

## ***In vitro* evaluation of cytotoxic activity of flower, leaf, stem and root extracts of five *Artemisia* species**

**B. Gordanian<sup>1</sup>, M. Behbahani<sup>2,\*</sup>, J. Carapetian<sup>1</sup> and M. Fazilati<sup>3</sup>**

<sup>1</sup>Department of Biology, Faculty of Science, Urmia University, Urmia, I.R. Iran.

<sup>2</sup>Department of Biotechnology, Faculty of Advanced Sciences and Technologies, University of Isfahan, Isfahan, I.R. Iran.

<sup>3</sup>Department of Biochemistry, Payam Nour of Tehran University, Tehran, I.R. Iran.

---

### **Abstract**

The present study was carried out to investigate cytotoxic activity of flower, leaf, stem and root extracts of five *Artemisia* species against breast cancer cell line (MCF7) and human embryonic kidney normal cell line (HEK293). The studied *Artemisia* species were *A. absinthium*, *A. vulgaris*, *A. incana*, *A. fragrans* and *A. spicigera*. The cytotoxic activity was measured by MTT assay at different concentrations (62.5, 125, 250, 500 µg/ml). Among these five species, methanol extracts of flower, leaf, stem and root of *A. absinthium* and *A. vulgaris* exhibited considerable cytotoxic activity. The flower extracts of these two species were found to have higher cytotoxic effect on MCF7 cell with an IC<sub>50</sub> value of 221.5 and >500 µg/ml, respectively. Leaf methanol extract of *A. incana* also showed cytotoxic activity. Cytotoxic activity of different extracts of *A. absinthium*, *A. vulgaris* and *A. incana* against MCF7 was 10%-40% more than HEK293 cells. Not only the extracts of *A. spicigera* and *A. fragrans* did not show any cytotoxic effect against both cell lines, but also increased the number of cells. This study revealed that *A. absinthium* and *A. vulgaris* may have a great potential to explore new anticancer drugs.

**Keywords:** *Artemisia*; MCF7; HEK293; MTT assay

---

### **INTRODUCTION**

*Artemisia* is a shrub or small herb that grows in dry and semi-dry regions. This genus chiefly is found in northern hemisphere and lowers in southern hemisphere. The genus *Artemisia* belongs to the Anthemideae tribe of Asteraceae family. There are about 500 species of herbs and shrubs in this genus (1). *Artemisia* species are mainly found in Asia, Europe and North America (2).

The greatest number of these species was attained in Asia (3). Thirty five species of this genus are found in Iran (4). The *Artemisia* species have been used in Iranian traditional medicine as anti-infectious, anti-bacterial, gastric tonic, digestive and stomachic (5). Recently, monoterpenes, sesquiterpenes, sesquiterpene lactones, flavonoids, coumarins, sterols, polyacetylenes have been isolated from *Artemisia* species (6-8). Previous studies on some *Artemisia* species have shown that most

species possess medicinal properties such as anti-bacterial and anti-cancer effects (9-12). Many *in vitro* and *in vivo* studies have been published on the anticancer activity of different species of *Artemisia* (13-15).

Some studies reported that fresh leaves of *A. absinthium* and flowers of *A. vulgaris* have strong cytotoxic effect against breast cancer cell line (MCF7) (16,17). But there is no scientific study available about anti-cancer activities of flower, leaf, stem and root of *A. absinthium* and *A. vulgaris*. The present study is focused to evaluate the cytotoxic activity of methanol extract prepared from various organs of *Artemisia* species on MCF7 cell line and human embryonic kidney normal cell line (HEK293).

### **MATERIALS AND METHODS**

#### ***Plant material***

Five species of *Artemisia* (*A. absinthium*, *A.*

---

\*Corresponding author: M. Behbahani  
Tel. 0098 937 5076829, Fax. 0098 311 6284782  
Email: ma.behbahani@ast.ui.ac.ir

*vulgaris*, *A. fragrans*, *A. spicigera* and *A. incana*) were collected from two different regions in Urmia city, West Azerbaijan Province, Iran, in October 2011. These species were recognized after a series of taxonomic revisions. The Voucher specimens were deposited at the Herbarium of National Botanical Garden of Iran.

