

Investigating the Effect of *Phlomis Lanceolata* Boiss and Hohen on Cancer Cell Lines

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Received: 4 Sep. 2012; Received in revised form: 20 Feb. 2013; Accepted: 27 Feb. 2013

Abstract- *Phlomis lanceolata* is a medicinal plant that has long been used to treat various conditions such as diabetes, gastric ulcer, hemorrhoids, inflammation and wounds. As most of *Phlomis* species have shown cytotoxic activity against proliferation of different cell lines, a biological investigation of *P. lanceolata* was carried out in this study. The aim of this study was to find out the in vitro cytotoxic activity of total extract and different fractions of *Phlomis lanceolata* on four cell lines. Cytotoxic activity of the metanolic total extract and partition fractions of chloroform, ethyl acetate and petroleum ether of flowering aerial parts of *Phlomis lanceolata* on the HT29, Caco2, T47D and NIH3T3 cell lines is examined by MTT. Petroleum ether fraction showed high cytotoxic activity against proliferation of all four cell lines. Presence of heavy triterpens and lipophil compounds recognized by TLC test in Petroleum ether fraction is responsible for high cytotoxic activity. The results emphasize the importance of phytochemical studies which could lead to the discovery of new active compounds.

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Acta Medica Iranica, 2014;52(5):333-336.

Keywords: Cell line; Cytotoxic activity; MTT assay; *Phlomis lanceolata*

Introduction

Medicinal Plants are believed to be an important source of new chemical substances with potential therapeutic effects. Herbal therapy is used to treat a large variety of ailments and symptoms such as inflammation, pain, cancer, and healing wounds; however, there is no adequate experimental evidences about their effectiveness (1). The genus *Phlomis* (Lamiaceae) comprises approximately 100 species in the world of which 10 species are endemic to Iran including *P. lanceolata* which is found only in Iran (2). Some *Phlomis* species are used in Iranian traditional medicine as stimulant, tonic (3), analgesic, ulcer and hemorrhoid healing and anti inflammation (4). Several *Phlomis* species are used in folk medicine, for diseases of the respiratory tract or externally for treatment of wounds (5). Plants belonging to the genus *Phlomis*, have been shown to contain different classes of iridoids, flavonoids, phenylpropanoids, phenylethanoids and diterpenoids (1).

Some phenylpropanoids are known to posses diverse biological properties including cytotoxic, cytostatic, anti inflammatory, antinociceptive, immunosuppressant and antibacterial effects (6). Until now *Phlomis lanceolata* has not been the subject of any cytotoxic assay. The aim of the present investigation is to screen out the cytotoxic activity of total extract and different fractions of *P. lanceolata* and see the possibility of utilization of the samples in the future, for isolation of active compounds. In this study cytotoxic activity of *P. lanceolata* was investigated toward three cancer cell lines and one non cancerous cell line by MTT assay.

Materials and Methods

Plant material

Flowering aerial parts of *P. lanceolata* were collected from Khansar district (Isfahan province, Iran) in July 2010. A voucher specimen (No. 823) has been deposited in the herbarium of the Department of Pharmacognosy,

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Extraction

The air dried flowering aerial parts of *P. lanceolata* (400 g) was exhaustively extracted by percolator apparatus using Methanol (3×3 lit). The extract was concentrated by Rotary Evaporator apparatus and the solvent removed to produce a dark green gummy solid (98 g). An adequate part of the resulting extract was kept in a sterile vial in a dark and cool place for further tests. The remains were partitioned between petroleum ether (17 g), CHCl₃ (48 g) and EtOAc (22 g) to yield different fractions.

Cytotoxicity assay

The colon carcinoma (HT29) and breast ductal carcinoma (T47D) cell lines were mentioned as exponentially growing cultures in RPMI 1640 cell culture medium (Gibco, England), supplemented with %10 fetal bovine serum (FBS) (Gibco, England) and colorectal carcinoma (Caco₂) cell line was mentioned in RPMI 1640 supplement with %15 fetal bovine serum.

The Swiss mouse embryo fibroblast (NIH3T3) cell line was kept in Dulbecco's modified Eagle's medium (DMEM; PAA, Germany) supplement with %10 FBS.

100 IU/ml penicillin and 100 µg/ml streptomycin (Gibco, England) were added to the all media.

All the cell lines were cultured at 37°C in air/carbon dioxide (95:5) atmosphere. Samples including total extract and three fractions were tested at different concentrations. The samples were dissolved in dimethyl sulfoxide (DMSO) and further diluted with cell culture medium. The DMSO final concentration was adjusted to %1 of the total volume of medium in all treatments, including blank.

