

Antiproliferative activity of aqueous extract from *Arictum lappa L.* root in human erythroleukemia cell line (k562) and lymphocyte cell

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Abstract: *Arictum lappa* is extensively used in traditional Iranian medicine to treat upper respiratory infections and pneumonia in Iran as well as other countries. The objective of this study was to determine the cytotoxic effect of the aqueous extract of the roots of the plant *Arictum lappa* against endothelial human bone marrow cells (line k562) and human lymphocytes as a model of cancerous cell and normal respectively under different concentrations using the MTT assay. Percentage cell viability of cell lines were carried out by using Trypan blue dye exclusion technique MTT assay was used to evaluate the reduction of viability of cell cultures in the presence and absence of the extract. The absorbance was measured at 570 nm immediately using an ELISA reader. The results showed that the extract, exhibited marked cytotoxic effect on the k562 cell line and lymphocyte at a maximum concentration of 10 mg/ml culture medium. In fact at the highest concentration tested (10 mg/ ml culture medium) with a 24-hrs incubation period, *A. lappa* extract resulted in 96% (in average) inhibition of cell proliferation in both lymphocyte and k562 cells. Therefore this plant could be considered as potential source of anticancer compounds. Further studies are necessary for chemical characterization of the active principles and more extensive biological evaluations.

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Introduction

Plants have been a source of medicine for thousands of years, and phytochemicals continue to play an essential role in medicine (1). In fact, plant-derived compounds and their semi-synthetic, as well as synthetic analogs, serve as major source of pharmaceuticals for human diseases. In developing countries, the practice of medicine still relies heavily on plant and herbal extracts for the treatment of human ailments. Dietary agents consist of a wide variety of biologically active compounds that are ubiquitous in plants, and many of them have been used as traditional medicines (2, 3). Some phytochemicals derived in spices and herbs as well as other plants possess substantial cancer preventive properties (4, 5). Plant diversity is an important source of new molecules. More than 60% of the anticancer agents used today are derived directly or indirectly from natural sources. Higher plants have been one of the largest sources of new compounds with pharmacological activity (6). Hence, for the treatment of disease states wherein drug therapy is a rational approach, plant materials represent legitimate starting materials for the discovery of new agents (7). In the case of human cancers, thus far, nine plant derived compounds have been approved for clinical use as anticancer drugs in Iran. For example, several of the current chemotherapeutic drugs like vinblastine, methotrexate, taxol, and so forth, were

first identified in plants (8). Ethnobotanical approach is a strategy that has successfully identified new bioactive molecules from diverse plants. In this approach, the information obtained from traditional communities about the use of medicinal plants is combined with chemical/ pharmacological studies performed in laboratories (9). This strategy has been helpful in plant pharmacological research and has yielded better results than the random approach used in different experimental models (10, 11). In fact, in the cancer drug discovery program, a paradigm based on ethnobotanical and ethnopharmacological data would be more economical and beneficial for identifying potential anticancer molecules than mass screening of plant species. It is estimated that approximately the most of prescriptions handled in Iran contain a plant-derived natural product and the most important drugs currently contain ingredients from plants used in traditional medicine. Plants of Asteraceae family (Compositae) have been frequently and widely used in traditional systems of medicine practiced in many Asian countries, and their medicinal functions have been broadly discussed and accepted in many traditional recipes. The Compositae plants contain a number of volatile and essential oils including terpenoids, phenylpropanoids, flavonoids, and sesquiterpenes, which have reported antitumor activity (12). As plants of compositae family are considered safe for

