeghi-Aliabadi, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Isfahan, Iran.
Tel: (+98)311-6688064, Fax: (+98)311-6680011
E-mail: sadeghi@pharm.mui.ac.ir

on the basis of references to traditional usage. These bio-evaluations resulted in finding new compounds that are biologically active against tumor cells. To our best knowledge there is no published data regarding the cytotoxicity of *E. macroclada* Boiss. Therefore following our investigations on the characteristics of Iranian native *Euphorbia*, it was decided to evaluate cytotoxic activity of different extracts and latex of this plant against MDA-MB-468 as a breast cancer cell line using the *in vitro* colorimetric MTT assay.

2. Materials and methods

2.1. Plant material

E. macroclada Boiss. was collected at full flowering stage from Isfahan province in May 2005 at altitude of 2400m. The plant was identified by Prof. Rahiminejad, Department of Biology, University of Isfahan, Isfahan,

Iran. A voucher specimen (no. 1117) is deposited in the Herbarium of Faculty of Pharmacy, Isfahan University of Medical Sciences, Isfahan, Iran.

2.2. Chemicals

All chemicals and reagents used in extractions were of analytical grade and obtained from Merck (Darmstadt, Germany). Chemicals used in tissue culture assays were purchased from Gibco (Scotland) via local vendors. Taxol was obtained from Bristol-Myers Squibb Co. (USA).

2.3. Preparation of the extracts

Aerial parts of the plant were air-dried in shade at room temperature. The dried parts were powdered mechanically and 170 g was extracted by 500 ml of dichloromethane using maceration method. The residue was subjected

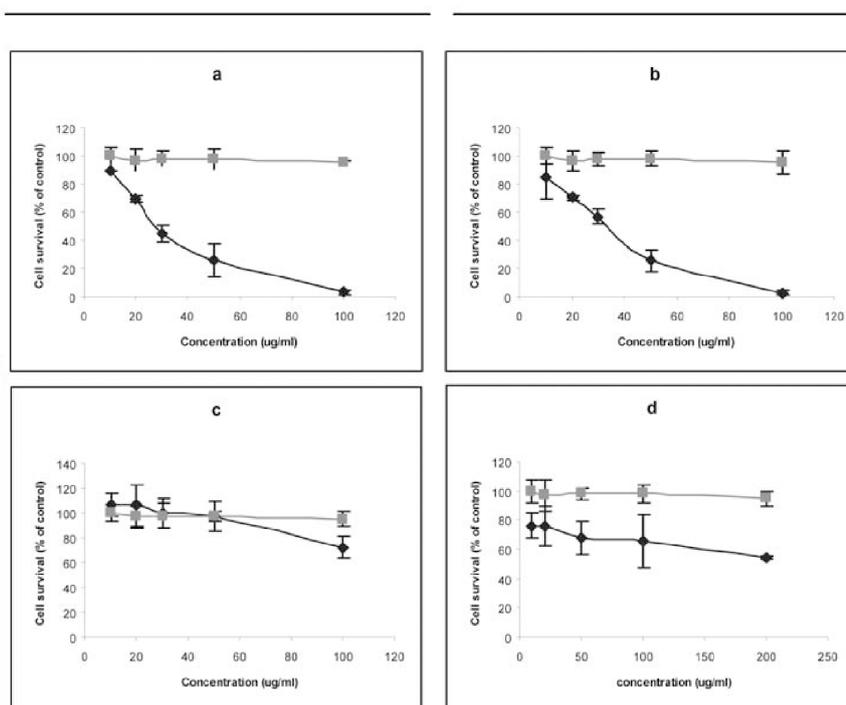


Figure 1. Dose response curves for MDA-MB-468 cell line following 72 h continuous exposure to dichloromethane (a), ethylacetate (b), methanol (c) extracts and latex (d) of *Euphorbia macroclada*. Cells were pre-incubated for 24 h prior to extract addition. Negative control: Grey line; cells (1×10^6 cell/ml) were incubated with extract solvent and assumed as 100 % survival. Results are the mean of five determinations (Mean \pm SD) and are expressed as % cell survival.

to more extraction using 500 ml of ethylacetate and methanol, respectively. The obtained extracts were evaporated to dryness under reduced pressure. Crude white milky latex of *E. macroclada* was obtained through cutting and squeezing the stem of the fresh plants and dried in vacuum oven at 40 °C. Dried latex and extracts were stored at 4 °C until used. To evaluate the biological activity, 1 mg of the dried crude extracts of dichloromethane, ethylacetate, methanol, and dried latex were dissolved in 100, 80, 50, and 100 µl of DMSO, respectively. RPMI 1640 was added to the total volume of 1 ml and stored at 4 °C as stock solutions. To evaluate the cytotoxicity of each extract 5 different concentrations (10, 20, 30, 50 and 100 µg/ml) were prepared by diluting the stock solution in RPMI 1640, so that the highest concentration was 1 mg/ml except for latex which the highest concentration was 2 mg/ml. For each individual experiment final solutions were prepared freshly from stock solution.