#### **Preparation and analysis of extract**

The samples were separated into flower, leaf, stem and root parts. The plant material was carefully dried in shadow and powdered. The dried plant samples (30 g) were separately placed in a stopped conical flask and macerated with 250 ml methanol (98% v/v, Merck, Germany) at room temperature (25–28°C) for 72 h with occasional stirring. The solvent was filtered and evaporated in a vacuum rotary evaporator (Stroglass, Italy) at 45°C. The residue was placed in freeze drier (Zirbus, Germany) to dry. The crude extract was stored in a well-closed container, protected from light and kept in a refrigerator at 4°C. 40 mg of the sample extract were dissolved in 1 ml 100% (v/v) of dimethyl sulfoxide (DMSO). Each experiment was performed in triplicate.

#### **Cell lines and culture medium**

Cell lines were purchased from the Cell Bank of Pasteur Institute in Tehran (Iran). MCF7 and HEK293 cells were cultured in Roswell Park Memorial Institute (RPMI-1640) and Dulbecco's Modified Eagle Medium (DMEM) respectively and supplemented with 10% (v/v) fetal calf serum (FCS), 100 U/ml penicillin and 100 µg/ml streptomycin, 2 mM/L glutamine and 1 mM sodium pyruvate. All of the reagents were purchased from Gibco (Scotland).

#### **Cytotoxicity assay**

The cellular toxicity of the methanol extracts of five species of *Artemisia* on cultured cells were measured using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay (18).

The cells were grown in 96-well plates at a density of  $5 \times 10^4$  cells per well. After 2 h, cells were treated with different concentrations

of samples (62.5, 125, 250, 500 µg/ml) and incubated for 48 h. Later, 25 µl of the MTT solution (5 mg/ml) was added to each well, and the plate was reincubated for 2 h. Finally, the medium was removed and 100 µl of DMSO was added to solve formazan crystals.

The amount of formazan crystal was determined by measuring the absorbance at 570 nm by using a micro plate spectrophotometer (Awareness Technology Inc., stat fax 2100).

The survival curves of each cell line were established based on extract concentration after the specified period.

#### **Statistical analysis**

Each experiment was performed in triplicate and repeated two times. The experiments were performed using completely randomized design (CRD) and results were analyzed using one way ANOVA. Statistical analyses were performed by using Software SAS, Version 6.12. Probability  $P < 0.05$  were considered significant.

## **RESULTS**

The different parts of five species of *Artemisia* were tested for cytotoxicity against MCF7 and HEK293. All extracts from species were tested under comparable conditions at different concentrations (62.5, 125, 250, 500 µg/ml).

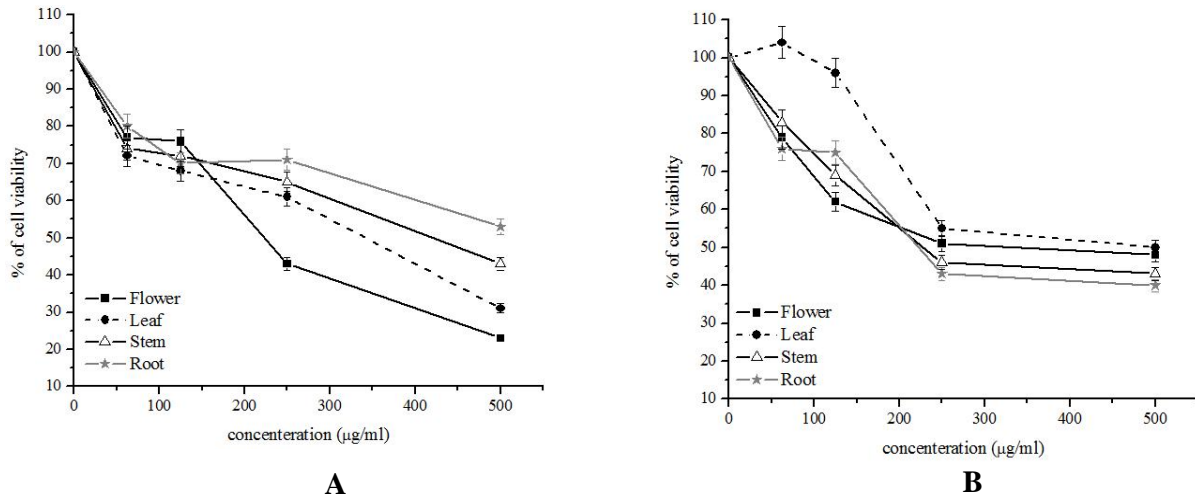
The plant extracts were included flower, leaf, stem and root. Among these five species, methanol extracts of different organs of *A. absinthium* and *A. vulgaris* strongly exhibited cytotoxic activity against MCF7 (Figs. 1, 2). The flower extracts of *A. absinthium* and *A. vulgaris* were shown higher cytotoxic effect against MCF7 cell with an  $IC_{50}$  value of 221 and  $>500$  µg/ml, respectively.