Cytotoxic activity has been measured using modified MTT assay (7), where 1×10⁴ cells/well have been plated in to 96-well plates (Nunc, Denmark) and incubated for 48h before the addition of drugs. After 48 hrs of

incubation 20ul of MTT (Sigma, USA) reagent (5 mg/ml) in phosphate buffered saline (PBS) was added to each well.

The plates have been incubated at 37°C for 4hrs. The median has been discharged and the foramazan blue which formed in the cells, were dissolved with 100 µl Dimethyl sulphoxide (DMSO). After incubation at 37°C for 10 min, absorbance at 570 nm at the dissolved solution has been detected by a microplate reader (Anthos, Austria).

For the trypan blue assay, 3 × 10⁴ cells/well cells were seeded in 24-well plates for 24h and were then treated with different concentrations of the samples. After the incubation period for each cell line, the medium was removed and cells were collected by trypsinization and counted in %0.4 trypan blue solution using a hemocytometer (8).

The cell viability in both MTT and trypan blue assays was calculated as a percentage of the control value (untreated cells). Cytotoxicity have been expressed as the concentration of extract inhibiting cell growth by %50(IC₅₀±SD), all tests and analysis were run in triplicate.

Statistical analysis

IC₅₀ (the median growth inhibitory concentration) values have been calculated from the IC₅₀ of dose-response curve in the Sigmaplot 10 software. Data representative of three independent experiments with similar results are presented as mean ± SD.

Results

The effects of these plant extracts on the proliferation of Caco₂, HT29, NIH3T3 and T47D cell lines have been analyzed by treating the cells with different concentrations of extracts. Table 1 shows the viability of these cell lines which treated by methanolic total extract and three fractions of flowering aerial parts of *P. lanceolata*. In this study we found that petroleum ether fraction has exhibited high cytotoxic activity on all four cell lines.

Table 1. IC₅₀ values (µg/ml) of total Methanolic extract and partition fractions of *Phlomis lanceolata* against tumor and normal cell lines. Results are expressed as mean±SD

Samples	Cell Lines			
	HT29	Caco2	T47D	NIH3T3
Total Methanolic extract	>1000	>1000	>1000	>1000
Chloroform fraction	>1000	>1000	>1000	>1000
Ethyl Acetate fraction	>1000	>1000	>1000	>1000
Petroleum Ether fraction	830.64±37.13	263.16±30.97	238.40±27.61	175.52±7.90
Methotrexate	0.23 ± 0.02	0.32 ± 0.04	0.16 ± 0.09	0.24 ± 0.013