human consumption, these species are excellent candidates for development of novel chemotherapeutics. *Arctium lappa*, a medicinal plant belonging to this family, locally known as burdock that has traditionally been used against arthritis, blood purifier, myelin, eczema, acne, dysphasia and ureic (13). Burdock in Iran is extensively used in traditional Iranian medicine to treat upper respiratory infections and pneumonia (14). In addition, arctium has also been found useful in the anti-inflammatory and radical scavenge effects and anti-oxidant (15, 16, 17). This plant has *in vitro* anti-HIV activity because of natural lignans (18). Chemical investigations of *A. lappa* have been shown that the plant contain tannin and flavonoid, mucilage, alkaloid, organic acids; aldehydes; carbohydrates; phytosterols, inulin, fructofuranan, caffeoylquinic acid (12). Seeds contain arctigenin which may help memory (19). Arctiin and its aglucone, arctigenin has shown potent *in vitro* antiviral activities against influenza virus in mice. Arctiin and arctigenin isolated from *A. lappa* root extract has been found to have substantial antitumor and anti-inflammatory activity against promyelocytic leukemic HL-60 cells (20, 21). In the other study, some novel lignin compounds like arctiin and arctigenin have been reported from the root, leaf extract of *Bardanae furctus* (Goboshi) extract which has cytotoxic, anti proliferation, inhibitory activity against human hepatoma HepG2 cells and mouse sarcoma 180 cells. These compounds showed strong cytotoxicity against HepG2 cells, but little toxicity against Chang liver cells (22). Uncontrolled proliferation is a universal property of tumor. These strategies were found to select of plants species in anticancer drug discovery. In fact, investigation of the cellular growth control mechanisms has contributed to the understanding of carcinogenesis and identification of compound with potential antitumoral properties for future studies. In this paper, we report the cytotoxic potential screening of *A. lappa* root extract to determine the cytotoxicity against human bone marrow cell line and lymphocyte as normal cell using MTT assay.

Materials and Methods

Plant material and extraction

The roots of *A. lappa* were collected from the campus of Agriculture Department, Bu-Ali Sina University, Hamedan, Iran. The plant materials were dried at room temperature in the dark and ground finely using blender. Exactly 20 g of powdered sample was extracted in a flask by adding distilled hot boiling water for 24 h at room temperature with constant shaking. Thereafter, the extract was filtered through a filter paper to remove particulate matter. The extracts were pooled and evaporated to dryness under reduced pressure at 40°C. Therefore the weight

of the residue was recorded and percent yield calculated. Then the stock solution was then prepared by dissolving the extract powder in distilled water and the experimental concentrations were diluted in basal medium.

In vitro cytotoxic activity assay

Cell Lines and Culture Conditions. Endothelial human bone marrow (line k562) cell lines were maintained in RPMI-1640 media supplemented with 10% fetal bovine serum, 100 IU/ml penicillin and 100 μ g/ml streptomycin (Invitrogen, Carlsbad, CA, USA). Cells were maintained in a humidified atmosphere of 5% CO₂ at 37°C in an incubator. Prior to use in the assay the cells were grown to 80 - 90% confluence and synchronized by incubation in the assay media for 4 hrs. Cell suspensions (5×10^3 cells/100 μ l/well) were then incubated with various concentrations (0.25, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 0.8, 1, 3, 5, 7.5, 10 mg) of aqueous plant extract solutions for 24 hrs.

Isolation of lymphocytes from whole blood

Three ml of blood were taken from normal healthy individuals and collected in heparinised test tube. Five ml of Phosphate Buffered Saline (PBS) were added and mixed well. Two ml of ficoll hypaque solution were taken and carefully layered blood PBS mixture on to the ficoll hypaque solution. It was centrifuged at 2000 rpm for 30 minutes. The opaque interface containing mononuclear cells was collected, mixed with PBS, and centrifuged at 1500 rpm for 10 minutes, and supernatant was discarded. The centrifugation was repeated thrice, and normal lymphocytes were resuspended in RPMI medium with 10% fetal bovine serum. Then the cells were plated in 96-well plates at 10^4 cells/100 μ l/well for the normal lymphocytes and used for cytotoxicity analysis.

In vitro cytotoxicity studies

Viability staining by trypan blue dye exclusion method. The aqueous extract was studied for short term *in vitro* cytotoxicity using human bone marrow cells (k562 cell line) and lymphocyte cells as normal cells. The cells were seeded in 96-well plates. Four wells for each concentration were seeded and triplicate plates were used the cell line. Then, the cells were incubated at 37°C. After 24 h the medium was replaced by fresh medium containing different concentrations of the plants extract. After incubation 0.1 ml trypan blue was added and number of dead cells determined by using haemocytometer.

$$\% \text{ viability} = (\text{live cell count} / \text{total cell count}) * 100$$