The cytotoxicity was followed by leaf, stem and root extracts with  $IC_{50}$  343, 430 for *A. absinthium* and  $IC_{50} > 500$ , µg/ml, for *A. vulgaris* respectively.

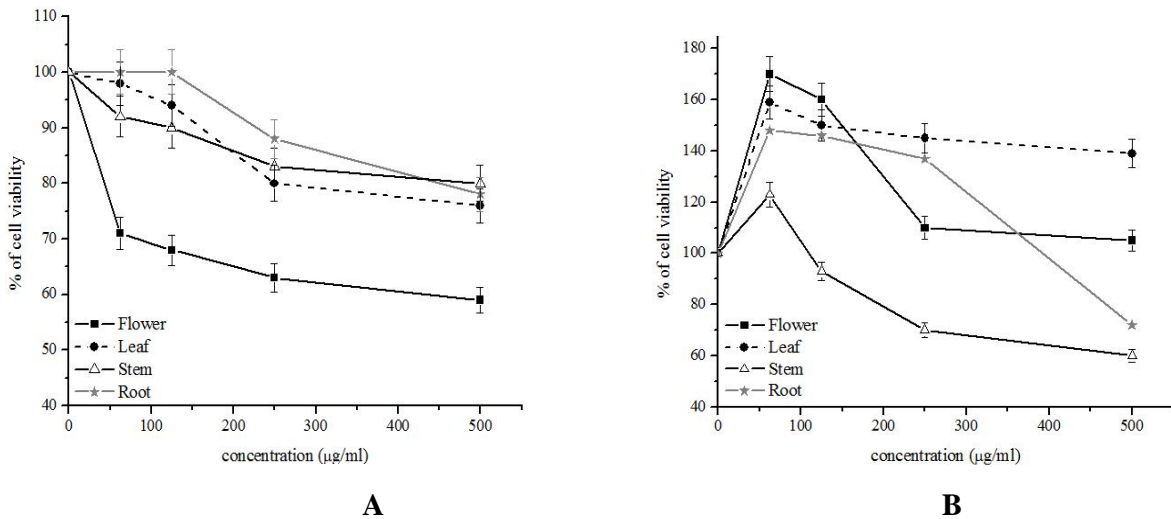
Based on these results, different organs of *A. absinthium* had stronger activity than *A. vulgaris*. Methanol leaf extract of *A. incana* showed cytotoxic activity at higher concentration (500 µg/ml) which decreased

cell viability up to 32% and the other organs did not have any cytotoxic effect (Fig. 3). In spite of above results, different parts of *A. spicigera* and *A. fragrans* significantly increased the number of MCF7 and HEK293 cell lines (Figs. 4, 5). So that methanol extract

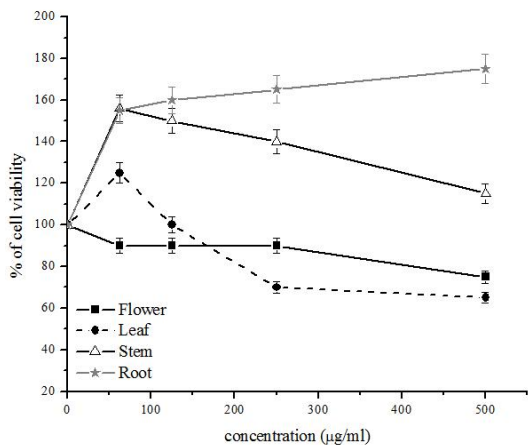
of root, leaf, flower and stem in *A. spicigera* increased MCF7 proliferation up to 93%, 86%, 79% and 60% respectively and similarly *A. fragrans* increased the number of cancer cells up to 54%, 50%, 31% and 11% at concentration of 500  $\mu\text{g/ml}$ .



