Original Article

An *in vitro* Study of Cytotoxic Activity of *Euphorbia macroclada boiss* on Mcf–7 Cells

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ABSTRACT

Objective: The study was aimed to evaluate the cytotoxic activity of acetone extract of leaves, flower and body of *Euphorbia macroclada boiss* on human breast cancer cell line (MCF-7). **Material:** The cells were plated at a cell density of $1x10^5$ cells in 96-well plates and grown with DMEM medium containing supplemented with 10% FBS and 1% penicillin. The cells were treated by different concentrations of acetone extract of *Euphorbia macroclada boiss* (10–1000 μg/mL) during 24, 48 and 72 h. The cytotoxic activities of the tested compounds were determined by cell proliferation analysis using standard (3-(4,5- dimethylthiazol-2-yl)- 2,5- diphenyltetrazolium bromide (MTT) assay. **Results:** After the evaluation of cytotoxicity assay results, it is determined that flower and body parts have a significant cytotoxic effect on MCF–7 breast cancer cell line. The values that obtained reading at 570 nm spectrophotometrically, were analyzed with GraphPad Prism7 and IC_{50} growth inhibition values was determined. **Conclusion**: The results of MTT assay showed that leaves, flower and body significantly reduced % cell viability comparative to the control. It was also shown that body had more growth inhibitory effect on MCF-7 cell compared to the leaves part.

Key words: *Euphorbia macroclada boiss, Cytotoxic activity, MCF-7, Breast cancer.*

INTRODUCTION

Breast cancer is the most common frequently diagnosed malignancy among women and leading cause of cancer death in women worldwide.[1] Drug discovery from medicinal plants has played a crucial role in the treatment of cancer.[2] Various plants, which serve as sources of medicinal compounds, have continued to play a dominant role in the maintenance of human health. Since ancient times medicinal plants have continued to be an important therapeutic aid for alleviating the ailments of human kind.[3] These medicinal herbs constitute indispensable components of the traditional medicine practiced worldwide due to their low cost, easy accessibility and ancestral experience.[4] Euphorbia species have been used in traditional medicine in many countries to treat cancer and warts.[5]

Euphorbia is a genus of plants in the Euphorbiaceae family. It contains at least 2,100 species and is one of the most diverse groups of flowering plants on earth.[6] Euphorbia is the richest genus represented by 80 species in Turkey.[7] Many plants of this genus have been used in folk medicine for centuries against various diseases including infections, skin diseases as well as cancer.[8-13] The effects of different Euphorbia species on pathogens microorganism have been studied by researchers in different parts of the world.[14-24] Recently *Battaloğlu F* showed that chemical characterization of *Euphorbia macroclada* Boiss plant that belongs Euphorbia family and grows in Nigde region of Turkey was examine and two different substances which were of diterpenoids

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(horminon) and aliphatic alcohol (1-octacosanol) were found.[25]

The study was aimed to evaluate the cytotoxic activity of acetone extract of flower, body and leaves of *Euphorbia macroclada* Boiss on *MCF-7* human breast cancer cell line.

MATERIAL AND METHODS

Plant material

Euphorbia macroclada Boiss was collected at full flowering stage from Kangal (Sivas-Turkey) province in May 2016 at altitude of 1540 m. The plant was identified by Prof. Dr. Akpulat HA, Cumhuriyet University Sivas, Turkey.

Preparation of the extracts

Dried and powdered leaves, body and flowers (5 g) were extracted 50ml of acetone, by maceration method for 24 h at room temperature with shaker separately. The extracts were filtered using Whatman filter paper (No. 1) and then concentrated in vacuo at 40°C using a rotary evaporator. The steps were reaped for two times. The obtained residues were stored in a freezer at −20°C until use.

Cell Culture

Cell lines including MCF–7 cells were maintained in DMEM medium, containing 10% fetal bovine serum (FBS), penicillin (100 U/mL) and streptomycin (10 mg/L). Cells were grown in at 37°C, 5% CO_2 and 95 % air in a humidified incubator. For each cell line, 70-80% confluent cell culture flask was trypsinized and cells were seeded in 96 well plates.

Cytotoxicity activities of acetone extracts of Euphorbia macroclada Boiss in MCF–7 cells

The *in vitro* cytotoxicity activities of acetone extracts of *Euphorbia macroclada* Boiss MCF–7 (HTB-22, human breast adenocarcinoma), and L-929 (normal cells adipose from mouse) was performed with the MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay according to the Skehan's method.[26] Briefly, cells were trypsinized and plated into 96-well plates (Corning, USA) in 0.1 mL of complete culture medium at a density of 1×10^5 cells per well and allowed to attach for 24 h. 2 µL of test substance at concentrations ranging between 10-1000 µg/mL were added into each well containing the cells. Test substance was diluted with acetone into the desired concentrations from the stock. The plates were incubated at 37°C with an internal atmosphere of 5% CO_2. After 24, 48 and 72 h incubation, with different concentrations of compounds, MTT (5 mg/ml dissolved in PBS) 10 µl/well was added directly to all the wells and incubated for 2 h at 37°C. The supernatant was carefully removed from each well and 100 mL of DMSO was added to each well to dissolve the formazan crystals. After mixing with a mechanical plate mixer for 15min, the absorbance of plates were recorded at 570 nm on a microplate reader (Bio-Tek, USA). All drug doses were parallel tested in triplicate and were performed at least 3 times; control samples were run with 2% acetone.

