



## Comparison of three extracts of *Fumeria indica* for the evaluation of cytotoxic and phytotoxic activities

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### Abstract

In present work, the *Fumeria indica* was collected from University of Peshawar. The plant was shade dried and three extracts were prepared from the powder. The powder was used to observe under ultra violet light and by change in colour, has shown the presence of secondary metabolites with their derivatives. The extracts were used against phytochemical screening test, cytotoxic and phytotoxic activities. Phytochemical screening has shown the presence of carbohydrates, alkaloids, flavonoids, tannins, anthraquinones, saponin and starch. In cytotoxic activity, the n-hexane was found more effective than chloroform and ethanol but in phytotoxic activity the n-hexane was highly significant than ethanol whereas chloroform was found non-significant. Further experiments on *Fumeria indica* is needed to investigate its therapeutic properties.

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## Introduction

From centuries, medicinal plants are used traditionally for curing various ailments but in developing countries where medical knowledge is inadequate, the medicinal plants still are used as medicines (Samie *et al.*, 2005). Each medicinal plant has its own pharmacological and pharmacological importance that can be evaluated by using phytochemical tests (Pandey *et al.*, 2006). Phytochemicals are natural secondary metabolites that's help plants to protect themselves against various pathogenic attacks and to make them healthy so that's they can survive safely (Chew *et al.*, 2009). Cytotoxic and phytotoxic activities were conducted on *Fumeria* species by using ethanolic extracts by many researchers (Camacho *et al.*, 2003; Farrukh *et al.*, 2010; Nikolova and Svetkova, 2007).

Fumeraceae consists of 46 species of *Fumeria* found in Europe and Asia (India, Afghanistan, Persia and Pakistan) out of which only three are found in Pakistan (*Fumeria parviflora*, *Fumeria vaillantii* and *Fumeria indica*). *Fumeria* species are known as fumitory, earth smoke, fumus or wax dolls (Kirtikar and Basu, 1985; Ali and Qaiser, 1993; Orhan *et al.*, 2010). *Fumeria indica* (Haausskn.) pugsley, is a small, delicately branched leafy herb with leaves usually glabrous, lanceolate-subulate and slightly acuminate. Flowers 6-15 in number with length 5-6 mm and are white or pale pinkish. Fruit about 2mm broad, sub-orbicular, quadrate with 1 seed. Stem light green, smooth hollow about 3-4 mm thick, root brown color (Ali and Qaiser, 1993). In Pakistan *Fumeria indica* is mostly use as fodder by cattle but it also possesses some therapeutic properties and because of this it is use traditionally by the local people for the treatment of various diseases e.g. as diuretic, diaphoretic and in leprosy, laxative, febrifuge, as a blood purifier and in treatments of digestive problems (Shinwari and Khan, 1999; Khatak, 2012; Zabiullah *et al.*, 2006).

In present work the cytotoxic and phytotoxic activities were conducted to evaluate the therapeutic properties of *Fumeria indica* but further investigation is required to check the use of plant in

medicines and to improve the economy of the country by checking weedicidal effects of the plant.

## Material and methods

*Fumeria indica* was collected from the campus of University of Peshawar and was identified by curator at herbarium in Department of Botany, University of Peshawar. The plant was shade dried for 10-15 days by spreading them on newspapers. After dring the powder was grinded by electric grinder. The ethanol, chloroform and n-hexane extracts were prepared for conducting various bioassays.

### Fluorescence study

Powder drug was macerated with different reagents like HCL, NaOH, H<sub>2</sub>SO<sub>4</sub>, methanol, ethanol, water, HNO<sub>3</sub>, Acetic acid and picric acid and observed their colors under ordinary and ultraviolet (UV) light of 255UV and 366UV (Kokoshi *et al.*, 1958).

### Phytochemical screening tests

Ethanol, Chloroform, n-hexane and aqueous extracts were used for phytochemical screening. Following are the tests.

### Tests for carbohydrate

Following tests were used to detect presence of any reducing sugar in *Fumeria indica* (Evans, 1989).

### Molisch's test

Extract solution was treated with few drops of alcoholic alpha-naphtol. Then 0.2ml of conc. H<sub>2</sub>SO<sub>4</sub> was slowly added through the sides of the test tube. The appearance of purple to violet ring at the junction indicates the presence of reducing sugars (Evans, 1989).

### Benedict's test

Extract solution was treated with few drops of Benedict's reagent and then boiled on water bath. The appearance of reddish brown precipitate indicates presence of carbohydrates (Evans, 1989).