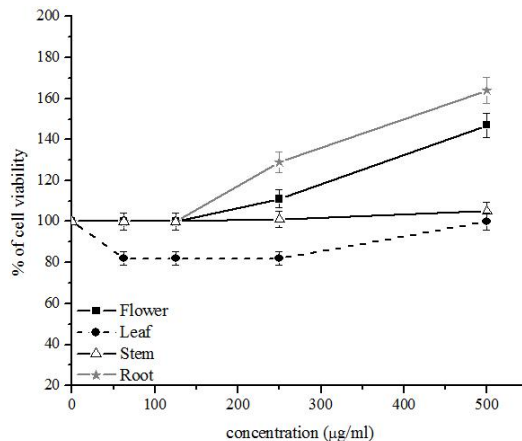
**Fig. 1.** Cytotoxic effect of flower, leaf, stem and root extracts of *A. absinthium* against A: MCF7 and B: HEK293 cells. Cell viability was assessed using MTT assay. Data are shown as Mean  $\pm$  SD, n=3.



**Fig. 2.** Cytotoxic effect of flower, leaf, stem and root extracts of *A. vulgaris* against A: MCF7 and B: HEK293 cells. Cell viability was assessed using MTT assay. Data are shown as Mean  $\pm$  SD, n=3.

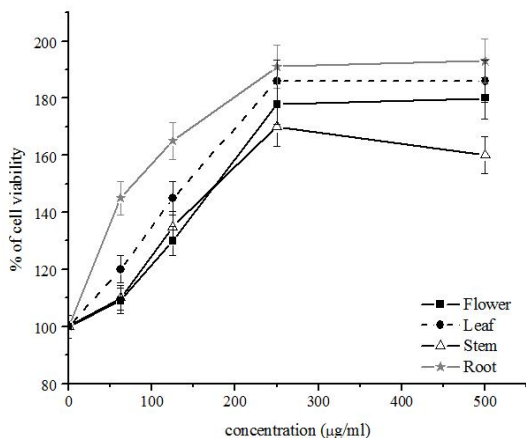


**A**

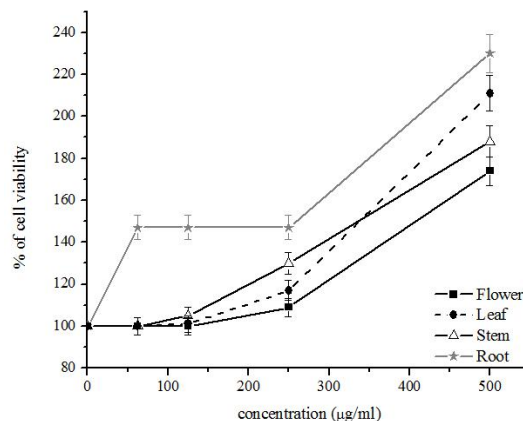


**B**

**Fig. 3.** Cytotoxic effect of flower, leaf, stem and root extracts of *A. incana* against A: MCF7 and B: HEK293 cells. Cell viability was assessed using MTT assay. Data are shown as Mean  $\pm$  SD, n=3.

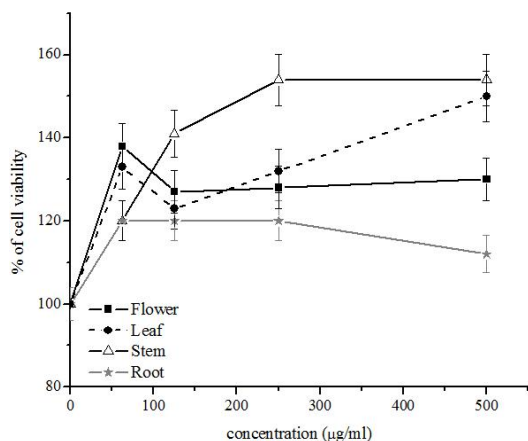


**A**

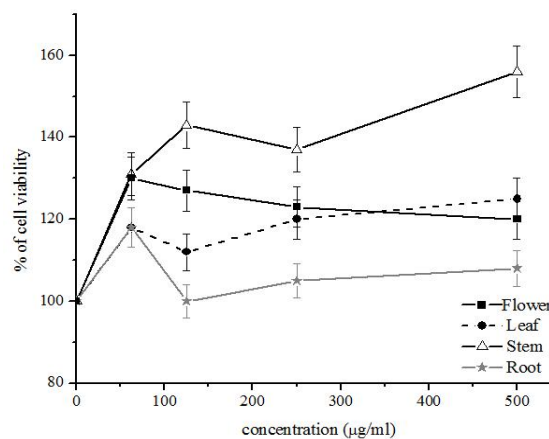


**B**

**Fig. 4.** Cytotoxic effect of flower, leaf, stem and root extracts of *A. spicigera* against A: MCF7 and B: HEK293 cells. Cell viability was assessed using MTT assay. Data are shown as Mean  $\pm$  SD, n=3.