Cytotoxicity assay

Cytotoxicity of sample on tumor cells was measured by microculture tetrazolium (MTT) assay (Mosmann, 1983). For the assays, 96-well microplates were seeded with 100 μ l medium

containing 5,000 cells. After 24 h incubation and attachment, the cells were treated with 6 fourfold dilution of crude extracts. Exactly from the stock solution (40 mg/ml), each extract sample was applied in a series of 6 dilutions (final concentrations ranging from 15.6 to 500 $\mu\text{g/ml}$) with a final DMSO concentration of 0.1% and was tested in quadruplicate. After 48 h incubation, cell viability was determined by adding (Sigma) tetrazolium salt as cytotoxicity indicator, so after 48h of incubation, 15 μl of MTT (5mg/ml) in phosphate buffered saline (PBS) was added to each well and incubated at 37°C for 4h. The medium with MTT was then flicked off and the formed formazan crystals were solubilized in 100 μl of DMSO and then measured the absorbance at 570 nm using micro plate reader. Tetrazolium salts are cleaved to formazan dye by cellular enzymes (only in the viable cells).

Light microscopy

Human bone marrow cell cancer and normal cells were grown to 70% confluence and treated with 5% aqueous plant extracts for 24h and the photographs were taken at 40X magnification using a phase-contrast inverse microscope (Olympus, Japan).

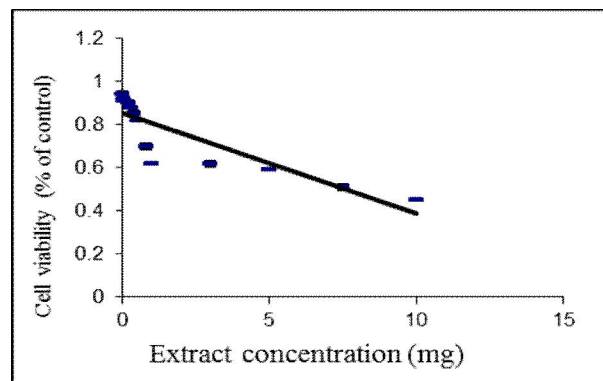
Statistical Analysis

All experiments were conducted with 4 replicates and the results expressed as mean \pm standard deviation. Differences between the control and the individual dosage group of each extract were analyzed by means of the Duncan's test of significance at the $P < 0.05$ level. The concentration that results in cell inhibition value of the extracts were calculated from the equation of the logarithmic line determined by fitting the best line to the curve formed from the data using Microsoft Excel.

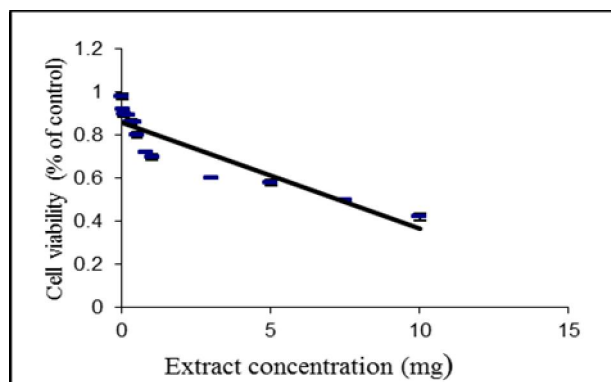
Results

The medicinal use of plants is probably as old as human kind itself. More than 150000 plant species have been studied, and many of them contain therapeutic substances (Ishii 1984). These substances can be extracted and used in the preparation of drug, or the plant itself can be used directly as a medication. Some medicinal plants that are used in the traditional medicine for various diseases, including cancer, were collected and evaluated for their cytotoxic activities. In this investigation the cytotoxic activity was evaluated on two human cells. The cytotoxic effect of aqueous plant extract on k562 cell line and lymphocyte cell was determined using the MTT assay. The cytotoxicity curves extract in human bone marrow cell line in comparison with normal human lymphocytes is presented respectively in Figures 1(A and B). Detection of Cell Viability by MTT Assay. Metabolic activity or cytotoxicity of sample on cells by measuring the activity of a mitochondrial enzyme succinate dehydrogenase was

measured by microculture tetrazolium (MTT) assay. In this study, we used the MTT test to evaluate the cytotoxic effect of aqueous plant extract on k562 cell line and lymphocyte cells. Therefore, cancer and normal cells were exposed to increasing concentrations (0.025-10 mg ml^{-1} of culture medium) of the aqueous of *A. lappa* extracts for 24 h.