### Fehling's test

Equal volume of Fehling's A and Fehling's B reagents were taken in equal quantities and were added to extract solution and boiled on water bath. Appearance of brick red precipitate indicates the presence of reducing sugars (Evans, 1989).

#### *Tests for proteins and amino acids*

##### *Ninhydrin test*

Extract solution was boiled with 0.2 % solution of ninhydrin. The appearance of violet color indicated the presence of proteins (Evans, 1989).

##### *Test for triterpenoids*

###### *Salkowski's test*

Extract was treated with chloroform along with few drops of conc. Sulphuric acid, shake well and allowed to stand for some time. Red color appearance in upper layer indicates the presence of sterols and if yellow color appears in lower layer, it indicates the presence of triterpenoids (Sofowara, 1982).

##### *Test for flavonoids*

###### *Alkaline reagent test*

Few drops of sodium hydroxide solution was mixed with extract, the formation of yellow red precipitate indicate the presence of Flavonoids (Trease and Evans, 2002).

##### *Tests for alkaloids*

###### *Mayer's test*

Few drops of Mayer's reagent were added to extract solution. Creamy white precipitate indicates presence of alkaloids (Trease & Evans, 2002).

###### *Dragendroff's test*

Few drops of Dragendroff's reagent were added to the extract. Reddish brown precipitate is produced, it indicate the presence of alkaloids (Trease and Evans, 2002).

###### *Wagner's test*

Few drops of Wagner's reagent were added to the extract. Reddish brown precipitate indicates presence of alkaloids (Trease and Evans, 2002).

##### *Hager's test*

Few drops of Hager's reagent were added. If yellow precipitate is produced, it indicates presence of alkaloids (Trease and Evans, 2002).

##### *Tests for tannin compounds*

###### *Ferric chloride test*

Few drops of  $FeCl_3$  were added to the extract solution. If blue-green color appears in the solution, it indicates the presence of tannin compounds (Kokate, 1994).

##### *Detection for anthraquinones*

A small amount of powder was mixed with small quantity of ether. If pink, red or violet color appears in aqueous layer, it indicates the presence of anthraquinones derivatives (Evans, 1989).

##### *Detection of calcium oxalate*

To the extract, HCL and  $H_2SO_4$  were added respectively. If no effervescence appears, it indicates the presence of crystals of calcium oxalate (Evans, 1989).

##### *Detection of saponin*

Powdered drug was taken in a test tube half filled with water and vigorously shaken. If frothing appears indicate the presence of saponin (Kokate, 1994).

##### *Detection of starch*

The powder drug was treated with few drops of iodine solution; if deep blue color appears indicate the presence of starch (Evans, 1989).

##### *Detection of fat and oil*

A small amount of powdered drug was taken on filter paper and was pressed between the folds of filter paper. The presence of an oily stain indicates the presence of fats and fixed oil (Kokate, 1994).

##### *Detection of catechin*

A matchstick was taken and was dipped in plant extract. Then was dried and moistened with conc. HCl and warmed near a flame, if pink or red color

appeared on matchstick, it indicate the presence of catechin (Evans, 1989).

#### *Tests for volatile oils*

Along with extract solution two drops of 1M alcoholic  $K_2Cr_2O_7$  and three drops of phenolphthaline were added in a clean test tube. Soap formation indicated the presence of essential oils (Brindha *et al.*, 1981).

#### *Biological activities*

These includes Cytotoxic and Phytotoxic activity. The ethanol, chloroform and n-hexane extracts were use for this "Brine shrimp assay" by following the method of Atta Ur Rehman (1991).

#### *Technique for hatching eggs*

A small plastic tray (4 ×6 ×2) was half filled with brine solution. The tray was divided into two unequal halves by a perforated portion. About 50mg eggs were sprinkled on the smaller half of the tray. This small half was then covered with a carbon paper to make it dark. An electric bulb was hung over the tray, which illuminated the larger half of the tray. After incubation for about 48hrs, the egg hatched into larvae, which actively swim and migrated to the larger illuminated half of the tray through the perforations of the partition.

#### *Brine shrimp lethality test*

By using Pasteur pipette ten hatched and matured larvae (now called nauplii) were placed in each vials (with concentrations of 10, 100 and 1000  $\mu\text{g/ml}$ ). By using squeezer the volume was made up to 5ml by adding sea water. After 24hrs the number of survivors was recorded in each concentration.