**A**



**B**

**Fig. 5.** Cytotoxic effect of flower, leaf, stem and root extracts of *A. fragrans* against A: MCF7 and B: HEK293 cells. Cell viability was assessed using MTT assay. Data are shown as Mean  $\pm$  SD, n=3.

## DISCUSSION

The results showed that methanol extract of different parts of *A. absinthium* and *A. vulgaris* has strong cytotoxic activities against MCF7. Aqueous, methanol and acetone extracts of *A. absinthium* have been reported to possess excellent anticancer activity against MCF7 by IC<sub>50</sub> values of 244.9, 14.31 and 6.11 µg/ml respectively (16). Nawab and coworkers, observed that *A. vulgaris* has significant dose-dependent inhibition of the proliferation and viability of the cancer cells such as colon carcinoma (RKO), prostate cancer (PC3) and human breast cancer (T47D ) (17). The other species like *A. princeps* have also been reported to inhibit the growth of breast cancerous cells generally in a concentration dependent way (12).

Phytochemicals screening of the methanol extract from aerial parts of *A. vulgaris* and *A. absinthium* revealed the presence of tannins and flavonoids (such as eupafolin, diosmetin, rhamnetin, apigenin and their glucosides, luteolin, quercetin, rutin and vitexin) which are characterized for their anticancer properties (17,19). Nibret and coworkers reported that the aerial parts of *A. absinthium* have cytotoxic activity against human promyelocytic leukemia cells (HL60) (20).

In the present study, flower extract of *A. vulgaris* and *A. absinthium* have the most cytotoxicity against MCF7 cells compared to those of leaf, stem and root extracts. It may be due to accumulation of artemisinin in flower extract of these two species in comparison with stem and roots. It has been reported that flower and leaf extracts of some *Artemisia* species are rich sources of artemisinin (21).

It has also been revealed a good correlation between the artemisinin accumulation in different parts of *Artemisia* species and its anticancer activity. artemisinin is a sesquiterpen lactones and demonstrated anticancer activities when tested *in vitro* and *in vivo* (22). Results of present research showed that cytotoxic activity of different parts of *A. absinthium* is more than that of *A. vulgaris*. The higher cytotoxicity of *A. absinthium* could be due to the high content of artemisinin in flowers, leaves, stems and roots which have been reported before (21).

In this work, the results of MTT assay showed that *A. spicigera* and *A. fragrans* have an increasing effect on number of MCF7 and HEK293 cell lines.

This increasing effect is due to occurrence of some compounds in these two species that can stimulates cell proliferation. Previous studies have reported that some ingredients in natural products can significantly increase proliferation of cancer and normal cell lines. The low concentration of essential oils isolated from *Artemisia princeps* has been shown to possess an increasing effect on proliferation of endothelial cells (23). Le Bail and coworkers, reported that flavonoids at low concentrations can significantly enhance the proliferation of human breast cancer cells MCF7 (24). These results indicated that the essential oils and flavonoids contained some low molecular-weight components stimulates the proliferation of vascular endothelial cells *in vitro* (25).

## CONCLUSION

In conclusion, these findings demonstrated *flower extracts of A. absinthium* and *A. vulgaris* and leaf methanol extract of *A. incana* can be used as cytotoxic agents, however, further investigations have to be performed.

## ACKNOWLEDGMENT

The authors acknowledge the hard work of Dr. Mozafarian for identification of plants and Mr. Sajad Nouri for collection of them.

## REFERENCES

1. Valles J, McArthur ED. *Artemisia* systematics and phylogeny: Cytogenetic and molecular in sights. USDA Forest Service Proceedings RMRS-P-21. 2001;67-74.
2. Bora KS and Sharma A. The genus *Artemisia*: A comprehensive review. Pharm Biol. 2011;49:101-109.
3. Jose AM, Miguel BL, Apaza L, Bermejo P. The *Artemisia* L. Genus: A review of bioactive essential oils. Molecules. 2012;17:2542-2566.
4. Mozafarian V. Flore of Iran. 1st ed. Research institute of forests and Rangelands. Tehran. 2008. p. 199-260.
5. Nezhadali M, Parsa M. Study of volatile compounds in *Artemisia Sagebrush* from Iran using HS/SPME/GC/MS. Int J Environ Sci Dev. 2010;3:287-289.