A



B

Figure 1. cytotoxicity activity of aqueous extract of *A. lappa* against k562 cells (A) and lymphocytes cells (B). Cells were incubated with different concentrations of the plant extracts (ranged from 0.025-10 mg ml^{-1} $\mu\text{g/ml}$ of culture medium) for 24 h. Cell viability was determined by the MTT assay (n=3). Viability curves: Percentage viability = the ratio (expressed as a percentage) of absorbance of treated cells to

The cell viability assay by MTT revealed that *A. lappa* is the active fraction with antiproliferative effects against lymphocytes and k562 cancer cell when treated for 24 hours. As shown in Fig. 1, the cytotoxic effect was dose dependent at concentrations of the crude extract in the medium. The cytotoxic effects were not found in the extracts on both cells as significantly at the same concentrations.

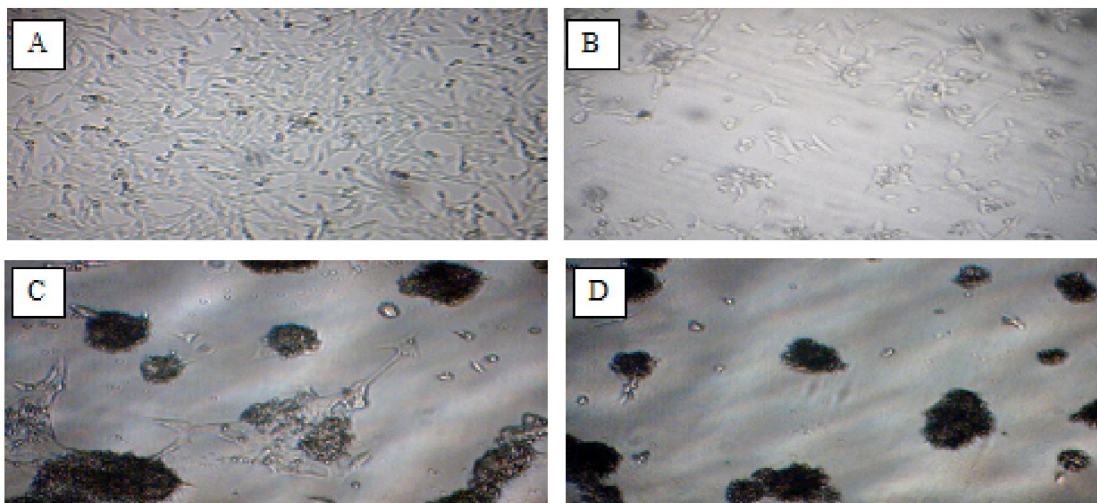


Figure 2. Morphological changes in k562 cells after 24 h of treatment with the plant extracts. (A) control, (B) 0.8 mg/ml (C) 7.5 mg/ml (D) 10 mg/ml. Cells treated with plant extracts were observed under inverted microscope and photographed in same magnification (40X).

Table 1. The result of analysis of variance of *Arctium lappa* extract on cancer cell line (k562) and lymphocyte cells.

Source of variation	Degree of freedom	Cytotoxic concentration
Cells	1	0.001 ^{ns}
Concentration	13	0.123**
Concentration × cells	13	0.001**
Experimental error	28	0.001
Coefficient of variation	-	1.91%