Statistical analysis

All experiments were carried out in triplicates and results are expressed as means ± standard error mean. Data were analyzed using one-way analysis of variance and differences were considered significant at $p < 0.05$. The IC_{50} were determined by statistical software, GraphPad Prism7 (GraphPad Software, San Diego, CA, USA).

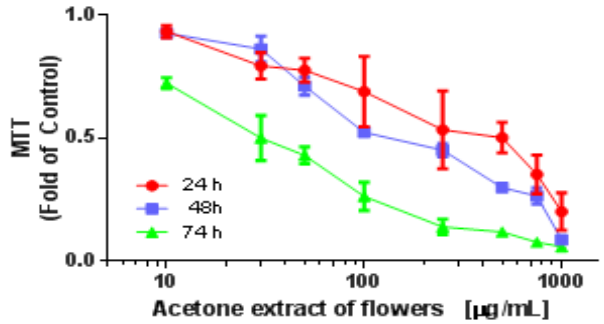
RESULTS AND DISCUSSION

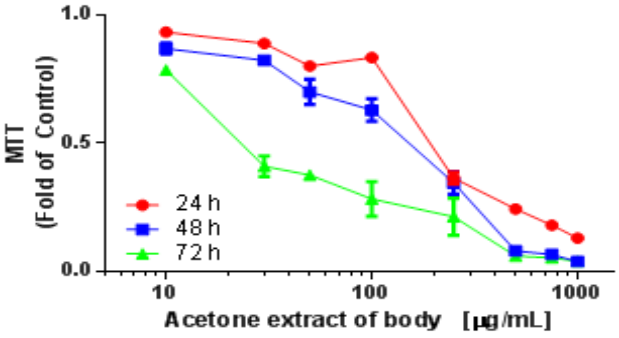
Euphorbia macroclada Boiss is a member of the Euphorbiaceae family and it grows abundantly in arid and semi-arid-land regions in Anatolia, Turkey7. Euphorbia species have been used in traditional medicine in many countries to treat cancer and warts.[5] The obtaining of the drug candidate herbal active ingredients remains important today. Kirbag *et al.* have proposed that some Euphorbia species, antibacterial and antifungal properties can be used as antimicrobial agents in the devel-

Table 1: Comparison of IC_{50} values between *Euphorbia macroclada* boiss flower, body and leaves extract on MCF-7 and Fibroblast cell lines (noncancerous cell lines) (L-929) after 24 h, 48 h and 72 h of incubation.

Extracts	IC_{50} (µg/mL±SD*)					
	MCF-7			L-929		
	24 h	48 h	72 h	24 h	48 h	72 h
Flowers	497,80 ± 0,24	154,90 ± 0,12	39,28 ± 0,06	202,30 ± 0,22	76,32 ± 0,12	45,49 ± 0,09
Body	211,80 ± 0,10	229,20 ± 0,06	24,52 ± 0,17	272,10 ± 0,09	133,50 ± 0,07	53,86 ± 0,05
Leaves	276,20 ± 0,11	127,00 ± 0,09	8,91 ± 0,10	168,70 ± 0,15	38,22 ± 0,09	9,37 ± 0,11

* Cytotixicity as IC_{50} for each cell lines is in average of three independent experiments after treatment for 24 h, 48 h and 72 h.





