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Research Article

Antiproliferative and Antioxidant potential of different extracts of *Fritillaria roylei*

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Abstract

Fritillaria roylei is one of the medicinal plant used in traditional system of medicine for ailment of various diseases. But scientific study of the plant has been less carried out for which the study was conducted for validation of prevailing medical practice. The soxhlet extraction of the plant were conducted in various solvents (hexane, chloroform, ethyl acetate, methanol and water) differing in polarity. The phytochemical screening of extracts revealed the presence of alkaloids, saponins and tanins. In vitro Cytotoxicity evaluation against five human cancer cell lines A549 (Lung), C6 (Glioma), T47D (Breast), MCF (Breast), and TH-1(Colon) showed that the extracts of *Fritillaria roylei* exhibited potent cytotoxic activity. The extracts of *Fritillaria roylei* also showed antioxidant activity.

Key words: Fritillaria roylei; cytotoxic; antioxidant

INTRODUCTION

Nature stands a golden mark and provides the store house of remedies to cure all ailments of mankind. Herbs have always been the principal form of medicine throughout the world as people strive to stay healthy in the face of chronic stress and pollution and to treat illness with medicines that work in count with the body's own defense. There is a widespread belief that green medicines are healthier and safer than synthetic ones [1]. In traditional medicine, there are many natural crude drugs that have the potential to treat many diseases and disorders and one of them is *Fritilaria. roylei*. *Fritillaria* is a genus of about 1000 species of bulbous plants belonging to the family Liliaceae. It is a perennial temperate herb that grows in mountain slopes and sub-alpine meadows, usually on open, stony and moist hill sides. *F. roylei* is a rare speciesfound at an altitude around 3800- 4700 m. In the West, *Fritillaria* is most commonly regarded as an ornamental garden plant. It is traditionally valued as an herbal remedy in Nepal and China. . *Fritillaria* affects the heart and lung meridians or energy pathways in the body and is used primarily to treat various lung diseases, including asthma, bronchitis, tuberculosis and coughs of any type. It is also used as a lymphatic decongestant to reduce swellings, nodules, fibrocystic breasts, goiter and swollen lymph glands. Previous research reveals that it is used for treatment of nervous system, prolonged decrease in blood pressure, stimulation of the heart muscle and dysfunction of breathing [2]. Plant contains phytochemicals to protect itself. But recent researches demonstrate that many phytochemicals can protect human and plants diseases [3]. Compounds theorized to be responsible for activity includes several bioactive isosteroidal alkaloids (verticine, verticinone, isoverticine, imperialine, hupe henine, ebeiedine, ebeienine and ebeiedinone) and 2 nucleosides (thymidine and adenosine) [2]. *Fritillaria roylei* possess peimine, peiminine, peimisine, peimiphine, peimidine, peimitidine, propeimin, sterol, etc and has been reported forantiasthmatic, antirheumatic, febrifuge, galactogogue, haemostatic, ophthalmic and cytotoxic properties, etc [4]. Very less pharmacological and anticancer study has been reported on *F. roylei* for which the study was designed to establish scientific basis for some of its therapeutic properties in traditional use. This investigation encourages for further findings of medicinal plants.

Hence, the study deals with the phytochemical screening, cytotoxic assay and antioxidant activity that helps in scientific evaluation of bioactive compounds and may prove beneficial for mankind.

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METHODOLOGY

Collection of samples: The plant material were collected from Gulmarg area in the month of May-June 2013. They were shade dried and pulverized in powder form. Voucher specimen of *F.roylei* bearing specimen no.27913, was deposited at KASH herbarium in centre of plant taxonomy, Kashmir University, Srinagar

Preparation of extract: The powdered sample were extracted by soxhlet extraction method using hexane, chloroform, ethyl acetate, methanol and water as solvent in the order of increasing polarity. Then, solvents from extracts were removed under reduced pressure with the aid of rotary vacuum evaporator.

Experimental:

Phytochemical Screening of Crude Extracts: The phyotchemical screening of the plant extracts was carried out according to standard procedure [5].