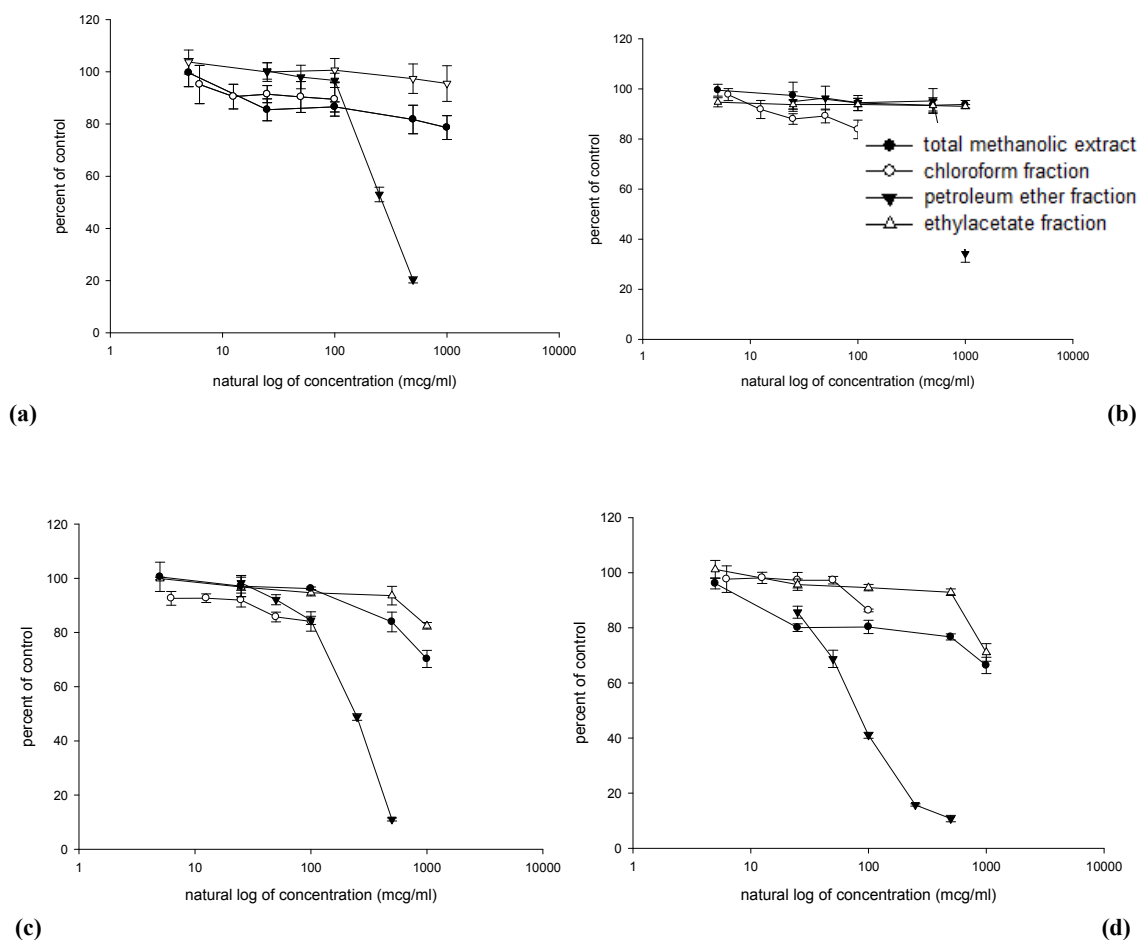


Figure 1. The effect of the Methanolic total extract and partition fraction of Chloroform, Ethylacetate and Petroleum ether of *Phlomis lanceolata* on cell growth and survival. Cells (1×10^4 cells/well) were cultured in the absence and presence of various concentrations of extracts. After the indicated time points, cell viability was determined by MTT assay. (a) Caco2, (b) HT29, (c) T47D, (d) NIH3T3 cells.

The results are presented as mean \pm SD (n=3)

Figure 1 shows the viability of these cell lines when treated by different concentrations of *Phlomis lanceolata*'s extract. Except petroleum ether fraction, other fractions have been demonstrated slightly cytotoxic effect toward tested cell lines, but methanolic total extract showed better cytotoxic effect on T47D and NIH3T3.

Discussion

In many countries, cancer is the second leading cause of death after heart diseases (9). The incidence of different carcinomas that estimated in worldwide is about 10 million; 50% of these are in developed countries (10). Among the patients with cancer in the USA, the use of complementary and alternative medicine, represented

mainly by plants ranges between 30-75% (11). This is acceptable reason for the interest in search of possible anticancer agents from the flora of different countries.

Phlomis lanceolata is a medicinal plant that has long been used to treat various medical conditions such as diabetes, gastric ulcer, hemorrhoids, inflammation and wounds. As most of *Phlomis* species have shown cytotoxic activity against proliferation of different cell lines, a biological investigation of *P. lanceolata* was carried out in this study. To the best of our knowledge, antiproliferative activity of *P. lanceolata* on cancer cell lines has never been established.

Cytotoxic activity of the methanolic total extract and partition fractions of chloroform, ethyl acetate and petroleum ether of flowering aerial parts of *Phlomis lanceolata* on the HT29, Caco2, T47D and NIH3T3 cell

lines has been investigated by MTT assay. Antiproliferative activity recorded in the present study revealed high potential cytotoxic activity of the petroleum ether fraction with concentrations of 830.64 ± 37.13 , 263.16 ± 30.97 , 238.40 ± 27.61 , 175.52 ± 7.90 $\mu\text{g/ml}$ against proliferation of HT-29, Caco2, T47D, NIH3T3 cell lines.

The real IC₅₀ values of petroleum ether fraction may be considerably lower than the positive control (Methotrexate) since its pharmacological active compounds are not pure and further researches are needed for defining potential component as cytotoxic natural medicines.

Recognition of heavy triterpens and lipophil compounds by TLC method in Petroleum ether fraction probably exposed the responsibility these types of compounds for high cytotoxic activity.

In addition the preliminary general phytochemical tests showed that the petroleum ether fraction of the flowering aerial parts of *P. lanceolata* could be considered as a rich source of different heavy triterpenoids and lipophil compounds. According to our data, the cytotoxic activity of petroleum ether fraction on Caco2, T47D and NIH3T3 cell lines were much stronger than that on HT29. This is maybe related to the ability of colon cancer cells to efflux toxic compounds from the cells but this ability is not uniform between colon cancer cells (12). This preliminary study is implied the high potency of petroleum ether fraction of *P. lanceolata* against the proliferation of cancerous and non cancerous cell lines which have not been reported from this plant so far.

The results emphasize the importance of isolation and characterization of the active components as well as the investigation of this specific cytotoxic pathway which may help to determine whether the fraction is valuable for antineoplastic effects.

Acknowledgment

Authors thank Mr. M. Asoudeh for collecting the plant material. Financial support from Pharmacology and Nanotechnology Research Center, Tehran University of Medical Sciences, Tehran, Iran is gratefully acknowledged.

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