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Research Article

ANTIOXIDANT AND *IN-VITRO* CYTOTOXIC ACTIVITY OF ROOT EXTRACTS OF *FRITILLARIA ROYLEI* USING HUMAN LYMPHOMA CELL LINES

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ABSTRACT

Drug discovery from medicinal plants has played an important role in the treatment of cancer. Cancer is considered as one of the dangerous disease associated with abnormal, uncontrolled growth of cell. There are about 13,000 plant species worldwide that are known to have been used as drugs. These plant species contain biologically active compounds that protect human health with respect to human carcinogenesis, acting against initiation, promotion or progression stages or destroying/blocking the DNA damaging mutagens, thus avoiding cell mutations. It is believed that herbs play vital role in the prevention and treatment of cancer. *Fritillaria roylei* (Kshirakakoli) contain different active compounds like peimine, peiminine, peimisine, peimiphine, peimidine, peimitidine, propeimin, sterol and these active compounds possess different pharmacological activity like galactogogue, haemostatic, ophthalmic and cytotoxic properties. So it was pertinent, to evaluate antioxidant and *in-vitro* cytotoxic potential of root extracts of *Fritillaria roylei* against human lymphoma cell lines. Antioxidant activity and *in-vitro* cytotoxic activity of the extracts were measured using DPPH radical scavenging method and lymphoma cell lines Jurkat and u937 respectively through MTT assay. The methanol extract of the plant showed potent antioxidant activity in a concentration dependent manner and decreased cell viability and cell growth inhibition in a dose dependent manner. Further studies are in progress to find out the active isolated compounds responsible for these activities.

INTRODUCTION

Cancer has been considered as the major threat all over the world. Specifically, in India it is highly prominent. It is the diseases caused by loss of cell cycle control and has been characterized by proliferation and differentiation of abnormal cells that give rise to undifferentiated mass of cells like lobe[1]. At present, chemotherapy and radiotherapy treatments are followed by doctors for the treatment of various types of cancers but even though the rate of survivability was found to be limited and also possess various side effects[2]. There is a widespread belief that green medicines are healthier and safer than synthetic ones[3]. Nature provides the store house of remedies to cure all ailments of mankind. Medicinal plants symbolize a huge probable source for anticancer compounds and also support the immune system, by improving body resistance against the disease[4]. Plants have long history in the treatment of cancer[5]. Recently, the use of traditional medicine information on plant research has again received considerable interest and worldwide efforts are made to discover new anticancer agent from the plants[6,7]. The National Cancer Institute has screened about 35,000 plant species that possess activity against cancer[8]. In traditional system of medicine, there are many natural crude drugs obtained from the plants that have the potential to treat various threat full diseases and disorders and one of them is *Fritillaria roylei* (Kashirkakoli).

Fritillaria roylei (Kashirkakoli) is a perennial herb that belongs to family Liliaceae. *Fritillaria roylei is* the one of the important plant out of eight plants of Ashtawarga. *Fritillaria roylei* is a rare species found at an altitude around 3800- 4700 m above the sea level. It is commonly regarded as an ornamental plant but traditionally used in a number of important ayurvedic formulations[9]. The best known Ayurvedic formulation is 'Chywanprash' which is used as a tonic to cure number of diseases. *Fritillaria roylei* is used primarily to treat various

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lung diseases like asthma, bronchitis, tuberculosis and coughs of any type. Previous research reveals that it is used for the treatment of heart diseases, decreased blood pressure, stimulation of the heart muscle, nervous system and dysfunction of breathing[10]. It has been reported that *Fritillaria roylei* contain various active compounds like peimine, peiminine, peimisine, peimiphine, peimidine, peimitidine, propeimin, sterol and these active compounds possess galactogogue, haemostatic, ophthalmic and cytotoxic properties[11]. As very less study was done on cytotoxic property of the plant or its parts. So, it was pertinent to evaluate antioxidant and *in-vitro* cytotoxic potential of root extracts of *Fritillaria roylei* plant against human lymphoma cell lines.

Experimental Section

Collection of plant material, chemicals and cell lines

The roots of *Fritillaria roylei* plant were procured from Himachal Pradesh and duly authenticated by Central Instrumentation Facility, National Botanical Research Institute, Lucknow vide letter no. NBRI/CIF/535/2017. Roots were washed, shade dried and stored in air tight container for future use. The chemicals and solvents used were of analytical grade. Lymphoma cell lines for the cytotoxic study i.e. Jurkat and u937 were procured from National Centre for Cell Science (NCCS), Pune.