Alkaloids: The methanolic extract (30ml) was evaporated to dryness in an evaporating dish on water bath. Five ml of 2 N HCl were added and stirred while heating on the water bath for 10min., cooled, fi..ltered, and the filtrated was treated with a few drops of Mayer reagent. The samples were thanobserved for the presence of turbidity or precipitation.

Flavonoids: The alcoholic extract (75 ml) of plant sample were evaporated to dryness on a water bath, cooled and the residue was defatted by washing several times with petroleum ether. The defatted residue was dissolved in 30ml 80% ethanol and filtered. The filtrated was treated with a few drops of concentrated HCl and magnesium turnings (0.5g). The presence of flavonoids was indicative if a pink or magneta red color developed within 3 min.

Tannins: The alcoholic extract (25ml) was evaporated to dryness on a water bath. The residue was extracted several times with n-hexane & filtered, the insoluble residue was stirred with 10ml of hot saline solution, the mixture was cooled, filtered and the volume of filtrate was adjusted to 10ml with more saline solution. To 5 ml of this solution, few drops of ferric chloride test reagent were added. An intense green blue or black colour was taken as an evidence for the presence of tannins.

Saponins: 1 gm of ethanol extract was dissolved in 10ml of distilled water in a test tube and shaked vigorously for 1-2 min. The presence of saponins was indicated by characteristic honeycomb froath at least 1cm in height, which persisted for 30 min

Anticancer activity

Human cell lines and culture: Cytotoxic assay was carried out by MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide, a tetrazole) protocol in order to evaluate the anti proliferative effect of extracts of *F. roylei*. For this purpose, a sufficient number of exponentially growing cells were used to avoid confluence of the culture during the treatment. The cell lines A549, C6, T47D, MCF, and TH-1 were seeded at 10^4cells/well and allowed to adhere for 12 h.

Cytotoxicity assay: In order to evaluate the optimum concentration at which the extracts inhibited the cell proliferation in all the five cell lines, cells were treated with the extracts at a concentration of 100 µg/ml. DMSO was used as a solvent for the dilution of extract, which was also used as an experimental control. Mitomycin-C was used as positive controls at a concentration of 1×10^{-5} µg/ml. After 48 h treatment, cell growth was evaluated by MTT assay [6]. MTT solution of 50 µl (5 mg/ml of PBS) was added to each well and the plates were incubated for 3 h at 37^oC in dark. The media was aspirated and 150 µl of MTT solvent (4 mM HCl, 0.1% Nondet P-40, all in isopropanol) was added to each well to solubilize the formazan crystals. The absorbances of plates were measured on ELISA reader (Benchmark, BioRad) at a wavelength of 570 nm. Each sample was performed in triplicate, and the entire experiment was repeated thrice

Antioxidant Activity: The free radical scavenging activity of the extract was measured in terms of hydrogen donating or radical- scavenging ability using the stable radical DPPH . 0.1 mM solution of DPPH in methanol was prepared and 1.0 ml of this solution was added to 1.0 ml of the test solution in methanol at concentrations of 100 µg/ml). Thirty minutes later, the absorbance was measured at 517 nm. Ascorbic acid was used as the reference compound. Lower absorbance of activity was expressed as the inhibition percentage of free radical by the sample and was calculated using the following formula:

% scavenging activity = $(A_0\text{-}A) / A_0 \times 100)$.

Where A_0 was the absorbance of the control (blank, without compound) and A was the absorbance of the the reaction mixture. All the tests were performed in triplicate and the graph was plotted with the mean values.

RESULTS AND DISCUSSION

The use of herbs for medicinal purpose is a universal phenomenon. Every culture and civilization on earth, through written or oral tradition, has relied on vast variety of natural chemistries found in plants for their therapeutic properties. All drugs from the plant are substances with a particular therapeutic action extracted from plants. Plant substances continue to serve as viable source of drugs for the world population and several plant-based drugs are in extensive clinical use [7].The curative properties of plants are perhaps due to the presence of various secondary metabolites which are non nutritive plant compounds. These classes (such as alkaloids, tannins, anthraquinones and flavonoids) of compounds are known to have curative activity against several pathogens and therefore could suggest the use of it traditionally for treatment of various illnesses [8] (Usman & Osuji 2007).