At the highest concentration tested (10 mg) with a 24-hrs incubation period, *A. lappa* extract resulted in 96% (in average) inhibition of cell proliferation in both lymphocyte and k562 cells. The lowest concentrations of extract were not cytotoxic to both of cells; they caused significant cell death in both sensitive as well as resistant human bone marrow cell line. Statistical results showed that the concentrations also differ significantly ($p < 0.05$). In particular, following pair wise comparisons, the first seven concentrations do not differ significantly but differ from the last five concentrations which also differ from each other. However, the last five concentrations the extract behaves similarly. However, what can be concluded from the graph is that cell viability decreases when using the two highest concentrations of extract in both cells. In addition, it showed that the % viability of both cells is approximately 43-93%, which is most suitable to perform cytotoxicity studies. Percentage cell viability of both of cells was carried out by using Trypan blue

dye Exclusion technique (Figure 2). The extract showed different anti-proliferative profiles regarding extract concentrations. There were different inhibitions produced by different concentrations of at 24-hours incubation. On the other hand, there was no difference in the level of inhibition produced by concentrations lower than 0.4 mg in both cells. The inhibition level of each concentration (7.5 and 10 mg) was significantly different from those in lower concentrations ($P < 0.05$ and $P < 0.001$ respectively) (Table1). There was however no significant difference between the inhibition produced by 7.5 and 10 mg.

Discussions

Since a long time medicinal plants maintain the health and vitality of individual and also cure and treat various human disease including cancer without causing toxicity. There are lots of medicinal plants available in nature which has the anticancerous properties and theirs Natural products discovered from medicinal plants have played an important role in treatment of cancer (23). So, considering the facts

for these plants to be discovered so that the cancer could be totally eradicated. The parts of *A. lappa* have been commonly used in traditional Iranian medicine for the treatment of various human ailments for many years. It is believed that extract of this medicinal plant contains a wide array of polyphenolic (caffeoylquinic acid derivatives) and butyrolactone lignans compounds which might possess cancer preventive, antioxidant and/or therapeutic properties, and also this compound can be protect cell walls against free oxygen radicals (16, 17, 24). This study was commenced in an attempt to elucidate some of the generally unexplored aspects inhibitory effect and death cell. To meet this objective a detailed cytotoxicity screening was performed in both cancer and normal cell after 24 h exposure using cell-based assays. Our results show that the extract of *A. kurdica* had dose dependent response. The results obtained from the present study unambiguously indicate that the cell lines are chemosensitive to the components of the crude extract, even though with varying degree. The performed phytochemical study has shown the cytotoxic activity may be due to the presence of alkaloids, terpenoid and flavonoids present in the root of *Arctium lappa*, to which its biological activity could be generally ascribed. In addition according to other studies arctiin and arctigenin isolated from *A. lappa* root extract can be effect agent to have substantial antitumor (17, 20, 22, 24). In conclusion, the *in vitro* data presented here suggests that consumption of the root extract of this plant as tea may impart anticancer effects but we propose the following suggestions for the plant surveyed in this study: 1) Further studies are required to identify the molecule(s) responsible for their biological activity and identification of the molecule(s) responsible for their biological activity which will allow the basis design for more effective molecules for the eventual use as cancer chemopreventive and/or therapeutic agents and targets for cell growth inhibition. 2) To do detailed pharmacodynamic study, it is necessary to perform *in vitro* experiments with different solvents and using different cancer cell lines. 3) For the species from which active extract with known chemical structure(s) have been isolated and characterized which have demonstrated significant activity *in vitro*, it is necessary to proceed with studies *in vivo*. 3) It is necessary to make an extensive *in vivo* biological evaluation and subsequently proceed with clinical evaluations.