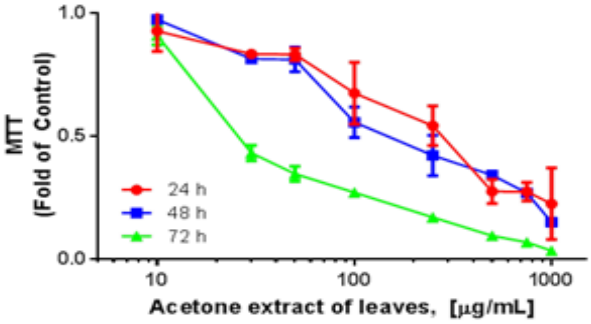
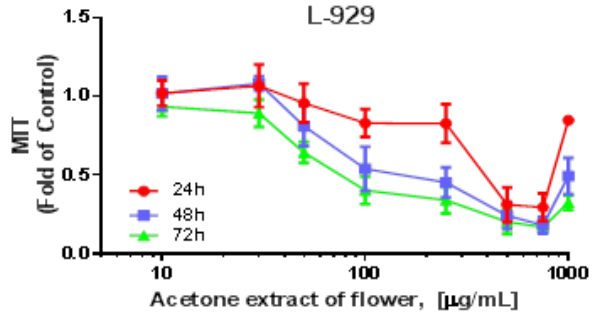
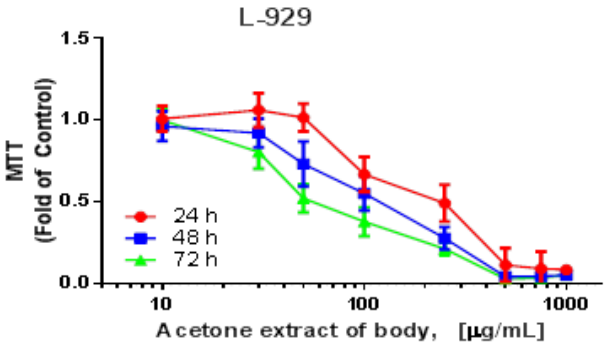


Figure 1: Cytotoxi activities of *Euphorbia macroclada boiss* flower, body and leaves extract on MCF-7.





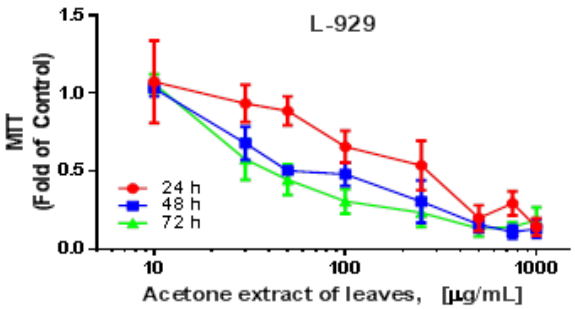


Figure 2: Cytotoxi activities of *Euphorbia macroclada boiss* flower, body and leaves extract on Fibroblast cell lines (L929 Mouse).

opment of new drugs for the treatment of infectious disease.[27]

In this study, acetone extracts of flower body and leaves of *Euphorbia macroclada* Boiss were prepared. These extracts were first applied to the MCF-7 cell line for 24 h, 48 h and 72 h. The cytotoxicity of the extracts was found to be concentration and time dependent and the cell viability decreased with increasing concentration of extracts (Figure 1-2). Results indicated that acetone extracts of leaves were more active in the MCF-7 cell line than other extracts after 24 h, 48 h and 72 h incubation. In the second step of the experiment, all extracts were applied to L-929 (normal cells adipose from mouse) and incubated for 24 h, 48 h and 72 h. Similar to MCF-7 cell lines, acetone extract of leaves was more active than the other extracts for all time point. Acetone extract of leaves of *Euphorbia macroclada* Boiss was found more cytotoxicity activity on MCF-7 cell lines compared to L-929 for after 72 h incubation Table 1.

CONCLUSION

As a result, in this study, acetone extract of leaves of *Euphorbia macroclada* Boiss was found to be more active in both cell lines. This extract was noted to be more effective in the MCF-7 cell line than the L-929 cell line. Analysis of the active ingredients of leaves extract with have highest cytotoxic activity will be performed.

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CONFLICT OF INTEREST

There are no conflicts of interest among the authors.

ABBREVIATIONS

MTT: (3-(4,5- dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; **FBS:** fetal bovine serum; **DMEM:** Dulbecco's Modified Eagle Medium; **MCF–7:** HTB-22, human breast adenocarcinoma; **L-929:** Normal cells adipose from mouse; **DMSO:** Dimethyl Sulfoxide.

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SUMMARY

- The study was aimed to evaluate the cytotoxic activity of acetone extract of leaves, flower and body of *Euphorbia macroclada* Boiss on human breast cancer cell line (MCF-7) and L-929. The cells were plated at a cell density of 1x105 cells in 96-well plates and grown with DMEM medium containing supplemented with 10% FBS and1% penicillin. The cells were treated by different concentrations of acetone extract of *Euphorbia macroclada* Boiss (10–1000 μg/mL) during 24, 48 and 72 h. The cytotoxic activities of the tested compounds were determined by cell proliferation analysis using standard (3-(4,5- dimethylthiazol-2-yl)- 2,5- diphenyltetrazolium bromide (MTT) assay. After the evaluation of cytotoxicity assay results, it is determined that flower and body parts have a significant cytotoxic effect on MCF–7 breast cancer cell line. The values that obtained reading at 570 nm spectrophotometrically, were analyzed with GraphPad Prism7 and IC_{50} growth inhibition values was determined. The results of MTT assay showed that leaves, flower and body significantly reduced % cell viability comparative to the control. It was also shown that body had more growth inhibitory effect on MCF-7 cell compared to the leaves part.

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