The extracts of *Fritillaria roylei* were phytochemically screened for the presence of Alkaloids, Flavonoids, Tannins and Saponins. However the results showed that the *Fritillaria roylei* contains Alkaloids, Tannins and Saponins in good amount. Since these alkaloids and tannins are responsible for broad pharmacological profile, including antiinflammation, antioxidant, antibacterial, anticancer, and anti-histamine activity.

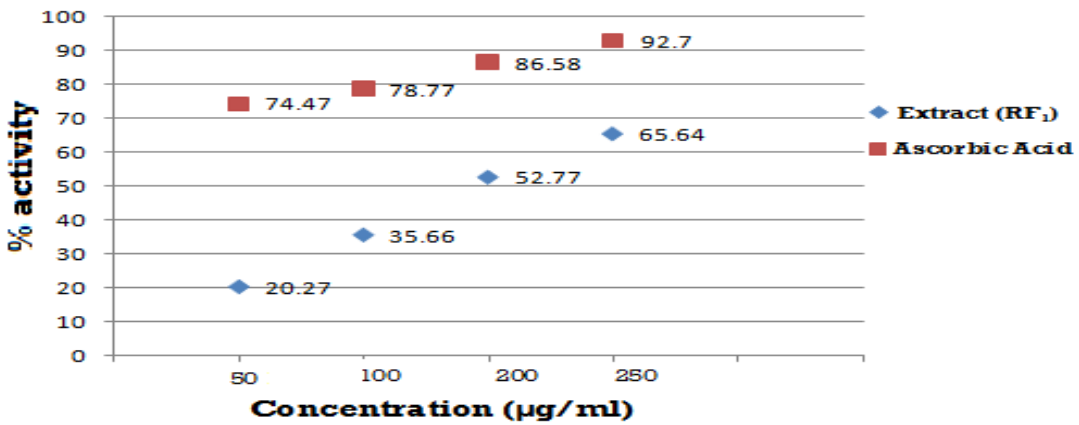
Cytotoxic activity Therefore the methanolic and ethylacetate extracts of the *Fritillaria roylei* were screened for their possible anticancer activity. In order to understand the effects of various extracts of *Fritillaria royle* on human cancer cell lines, experiments were carried using cultured A549(lung), C6(Glioma), T47D (Breast) , MCF (Breast) and TH-1(Colon) cell lines by MTT assay. All cell lines were submitted to 100µg concentration of extracts, which reduced the viability of these cell lines. As shown in **Table 1** the extract was active against all the five cancer cell lines tested. The concentration of Mitomycin C was taken as 1×10^{-6}µg/ml.

Antioxidant Activity: DPPH free radical scavenging capacity of methanolic extract of *Fritillaria roylei* was measured by DPPH assay under in vitro conditions. The ability of the examined extract to act as donor for hydrogen atoms in the transformation of DPPH radical into its reduced form DPPH2 was investigated. The examined samples were able to reduce the stable purple colored DPPH radical into yellow colored DPPH2.The more rapidly the absorbance decreases, the more potent the antioxidant activity of the sample in terms of its hydrogen atom-donating capacity [9]-[10]. The aforementioned extract showed most promising radical scavenging activity at concentration of 150µg/ml. The results are plotted in the form of graph **(fig.1).**

Table:1 In-vitro cytotoxicity of the extracts of *Fritllaria roylei*

Cell-type	A549	C6	T47D	MCF	TH-1
DMSO	6	6	4.5	6.5	7
Mitomycin C	92	89	91	78	83
Methanolic extract	65	71	53	62	76
EthylAcetate extract	55	43	77	78	57

Fig 1: Antioxidant activity of methanolic extract of *Fritillaria roylei*



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