Preparation of extracts

Roots of *Fritillaria roylei* were coarsely powdered and defatted with petroleum ether followed by extraction with methanol and distilled water through continuous hot extraction process. The extracts thus obtained were filtered with Whatman paper no. 1, concentrated to dryness to obtain a semisolid mass and were stored in vacuum desiccator for further use. Different concentrations of the methanol extract (12.5, 25, 50, 100 µg/ml) and aqueous extract (12.5, 25, 50, 100 µg/ml) were prepared for determining antioxidant activity.

Phytochemical screening

The preliminary phytochemical screening of the methanol and aqueous extracts of *Fritillaria roylei* for detection of alkaloids, glycosides, steroids, terpenoids, flavonoids, tannins, phenolics, saponins, carbohydrates, proteins and amino acids were done using standard procedure[12-14].

Antioxidant activity

The evaluation of the antioxidant activity of methanol and aqueous extracts of *Fritillaria roylei* were done by 1, 1-Diphenyl-2-Picrylhydrazyl (DPPH) radical scavenging activity[15]. The free radical scavenging activity of different extracts of *Fritillaria roylei* and gallic acid (Standard) was measured in terms of hydrogen donating or radical-scavenging ability using the stable radical DPPH. About 0.1 mM solution of DPPH in methanol was prepared and 1.0 ml of this solution was added to 3.0 ml of extract having different concentrations (12.5, 25, 50, 100 µg/ml). After the incubation of 30 minutes absorbance was measured at 515 nm. The decrease in absorbance of the reaction mixture indicates higher free radical scavenging activity. The antioxidant activities of the extracts were expressed as IC_{50} (the concentration of extracts in µg/ml that inhibits the formation of DPPH radicals by 50%).

Preparation of Herbal extract for cytotoxic activity

Stock solution of methanol and aqueous extract of *Fritillaria roylei* were prepared by dissolving 100 mg extract in 20ml of DMSO and then final volume was made up to 100ml with DMSO. Four different concentrations of methanol and aqueous extracts (12.5, 25, 50 and 100 µg/ml) were then prepared from stock solutions. All solutions were sterilized by passing through 0.22 µm syringe-adapted filters and stored at -20°C until use.

Cell culture maintenance

Cytotoxicity of the extracts were evaluated on Jurkat (P. No. 35) and u937 (P. No-40) human lymphoma cell lines. Cells were grown in RPMI-1640 medium with L-glutamine and supplemented with 2 mg/ml NaHCO3, 10 µg/ml hypoxanthine, 11.1 mM glucose, 10% FBS and 5µg/ml antibiotic solution. The cells were incubated at 5% CO_2 in humidified incubator at 37°C until confluent stage before use for cytotoxicity assay. After incubation, as the suspended cells reach to confluency stage $1/3^{rd}$ of medium was removed and replaced aseptically with the same amount of fresh medium.

Cytotoxic analysis

Healthy cells of the lymphoma cell lines Jurkat and u937 were transferred to 96 well plates at concentration of 10,000 cells/100 µl/well and incubated for 48 h at 37°C to allow them to grow before addition of the extracts. This was followed by addition of 100µl of different working concentrations of respective extracts (12.5, 25, 50 and 100 µg/ml) to 96 well plate. The wells having only cell suspensions without plant extract were designated as controls. The anticancer drug methotrexate was used as the standard drug. The cells with or without extract along with the standard were incubated at 37°C for 72h before determining their viability, turbidity and toxicity. Each concentration level was tested in triplicate[16].

MTT assay

Cell viability was determined using MTT assay[17]. After examination of the 96 well plate, the medium from the well was removed carefully and replaced aseptically with the same amount of fresh RPMI-1640 medium. Thereafter, to each well of the plate, 10µl of MTT solution (5mg MTT/ml of PBS, pH 7.2) was added and incubated at 37°C for 3h in a CO_2 incubator with 5% CO_2. Purple formazan crystals were formed which were then solubilised by adding 1ml DMSO to each well followed by 1h incubation. The optical density of 96 well plate was then measured at 540nm using DMSO as blank. This formazan production is directly proportional to the viable cell number and inversely proportional to the degree of cytotoxicity. The % cell viability was calculated with the following formula:

Cell viability % = Mean OD of wells receiving each plant extract concentration/ Mean OD of control wells x 100.

