Cytotoxic Activity of *Lythrum salicaria* L.

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**Abstract:** The aerial parts of *Lythrum salicaria* L. from Lahijan in Gillan province have been collected in June 2009. Total extract were obtained by MeOH/H2O (80/20) and then partitioned between CHCl3, EtOAc and MeOH. These fractions and total extract have been investigated for in vitro cytotoxic activity against colon carcinoma (HT-29), leukemia (K562), breast ductal carcinoma (T47D) and Swiss mouse embryo fibroblast (NIH 3T3) cell lines by MTT assay (3-(4,5-di methyl thiazol-2-yl)-2,5-di phenyltetrazolium bromide). At each cell line doses of 50, 100, 200, 400 and 800 μg mL−1 in 1% (v/v) DMSC of all samples were tested. Ethyl acetate fraction against proliferation of T47D cell line has been represented high cytotoxic activity (IC50<70). Chloroform fraction and total extract against proliferation of T47D cell line and chloroform fraction toward K562 cell line and total extract against HT-29 cell line have been demonstrated slight cytotoxic activity (IC50=200). The other fractions have been demonstrated no effective cytotoxic activity. IC50 values confirmed that the growth and proliferation of T47D cells were most affected by ethyl acetate fraction.

**Key words:** Cytotoxic activity, *Lythrum salicaria* L., MTT assay, fraction, chloroform, cell, Iran

**INTRODUCTION**

The genus *Lythrum* belongs to the plant family lythraceae, this genus is spread throughout the world. It is well represented in the flora of Iran, at least with 30 species in the world (Zargari, 1996) and 8 species in Iran. It is known as khon fam in Farsi (Mozaffarian, 1996). Its English name is red sally, purple lythrum, Grgass-polly and loessestrife. It is known in German as blut-weiderich in French Salicaire (Zargari, 1996). Phytochemical investigation of *Lythrum salicaria* L. has been demonstrated phenolic acids, flavonoids, tannins, anthocyanins, glycosides (salicairine), triterpenoids and organic acids (Rauha et al., 2001; Zargari, 1996; Becker et al., 2005).

In addition, vescalagin, pedunculagin, vanoileic acid dilactone, 1, 6-di-O-galloylglucose, 1-O-galloylglucose and 6-O-galloylglucose were identified. Sterols as β-sitosterol were also detected in this plant (Gruenwald et al., 2000; Rauha et al., 2001). There are some pharmacological activities of this species including Antioxidant, anti-inflammatory, anti-nociceptive and hypoglycemic (Tunalier et al., 2007; Lopez et al., 2008; Lamela et al., 1985, 1986). *Lythrum salicaria* L. is used for diarrhea, chronic intestinal catarrh, hemorrhoid and eczema (Tunalier et al., 2007). *L. salicaria* L. extracts showed activity against the Cladosporium cucumerinum, *S. aureus*, Proteus mirabilis and Micrococcus luteus (Becker et al., 2005). More than half of drugs in cancer therapy were obtained from natural products or are related to them (Newman and Cragg, 2007) so in this study cytotoxic activity of *Lythrum salicaria* L. toward three cancer cell lines by MTT assay have been investigated.

**MATERIALS AND METHODS**

**Plants:** The aerial parts of *Lythrum salicaria* L. from Lahijan in Gillan province were collected in June 2009. The plants have been identified and deposited at the Central Herbarium of Medical Plants (ACERC), Karaj, Iran.

**Extraction:** Freshly collected aerial parts of *L. salicaria* L. were cleaned, shade dried. These parts were coarse powdered in a hand mill and stored at room temperature.

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100 g of powdered plants have been extracted by percolation method with 80% aq. MeOH three times at room temperature. The extract was evaporated by rotary evaporator and consequently partitioned between CHCl₃, EtOAc and MeOH. Each fraction evaporated by rotary evaporator and has been stored at refrigerator for investigation of cytotoxic activity.