2.4. Cell line

MDA-MB-468 (human breast adenocarcinoma) was purchased from Pasture Institute of Iran, Tehran. It was grown in RPMI 1640 supplemented with 10% (v/v) heat inactivated fetal calf serum, penicillin-streptomycin (100 IU/ml and 100 µg/ml, respectively), sodium pyruvate (1 mM), NaHCO₃ (1 g) and L-glutamine (2 mM) and sterilized using 0.22 µm microfilters and stored at 4 °C before use.

2.5. Cytotoxic assay

The cytotoxic effects of extracts were determined against MDA-MB-468 cell line by a rapid colorimetric assay using 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) and compared with the untreated control [16]. This assay is based on the metabolic reduction of soluble MTT by mitochondrial enzyme activity of viable cells

into an insoluble colored formazan product, which can be measured spectrophotometrically after dissolving in DMSO [17]. For experiments, cells were plated in 96-well micro plates (8000 cells / well in 200 µl of medium) and incubated for 24 h (37 °C, 5% CO₂, air humidified), then 20 µl of prepared concentration of each extract was added and incubated for further 72 h in the same condition.

Taxol was used as a positive control. The second column of each microplate was assumed as negative control (containing cell suspension with a concentration of 1×10⁶ cell/ml incubated with extracts solvent). To evaluate cell survival, 150 µl of old media was replaced by fresh RPMI and 20 µl of MTT solution (5 mg/ml in phosphate buffer solution) was added to each well and incubated for 3 h. Then supernatant was removed and 150 µl of DMSO was added and pipetted to dissolve formazan crystals formed. Absorbance was then determined at 540 nm using an ELISA plate reader (startfix-2100, Awareness, USA). Each extract and latex concentration was assayed in 6 wells and each experiment was repeated at least 4 times (n ≥ 4).

Cell survival percentage was calculated using MTT assay, via following Equation.

$$\% \text{ survival} = \frac{\text{Abs in drug treated wells} - \text{Abs in blank}}{\text{Abs in control well} - \text{Abs in blank}} \times 100$$

Percentage of cell survival in the negative control was assumed as 100.

2.6. Statistical analysis

Sigmastat™ was used to perform statistical test. Analysis of variance followed by post Hoc test was used to distinguish the differences among groups. Significance was assumed at 5% level.

3. Results

Extracts which caused at least 50% growth inhibition were accounted as cytotoxic. The

dichloromethane and ethylacetate extracts had shown cytotoxic effects on MDA-MB-468 cell line at concentrations of 30 and 50 µg/ml respectively (Figure 1); whereas methanol extract and latex were not cytotoxic even at highest applied concentrations (100 and 200 µg/ml, respectively). In these studies dichloromethane extract showed most cytotoxic effect against tested cell line (IC₅₀ = 30 µg/ml).

4. Discussion

Cytotoxic effects of some *Euphorbia* species have been already evaluated on different cell lines. Betancur-Galvis and co-workers [6] showed that dichloromethane extract (using soxhlet) of leaves of *E. cotinifolia* with IC₅₀ of 35 and 18 µg/ml had most cytotoxic effect on HEP-2 and CHO cell lines, respectively, which is consistent with the results of this study. Our results showed that methanol extract of *E. macroclada* was not cytotoxic. This was consistent with Javidnia and co-worker's results [18] that showed the methanol extract of *E. hebecarpa*. was not cytotoxic against KB cell line, although this extract inhibited K₅₆₂ and U₉₃₇ growth by 66.3 and 56.1%, respectively, that can be related to various sensitivity of different cell lines.

Jassbi showed that polycyclic diterpenoids such as ingenol-type diterpenoids and triterpenoids isolated from some Iranian *Euphorbia* species are biologically active constituents that contribute to cytotoxic activity of *Euphorbia* genus [19]. Ravikanth *et al.* [20] revealed that from 8 indol diterpenes isolated from the latex of *E. nivulia*, only 3 of them were cytotoxic against 3 tested cell lines, using MTT assay. Darwish *et al.* [21] in their studies used *E. macroclada* Bioss. latex against two different strains of *Staphylococcus aureus* (standard and resistant) and showed it was effective against standard strain but the growth of *S. aureus* was not affected significantly. Furthermore, Mucsi *et al.* [22] demonstrated that cytotoxicity of diterpenes was in a dose dependent manner.

al. [22] demonstrated that cytotoxicity of diterpenes was in a dose dependent manner.

Terpenoids as lipophilic compounds are efficiently extracted by nonpolar solvents like ethyl acetate, hexane and dichloromethane [23]. This is consistent with our results showing cytotoxicity of ethyl acetate and dichloromethane extracts of *E. macroclada* with lower IC₅₀.

5. Conclusion

In summary, cytotoxic compounds in this plant mostly belong to nonpolar constituents such as terpenoids and can be extracted by nonpolar solvents. Further investigations are underway for the isolation and identification of the active constituents of the extracts.