#### *Phytotoxic activity*

Ethanol, chloroform and n-hexane extracts of *Fumeria indica* were used to conduct phytotoxic activity against *Lemna minor* by following the method of Atta-ur-Rehman, (1991).

First E-Medium was prepared then the pH was adjusted at 5.5. The medium was autoclaved at 121°C for about 15 minutes. Three sterilized flasks each

were inoculated with 1000ppm, 100ppm and 10ppm of solution. The flasks were kept overnight in sterilized condition for evaporation of solvent. 20ml of E-Medium was added to each flask. Ten plants of *L. minor* each having three fronds was added. Other flasks were supplemented with E-medium and standard plant growth inhibitors serving as negative and positive controls respectively. Plants were examined daily during incubation. Flasks were placed for 7 days in growth cabinet. Number of fronds were counted and recorded on day 7.

## **Results and discussion**

#### *Fluorescence study*

Ultraviolet light from sun show some of the chemicals with their derivatives that are present in plants (Vass *et al.*, 1996). Hence fluorescence study is effective to study the plant chemical composition, when all other methods cannot prove to be effective the fluorescence study can be proven effective in this respect (Kokoshi *et al.*, 1958). In present study the powder drug of *Fumeria indica* while studying in UV (255 and 366) and day light, has shown difference in their color. The results are shown in table.

#### *Phytochemical screening*

In the present study phytochemical screening was carried out of *Fumeria indica* by using four solvents e.g. aqueous, ethanol, chloroform and n-hexane. The results are shown in Table 1. All the extracts have shown almost the same results like carbohydrates, starch, flavonoids, tannins, alkaloids, anthraquinones and saponin were present in all the extracts but fats, proteins and amino acid (except ethanol and chloroform), volatile oils (except ethanol and chloroform), triterpenoids (except chloroform), gums and mucilage, volatile oils, calcium oxalate were absent in all the extracts.

**Table 1.** Fluorescence study of *Fumeria indica*.

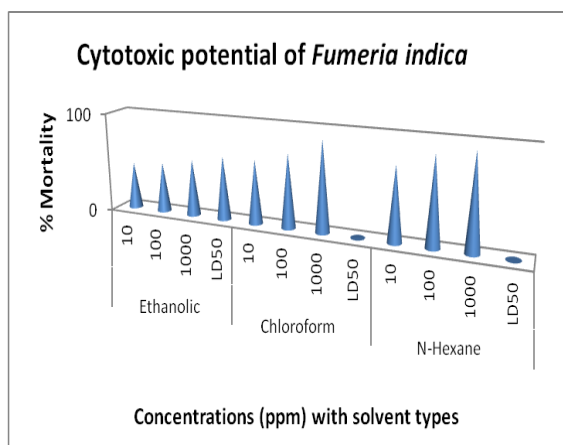
Reagents	Day light	255 UV	366 UV
Powder without treatment	Grass Green	Light Brown	Dark Brown
Powder + HCl	Green	Purplish Green	Blackish
Powder + NaOH	Brown	Blackish Brown	Dark Brown
Powder + 50% HNO <sub>3</sub>	Blackish Brown	Dark Brown	Light Brown
Powder + 50% H <sub>2</sub> SO <sub>4</sub>	Greenish Brown	Dark Green	Purplish
Powder + methanol	Florescent Green	Dark Green	Green Blue
Powder + Acetic acid	Dull Green	Dark Green	Dark Brown
Powder + Picric acid	Dark Brown	Dark Green	Greenish Brown
Powder + Water	Light Green	Dark Green	Dark Green
Powder + NaOH in methanol	Dark Green	Black	Greenish Black
Powder + ethanol	Spring Green	Dark Green	Grey