RESULTS AND DISCUSSION

The phytochemical screening of methanol and aqueous extracts of *Fritillaria roylei* showed the presence alkaloids, phytosterol, flavonoids, proteins and amino acids in good amount. The review of literature showed that presence of alkaloids, phenolics and flavonoids are responsible for a number of

pharmacological activities like anti-inflammatory, antioxidant, antibacterial, anticancer and anti-histaminic activity. The antioxidant activity of both the extracts using DPPH was found to increase in concentration dependent manner. Both the extracts (methanol and aqueous) exhibited potential antioxidant activity with an IC_{50} value of 28.4µg/ml and 33.7µg/ml respectively as compared to standard Gallic acid having IC_{50} value of 11.1µg/ml. The results of antioxidant activity of extracts of *Fritillaria roylei* using DPHH free radical scavenging method is shown in Figure 1

The methanol and aqueous extracts of the *Fritillaria roylei* were screened for their possible cytotoxic activity on human cell lines Jurkat and u937. The results showed decreased cell viability and cell growth inhibition in a dose dependent manner. The IC_{50} value of methanol extract, aqueous extract and standard (methotrexate) were 84.4µg/ml, 248.9µg/ml, 22.1µg/ml respectively for Jurkat and 51.4µg/ml, 209.9µg/ml and 26.1µg/ml respectively for u937 human cell lines. Methanol extracts of *Fritillaria roylei* demonstrated strong antioxidant and anti-proliferative activities. Accumulating evidences clearly indicate that apoptosis is a critical molecular target by dietary bioactive agents in the prevention of cancer. The cytotoxic activity of methanol and aqueous extracts of *Fritillaria roylei* was done on Jurkat and u937 human cell lines is shown in Figure 2 and Figure 3.

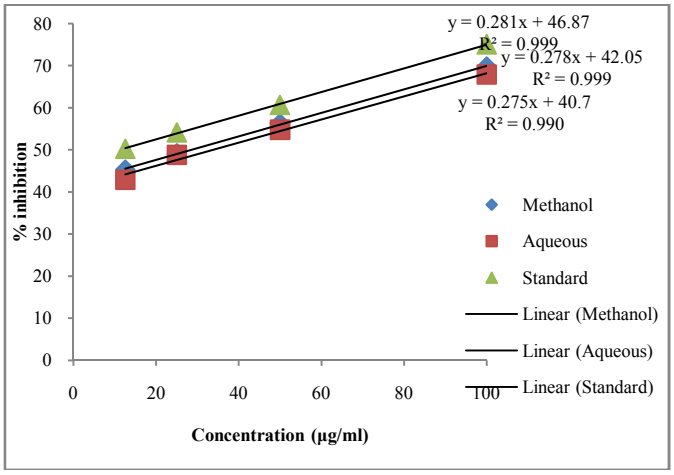

Figure 1 Antioxidant activity of *Fritillaria roylei* extracts of using DPPH free radical scavenging method

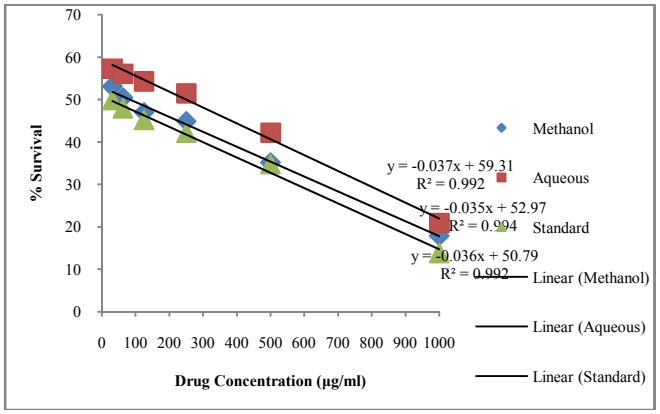

Figure 2 *In-vitro* cytotoxic activity of methanol and aqueous extracts of *Fritillaria roylei* on Jurkat cell lines

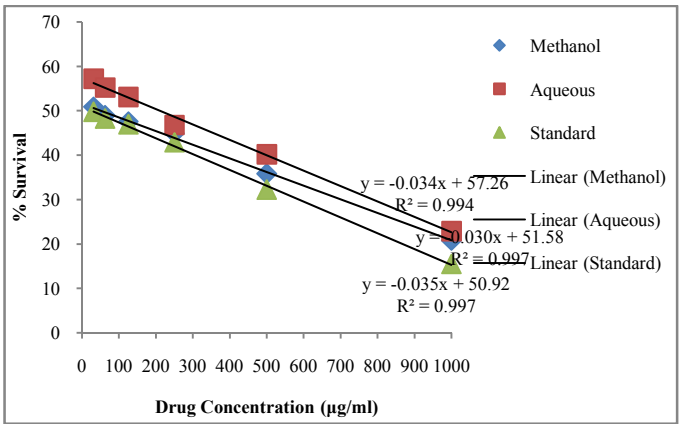

Figure 3 *In-vitro* cytotoxic activity of methanol and aqueous extracts of *Fritillaria roylei* on u937 cell lines