**Cytotoxicity assay:** The colon carcinoma (HT-29), leukemia (K562), breast ductal carcinoma (T47D) cell lines were mentioned as exponentially growing cultures in RPMI 1640 cell culture medium (PAH, Germany), supplemented with 10% fetal bovine serum (FBS; Gibco, USA).

The Swiss mouse embryo fibroblast (NIH 3T3) cell line was kept in Dulbecco’s Modified Eagle’s Medium (DMEM; PAH, Germany) supplemented with 10% FBS. 100 IU mL⁻¹ penicillin and 100 µg mL⁻¹ streptomycin (Roche, Germany) were added to the all media. All the cell lines were cultured at 37°C in air/carbon dioxide (95:5) atmosphere. Cytotoxic activity has been measured using modified MTT assay (Atta-ur-Rahman et al., 2001). Where 1×10⁴ cells well⁻¹ have been plated in to 96-well plates (Nunc, Denmark) and incubated for 24 h before the addition of drugs. After 72 and 96 h of incubation for HT-29 and T47D cells respectively, 20 µL of MTT (Merek, Germany) reagent (5 mg mL⁻¹) in Phosphate Buffered Saline (PBS) was added to each well.

The plates have been incubated at 37°C for 4 h. The medium has been discharged and the formazan 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 solutions has been detected by a micro plate reader (Arthos, Austria). The cell viability in MTT assay was calculated as a percentage of the control value. Methotrexate was used as a positive control. 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 IC₃₀ of dose-response curve in the sigma plot 10 software. Data representative of three independent experiments with similar results are presented as mean±SD.

<table>
<thead>
<tr>
<th>Table I: Cytotoxic activity of total extract and fractions of Lysimachia salicaria L.</th>
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<td>Samples</td>
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<tr>
<td>Lysimachia salicaria L.</td>
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<tr>
<td>Total extract</td>
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<tr>
<td>Methanol fr.</td>
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<td>Ethyl acetate fr.</td>
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<td>Chloroform fr.</td>
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<td>Methotrexate</td>
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Results are expressed as IC₅₀ values (µg mL⁻¹). Key to cell lines employed: HT-29 and Caco-2 (colon Adenocarcinoma), T47D (breast carcinoma), NIH 3T3 (Swiss mouse embryo fibroblast).

Concentrations of the extracts and significant decrease in cell lines proliferation were observed. The ethyl acetate fraction has shown high cytotoxic activity on T47D.

Chloroform fraction was better than other fractions on K562. Chloroform and ethyl acetate and methanol fractions did not show good effect on HT-29. IC₅₀±SD are shown in Table 1.

Among all the samples, ethyl acetate and chloroform fractions have been exhibited better cytotoxicities on T47D and K562 cell lines compared with polar fraction and total extract. In comparison with another fraction methanicol and total fractions of all samples have been demonstrated slightly cytotoxic effect toward tested cell line. Two antifungal triterpenoids oleanolic acid and ursoic acid was isolated by bioautography on thin-layer chromatograms (Becker et al., 2005). Ulsoric acid and oleanolic acid have significant activity against human colon carcinoma cell line HCT15. The possible mechanism of action is that both compounds have an inhibitory effect on proliferation of tumor cell through cell-cycle arrest (Li et al., 2002). There were some reports about oleanolic acid and some of its derivatives induce apoptosis in human prostate cancer and leukemia cells (Hyer, 2008; Zhang et al., 2007).

**CONCLUSION**

The study shows that the ethyl acetate and chloroform fractions of L. salicaria L. have been exhibited cytotoxic activity toward cancer cell lines because of these triterpenoids.

The real IC₅₀ values of fractions of L. salicaria L. may be considerably higher compared to the positive control (Methotrexate) because its pharmacological active compounds are not pure.

Isolation and characterization of the active components as well as investigation of the specific cytotoxic pathway may help to determine whether the extract is valuable for antineoplastic effects.
REFERENCES