Acknowledgments

This study was financially supported by the research council of Isfahan University of Medical Sciences (Project No. 384110), Isfahan, Iran.

References

- [1] [8] Heywood VH. *Flowering plants of the world*. London: Croom Helm, 1985; p. 185.
- [2] Evans WC. *Trease and Evans' pharmacognosy*. 13th ed.. London: Bailliere Tindall, 1989; p. 191.
- [3] Mozaffarian V. *A dictionary of Iranian plants names*. Tehran: Farhang Moaser, 1996; p. 219.
- [4] Tyler VE, Brady LR, Robbers JE. *Pharmacognosy*. 9th ed., Philadelphia: Lea & Febiger, 1988; p. 462.
- [5] Newall CA, Anderson LA, Phillipson JD. *Herbal medicines*. London: The Pharmaceutical Press, 1996; p. 109.
- [6] Bruneton J. *Pharmacognosy, phytochemistry, medicinal plants*. Paris: Lavoisier, 1995; p. 524.
- [7] Duke JA. *CRC handbook of medicinal herbs*. Boca Raton: CRC Press, 1989; pp. 189-93.
- [8] Yu FR, Lian XZ, Guo HY, Mc Guire PM, Li RD, Wang R, Yu FH. Isolation and characterization of methyl esters and derivatives from *Euphorbia kansui* (Euphorbiaceae) and their inhibitory effects on the human SGC-7901 cells. *J Pharm Pharm Sci* 2005; 8: pp. 528-35.
- [9] Betancur-Galvis LA, Morales GE, Forero JE, Roldan J. Cytotoxic and antiviral activities of

- Colombian medicinal plant extract of the *Euphorbia* genus. *Mem Inst Oswaldo Cruz* 2002; 97: 541-6.
- [10] Yang CM, Cheng HY, Lin TC, Chiang LC, Lin CC. *Euphorbia thymifolia* suppresses herpes simplex virus-2 infection by directly inactivating virus infectivity. *Clin Exp Pharmacol Physiol* 2005; 32: 346-9.
- [11] Singla A, Pathak K. Phytoconstituents of *Euphorbia* species. *Fitoterapia* 1990; 61: 483-516.
- [12] Kong LY, Li Y, Wu XL, Min ZD. Cytotoxic diterpenoids from *Euphorbia pekinensis*. *Planta Med* 2002; 68: 249-52.
- [13] Liu W, He F, Ruan Z, Gu X, Wu X, Qin G. Studies on *Euphorbia fischeriana* diterpenoid lactones inhibitory effect on human tumor cells *in vitro*. *Zhang Yao Cai* 2000; 23: 623-25.
- [14] Shi HM, Williams ID, Sung HH, Zhu HX, Ip NY, Min ZD. Cytotoxic diterpenoides from the roots of *Euphorbia ebracteolata*. *Planta Med* 2005; 71: 349-54.
- [15] Cai Y, Wang J, Liang B. Antitumor activity of the root of *Euphorbia helioscopic in vitro*. *Zhong Yao Cai* 1999; 22: 85-7.
- [16] Gerlier D, Thomasset N. Use of MTT colorimetric assay to measure cell activation. *J Immunol methods* 1986; 94: 57-63.
- [17] Mosmann T. Rapid colorimetric assay of cellular growth and survival: Application to proliferation and cytotoxicity assays. *J Immunol Methods* 1983; 65: 55-63.
- [18] Javidnia K, Miri R, Amirghofran Z. Cytotoxicity and antimicrobial assessment of *Euphorbia hebecarpa*. *Iran J Pharm Res* 2004; suppl 2: 75.
- [19] Jassbi AR. Chemistry and biological activity of secondary metabolites in *Euphorbia* from Iran. *Phytochemistry* 2006; 67: 1977-84.
- [20] Ravikanth V, Lakshmi Niranjana Reddy V, Vijender Reddy A, Ravinder K, Prabhakar Rao T, Siva Ram T, Anand Kumar K, Prakesh Vamanarao D, Venkateswarlu Y. Three new ingol diterpenes from *Euphorbia nivulia*: evaluation of cytotoxic activity. *Chem Pharm Bull (Tokyo)* 2003; 51: 431-4.
- [21] Darwish RM, Aburjai T, Al-khalil S, Mahafzah A. Screening of antibiotic resistant inhibitors from local plant materials against two different strains of *Staphylococcus aureus*. *J Ethnopharmacol* 2002; 79: 359-64.
- [22] Mucsi I, Molnar J, Hohmann J, Redei D. Cytotoxicities and anti-herpes simplex virus activities of diterpenes isolated from *Euphorbia* species. *Planta Med* 2001; 67: 672-4.
- [23] Corea G, Fattorusso C, Fattorusso E, Lanzotti V. Amygdaloidins A-L, twelve new 13 α -OH jatrophane diterpenes from *Euphorbia amygdaloides* L. *Tetrahedron* 2005; 61: 4485-94.

ONLINE SUBMISSION

ijps.sums.ac.ir