CONCLUSION

The results of the present study demonstrate the potential cytotoxic activity of *Fritillaria roylei*. Flavonoids being the major phyto-constituents may be responsible for this potential antioxidant and cytotoxic activity against cell lines. Further research has to be carried out with other leukemia cell lines to elucidate the possible mechanism of action.

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Conflict of interest

The authors confirm that this article content has no conflict of interest.

References

1. Dantu AS, Shankarguru P, Devi DR, Hari BN. Evaluation of *in vitro* anticancer activity of hydroalcoholic extract of *Tabernaemontana divaricata. Asian J. of Pharma. and Clinical Resh*. 2012; 5(3): 59-61.
2. Gandhiappan J, Rengasamy R. Antiproliferative activity of *Solanum anguivi* against cancer cell lines. Der Pharmacia Lettre. 2012; 4(3): 875-880.
3. Sindhia VR, Bairwa R. Plant review: *Buteamonosperma*. *Int. J. of Pharm. and Clinical Resh*. 2010; 2: 90-94.
4. Bachrach ZY. Contribution of selected medicinal plants for cancer prevention and therapy. *Scientific J. of the Faculty of Med. in Nis*. 2012; 29(3): 117-123.
5. Patel S, Gheewala N, Suthar A, Shah A. *In-vitro* cytotoxicity activity of *Solanum nigrum* extract against *HeLa* cell line and *Vero* cell line. *Int. J. of Pharm. and Pharma. Sci*. 2009; 1(1): 38-46.
6. Daniel JF, Alves CC, Grivicich I, da Rocha AB, de Carvalho MG. Antitumar activity of biflavonoides from *Ouratea* and *Luxemburgia* on human cancer cell lines. Indian J. of Pharma. 2007; 39(4): 184-186.
7. Kong JM, Goh NK, Chia LS, Chia TF. Recent advances in traditional plant drugs and orchids. Acta Pharmacol Sin. 2003; 24(1): 7-21.
8. Moustafa MA, Menshawi MB, Wassel MG, Mahmoud K, Mounier MM. Screening of some plants in Egypt for

their cytotoxicity against four human cancer cell lines. *Int. J. of Pharm. Tech. Resh*. 2014; 6(3): 1074-1084.
9. Kaur G, Gupta V, Bansal P. *Fritillaria roylei:* A comprehensive monograph. Lap Lambert Academic Publishing: Mauritius; 2018.
10. Erika L, Rebecca JF. Screening of antimicrobial and cytotoxic activities of Panamanian plants. Phytomedicine. 2005; 1: 149-153
11. Fong CY, Kao YS, Chang KJ. The Alkaloids of Fritillaria Roylei. Isolation of Peimine. J. *of American Chem. Soc*. 1936; 58: 1306–1307.
12. Banu KS, Cathrine L. General Techniques Involved in Phytochemical Analysis. *Int. J. of Adv. Resh*. In Chem. Sci. 2015; 2(4): 25-32.
13. Harborne JB. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis.2nded. Chapman & Hall: London; 1988.
14. Iqbal E, Salim KA, Lim LBL. Phytochemical screening, total phenolics and antioxidant activities of bark and leaf extracts of Goniothalamus velutinus (Airy Shaw) from Brunei Darussalam. *J. of King Saud Uni.–Sci*. 2015; 27: 224-232.
15. Panda BR, Mohanta SR, Manna AK, Si S. *In-vitro* antioxidant activity on the aerial parts of Cocculus hirsutus Diels. *J. of Adv. Pharm. Resh*. 2011; 2(1): 18-23.
16. Niles AL, Moravec RA, Riss TL. Update on in-vitro cytotoxicity assays for drug development. Expert Opin. on Drug Disc. 2008; 3(6): 655-669.
17. Niles AL, Moravec RA, Riss TL. In vitro viability and cytotoxicity testing and same-well multi-parametric combinations for high throughput screening. Curr. Chem. Genomics. 2009; 11(3): 33-41.

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