Research Journal of Biological Sciences 6 (2): 55-57, 2011
ISSN: 1815-8846

Cytotoxic Activity of *Lythrum salicaria* L.

[1]Mahnaz Khanavi, [1]Mana Moshteh, [1]Azadeh Manayi, [1]Mohamad Reza Shams Ardekani,
[1]Mahdi Vazirian, [3]Yousef Ajani and [2]Seyed Nasser Ostad
[1]Department of Pharmacognosy, Faculty of Pharmacy,
Traditional Medicine and Pharmaceutical Sciences Research Center,
[2]Department of Toxicology, Pharmacology and Nanotechnology Research Center,
Tehran University of Medical Sciences, P.O. Box 14155-6451, Tehran, Iran
[3]Institute of Medicinal Plants, Iranian Academic Centre for Education, Culture and Research,
P.O. Box 33651-66171, Karaj, Iran

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/H_2O (80/20) and then partitioned between $CHCl_3$, EtOAc and MeOH. These fractions and total extract have been investigated for in vitro cytotoxic activity against colon carcinoma (HT-29), lukemia (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) DMSO of all samples were tested. Ethyl acetate fraction against proliferation of T47D cell line has been represented high cytotoxic activity (IC_{50}<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 slightly cytotoxic activity (IC_{50}<200). The other fractions have been demonstrated no effective cytotoxic activity. IC_{50} 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 loosestrife. 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, vanoleic 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 (ACECR), 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.

Corresponding Author: Seyed Nasser Ostad, Department of Toxicology, Faculty of Pharmacy,
Pharmacology and Nanotechnology Research Center, Tehran University of Medical Sciences,
P.O. Box 14155-6451, Tehran, Iran

100 g of powdered plants have been extracted by perculation method with 80% aq. MeOH three times at room temperature. The extract was evaporated by rotary evaporator and consequently partitioned between $CHCl_3$, 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 (PAA, 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; PAA, 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 1×10^4 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 (Merck, 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 (Anthos, 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% (IC_{50}±SD), all tests and analysis were run in triplicate.

Statistical analysis: IC_{50} (the median growth inhibitory concentration) values have been calculated from the IC_{50} of dose-response curve in the sigma plot 10 software. Data representative of three independent experiments with similar results are presented as mean±SD.

RESULTS AND DISCUSSION

The effects of these plant extracts on the proliferative response of the HT-29, K562 and T47D cell lines have been analyzed by treating the cells with different concentrations of the extracts and significant decrease in cell lines proliferation were observed. The ethyl acetate fraction has been showed high cytotoxic activity on T47D.

Table 1: Cytotoxic activity of total extract and fractions of *Lythrum salicaria* L.

Samples	Cell lines[a] (MTT assay)			
Lythrum salicaria L.	HT-29	K562	T47D	NIH3T3
Total extract	175.57±12.34	>800	163.99±24.11	144.01±32.2
Methanol fr.	461.63±8.06	312.48±46.23	405.71±13.26	573.32±84.52
Ethyl acetate fr.	217.33±14.27	332.7±1.23	63.1±1.32	80.98±4.05
Chloroform fr.	246±9.4	178.23±9.04	108.23±11.11	71.45±12.01
Methotrexate	0.23±0.02	0.32±0.04	0.16±0.09	0.24±0.013

[a]Results are expressed as IC_{50} values (μg mL^{-1}), Key to cell lines employed: HT-29 and Caco-2 (colon Adenocarcinoma), T47D (breast carcinoma), NIH 3T3 (Swiss embryo fibroblast)

Chloroform fraction was better than other fractions on K562. Chloroform and ethyl acetate and methanol fractions did not show good effect on HT-29. IC_{50}±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 methanolic and total fractions of all samples have been demonstrated slightly cytotoxic effect toward tested cell line. Two antifungal triterpenoids oleanolic and ursolic acid was isolated the by bioautography on thin-layer chromatograms (Becker *et al*., 2005). Ursolic acid and oleanolic acid have significant anti-tumor activity on the 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 IC_{50} 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

Atta-ur-Rahman, M.I. Choudhary and W.J. Thomsen, 2001. Bioassay Techniques for Drug Development. Harwood Academic Publishers, The Netherlands, pp: 223.

Becker, H., J.M. Scher, J.B. Speakman and S. Zapp, 2005. Bioactivity guided isolation of antimicrobial compounds from *Lythrum salicaria* L. Fitoterapia, 76: 580-584.

Gruenwald, J., T. Brendler and C. Jaenicke, 2000. PDR for Herbal Medicines. 2nd Edn., Medical Economics Company, Montvale, New Jersey, USA.

Hyer, M.L., R. Shi, M. Krajewska and J.C. Reed, 2008. Apoptotic activity and mechanism of 2-cyano-3, 12-dioxoolean-1, 9-dien-28-oic-acid and related synthetic triterpenoids in prostate cancer. Cancer Res., 68: 2927-2933.

Lamela, M., I. Cadavid, A. Gato and M.J. Callega, 1985. Effects of *Lythrum salicaria* in normoglycemic rats. J. Ethnopharmacol., 14: 83-91.

Lamela, M., I. Cadavid and J.M. Calleja, 1986. Effects of *Lythrum salicaria* L. extracts on hyperglycemic rats and mice. J. Ethnopharmacol., 15: 153-160.

Li, J., W.J. Guo and Q.Y. Yang, 2002. Effects of ursolic acid and oleanolic acid on human colon carcinoma cell line HCT15. World J. Gastroenrerol., 8: 493-495.

Lopez, V., S. Akerreta, E. Casanova, J.M. Garsia-Mina, R.Y. Cavero and M. Calvo, 2008. Screening of Spanish medicinal plants for antioxidant and antifungal activities Pharmaceut. Biol., 46: 602-609.

Mozaffarian, V., 1996. A Dictionary of Iranian Plant Names. Farhang Moaser, Tehran, Iran, pp: 198.

Newman, D.J. and G.M. Cragg, 2007. Natural products as sources of new drugs over the last 25 years. J. Nat. Prod., 70: 461-477.

Rauha, J.P., J.L. Wolfender, J.P. Salminen, K. Pihlaja, K. Hostettmann and H. Vourela, 2001. Characterization of the polyphenolic composition of purple loosestrife (*Lythrum salicaria* L.). Z. Naturforsch, 56: 13-20.

Tunalier, Z., M. Kosar, E. Kupeli, I. Calis and K.H.C. Baser, 2007. Antioxidant, anti-inflammatory, anti-nociceptive activities and composition of *Lythrum salicaria* L. extracts. J. Ethnopharmacol., 110: 539-547.

Zargari, A., 1996. Medical Plants. Tehran University Press, Tehran, Iran.

Zhang, P., H. Li, D. Chen, J. Ni, Y. Kang and S. Wang, 2007. Oleanolic acid induces apaptosis in human leukemia cells through caspase activation and poly (ADP-ribose) polymerase cleavage. Acta Biochim. Biophys. Sinica, 39: 803-809.