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References

- 1) Aggarwal B, Kumar A, Bharti A. Anticancer potential of curcumin: preclinical and clinical studies. *Anticancer Research* 2003; 23 (1): 363–398.
- 2) Aggarwal BB, Shishodia S. Suppression of the nuclear factor- κ B activation pathway by spice-derived phytochemicals: reasoning for seasoning. *Annals of the New York Academy of Sciences* 2004; 1030: 434–441.
- 3) Singhal MK. Jungles: rich sources of medicinal plants. *Natural Product Radiance* 2004; 3: 203.
- 4) Lai PK, Roy J. Antimicrobial and chemopreventive properties of herbs and spices. *Current Medicinal Chemistry* 2004; 11 (11): 1451–1460.
- 5) Surh YJ, Lee E, Lee JM. Chemoprotective properties of some pungent ingredients present in red pepper and ginger. *Mutation Research* 1998; 402 (1-2): 259–267.
- 6) Cragg, G, Newman D, Snader K.. Natural products in drug discovery and development. *Journal of Natural Products* 1997; 60 (1): 52–60.
- 7) Wang HK, Morris-Natschke SL, Lee KH. Recent advances in the discovery and development of topoisomerase inhibitors as antitumor agents. *Med Res Rev* 1997; 17: 367-425.
- 8) Lee KH. Novel antitumor agents from higher plants. *Med Res Rev* 1999. 19: 539-596.
- 9) Elisabetsky E, Souza G. Etnofarmacologia como ferramenta na busca de substâncias ativas. In: *Farmacognosia*, Simões CM, Schenkel EP, Gosmann G, Mello GCP, Mentz LA, Petrovick PR, eds Editora da UFRGS/ Editora da, UFSC. 2004: 107–122.
- 10) Khafagi IK, Dewedar A. The efficiency of random versus ethno-directed research in the evaluation of Sinai medicinal plants for bioactive compounds. *Journal of Ethnopharmacology* 2000; 71 (3): 365–376.
- 11) Shishodia S, Ueda H, Arvigo R, Balick MJ. Ethnobotany in the search for vasoactive herbal medicines. *Journal of Ethnopharmacology* 1999; 66 (2): 159–165.
- 12) Maruta Y, Kawabata J, Niki R. Antioxidative Caffeoylquinic Acid Derivatives in the Roots of Burdock (*Arctium lappa* L.). *J Agric Food Chem* 1995; 43(10): 2592-2595.
- 13) Rodriguez P, Blanco J, Juste S, Garces M, Perez R, Alonso L, Marcos M. Allergic contact dermatitis due to burdock (*Arctium lappa*). *Contact Dermatitis* 1995; 33(2):134-135.
- 14) Chan YS, Cheng LN, Wu JH, Chan E, Kwan YW, Lee SMY, Leung GPH, Yu PHF, Chan

- SW. A review of the pharmacological effects of *Arctium lappa* (burdock). *Inflammopharmacology* 2010
- 15) Lin CC, Lu JM, Yang JJ, Chuang SC, Ujiie T. Anti-inflammatory and radical scavenge effects of *Arctium lappa*. *J ethnopharmacology* 1996; 24(2): 127-37.
- 16) Kardosova A, Machova E. Antioxidant activity of medicinal plant polysaccharides. *Fitoterapia* 2006; 77(5): 367 - 73.
- 17) Zhao F, Wang L, Liu K. *In vitro* anti-inflammatory effects of arctigenin, a lignan from *Arctium lappa* L, through inhibition on iNOS pathway. *Journal of Ethnopharmacology* 2009; 122 (3): 457-462.
- 18) Schroder HC, Merz H, Steffen R, Muller WE, Sarin PS, Trumm S, Eich E. Differential *in vitro* anti-HIV activity of natural lignans. *Z Naturforsch C* 1990; 45: 1215 -21.
- 19) Lee IA, Joh EH, Kim DH. Arctigenin Isolated from the Seeds of *Arctium lappa* Ameliorates Memory Deficits in Mice. *Planta Med* 2011.
- 20) Hirano T, Oka K, Gotoh M. Natural flavonoids and lignans are potent cytostatic agents against human leukemic HL-60 cells. *Life Sci* 1994; 55(13): 1061-9.
- 21) Liu S, Chen K, Schliemann W, Strack D. Isolation and identification of arctiin and arctigenin in leaves of Burdock (*Arctium lappa*) by polyamide column chromatography in combination with HPLC- ESI/MS. *phytochem anal* 2005; 16(2):86-9.
- 22) Moritani S Miyamoto K Takeda Y ‘ Nomura M. Cytotoxic components of *bardanae fructus*. *Biol Pharm Bull* 1996; 19(11): 1515-7.
- 23) Rajandeeep K, Karan K, Harpreet K. Plants as a source of anticancer agents. *J. Nat. Prod. Plant Resour* 2011; 1 (1): 119-124.
- 24) Matsumoto T, Hosono-Nishiyama K., Yamada H. Antiproliferative and apoptotic effects of butyrolactone lignans from *Arctium lappa* on leukemic cells. [Planta Medica](#) 2006; 72(3): 276-278.

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