6. Hoffman E J. Cancer the search for selective biochemical inhibitors. 2nd ed. CRC Press, Boca Raton. 1999. p. 95-97.
7. Sengul M, Ercisli S, Yildiz H, Gungor N, Kavaza A, Cetin B. Antioxidant, antimicrobial activity and total phenolic content within the aerial parts of *Artemisia absinthium*, *Artemisia santonicum* and *Saponaria officinalis*. Iran J Pharm Res. 2009;10:49-56
8. Rustaiyan A, Ezzatzadeh E. Sesquiterpene lactones and penta methoxylated flavone from *Artemisia kulbadica*. Asian J Chem. 2011;23:1774–1776.
9. Akrouf A, Gonzalez LA, Hajer EJ. Antioxidant and antitumor activities of *Artemisia campestris* and *Thymelaea hirsuta* from southern Tunisia. Food Chem Toxicol. 2011;49:342-347.
10. Zahi D, Supaibulwatana K, Zhong J. Inhibition of tumor cell proliferation and induction of apoptosis in human lung carcinoma 95-D cells by a new sesquiterpene from hairy root cultures of *Artemisia annua*. Phytomedicine. 2010;17:856-861.
11. Devmurari VP, Jivani NP. Anticancer evaluation of *Artemisia nilagirica*. Int J Pharm Tech Res. 2010;2:1603-1608.
12. Sarath VJ, So CS, Young DW, Gollapudi S. *Artemisia princeps* var *orientalis* induces apoptosis in human breast cancer MCF-7 cells. Anticancer Res. 2007;27:3891-3898.
13. MCGovern PE, Chirstofidou-Solomidou M, Wang W, Dukes F, Idson T, El-Deiyr WS. Anticancer activity of botanical compounds in ancient fermented beverages (Review). Oncology. 2010;37:5-14.
14. He L, Wu Y, Lin L, Wang J, Wu Y, Chen Y, et al. Hispidulin a small flavonoid molecule, suppresses the angiogenesis and growth of human pancreatic cancer by targeting vascular endothelial growth factor receptor 2-mediated PI3K/Akt/mTOR signaling pathway. Cancer Sci. 2011;102:219-225.
15. Perumal P, Rajesh V, Sekar V, Gandhimathi S, Sampathkumar R, Jayaseelan S, et al. Antitumor and antioxidant activity of *Artemisia nilagirica* (Clarke) against Ehrlich's ascites carcinoma in swiss albino mice. Inter J Pharm. 2010;9:1.
16. Feidberg R. An investigation into the antimicrobial and anticancer activities of *Geranium incanum*, *Artemisia afra* and *Artemisia absinthium* [dissertation]. Faculty of Health Science, Nelson Mandela Metropolitan University. 2009;95-205.
17. Nawab A, Yunus M, Mahdi A, Gupta S. Evaluation of anticancer properties of medicinal plants from the Indian sub-continent. Mol Cell Pharm. 2011;3:21-29.
18. Mosmann T. Rapid colorimetric assay for cellular growth and survival Application to proliferation and cytotoxicity assays. J Immunol Meth. 1983;65:55-63.
19. Blagojevic P, Radulovic N, Palic R, Stojanovic G. Chemical composition of the essential oils of Serbian wild-growing *Artemisia absinthium* and *Artemisia vulgaris*. J Agric Food Chem. 2006;54:4780-4789.
20. Nibret E, Wink M. Volatile components of four Ethiopian *Artemisia* species extracts and their *in vitro* antitrypanosomal and cytotoxic activities. Phytomedicine. 2010;17:369-374.
21. Mannan A, Ahmed I, Arshad W, Asim FM, Qureshi AR, Hussain I, et al. Survey of artemisinin production by diverse *Artemisia* species in northern Pakistan. Malaria journal. 2010;9:310.
22. Ferreira JFS, Luthria DL, Sasaki T and Heyerick A. Flavonoids from *Artemisia annua* L. as antioxidants and their potential synergism with artemisinin against malaria and cancer. Molecules. 2010;15:3135-3170.
23. Kaji T, Kaga K, Miezi N, Ejiri N, Sakuragawa N. A stimulatory effect of *Artemisia* leaf extract on the proliferation of cultured endothelial cells. Chem Pharm Bull. 1990;38:538-540.
24. Le Bail JC, Varnat F, Nicolas JC, Habrioux G. Estrogenic and antiproliferative activities on MCF-7 human breast cancer cells by flavonoids. Cancer Lett. 1998;14:209-216.