**Table 2.** Preliminary phytochemical tests for *Fumeria indica*.

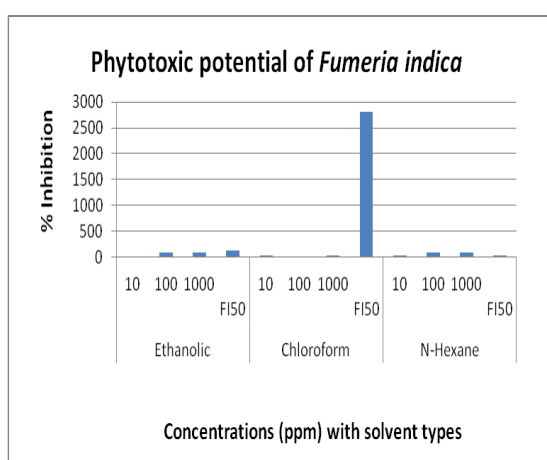
Constituents	Tests	Aqueous extract	Ethanolic extract	Chloroform	N-Hexane
Carbohydrate	Molisch's test	+	+	+	+
	Benedict's test	+	+	+	+
	Fehling's test	+	+	+	+
Proteins and Amino acids	Ninhydrin test	-	+	+	-
Triterpenoids	Salkowski's test	-	-	+	-
	Mayer's test	+	+	+	+
Alkaloids	Dragendroff's test	+	+	+	+
	Wagner's test	+	+	+	+
	Hager's test	+	+	-	+
Flavonoides	Flavonoides	+	+	+	+
Tannin	Ferric chloride test	+	+	+	+
Anthraquinone	Anthraquinone	+	+	+	+
Calcium Oxalate	HCl + Conc. H <sub>2</sub> SO <sub>4</sub>	-	-	-	-
Saponin	Frothing test	+	+	+	+
Starch	Starch	+	+	+	+
Fat and Oil	Paper test	-	-	-	-
Catechin	Flame test	-	-	-	-
Volatile oils	Volatile oils test	-	+	+	-
Gums and Mucilage	Mucilage	-	-	-	-

**Table 3.** Cytotoxic activity of *Fumeria indica*.

Treatments	Conc. (µg/ml)	Total shrimps	Shrimps Survived	Shrimps Dead	% inhibition	Intercept	Slope	R-Square	LD <sub>50</sub>
Ethanolic	10	30	16	14	46.66	2.363	0.034	0.972	63.66
	100	30	15	15	50.00				
	1000	30	13	17	56.66				
Chloroform	10	30	11	19	63.33	2.440	0.064	0.990	0.57
	100	30	8	22	73.33				
	1000	30	3	27	90.00				
N-Hexane	10	30	8	22	73.33	2.521	0.044	0.954	0.01
	100	30	4	26	86.67				
	1000	30	2	28	93.33				



**Fig. 1.** Cytotoxic potential of *Fumeria indica*.



**Fig. 2.** Phytotoxic potential of *Fumeria indica*.

Phytochemicals are responsible for various bioactivities such as antimutagenic, anticarcinogenic, antioxidant, antimicrobial and anti-inflammatory properties etc (Yen *et al.*, 1996). flavonoids shows astringent properties to protect against skin diseases (Tyler *et al.*, 1981). Similarly presence of glycosides, alkaloids and tannins shows the hypoglycemic potential of the plant (Cherian and Augustin, 1995).

Many researchers have done phytochemical screening of medicinal plants to evaluate their therapeutic properties like *Fumeria parviflora* (Naz *et al.*, 2012), *Acanthus ilicifolius* (Poorna *et al.*, 2011) and *Vitex trifolia* (Thenmozhi *et al.*, 2011).

#### Cytotoxic effects

Ethanol, chloroform and n-hexane extracts were used for evaluation of *Fumeria indica*. It was found

effective in all the extracts with sequence of n-hexane > chloroform > ethanol. These results were shown in table 3 and figure 1. With increase in concentration the extract shows more significant results. 1000 ug/ml concentration has shown in ethanol 56.6%, chloroform 90% and in n-hexane 93% inhibition. The brine shrimp bioassay was used as an indicator for evaluating the toxicity of a plant and also as a tool for the detection of antitumor properties (Laughlin, 1991).

Erdogen. T. F, 2009 work on *Fumeria densiflora* and *Fumeria officinalis* and reported that the both plants have shown more significant results in n-hexane extract than in aqueous and ethanol extracts. Farrukh *et al.*, 2010 work on the members of polygonaceae in which they clearly showed that with increase in concentrations the *Rumex* species showed significant results. Our findings were in line with the above researchers work.

#### Phytotoxic effects

Phytotoxic activity was not that much effective in chloroform than the ethanol and n-hexane. The results were shown in table 4 and figure 2. In ethanol the % inhibition was less in low concentration whereas with increase in concentrations the activity was significant. 1000 ug/ml concentration has shown in ethanol 86%, chloroform 26% and in n-hexane 100% inhibition. This shows that the n-hexane extract was more effective than the other two extracts (n-hexane > ethanol > chloroform).

Farrukh *et al.*, 2010 conducted his work on the members of polygonaceae and found that all the species of *Rumex* were showing significant inhibition in n-hexane extract solution at 1000 ug/ml then the aqueous and ethanolic extracts. Hussain *et al.*, 2009 work on *Nepeta juncea* and showed that chloroform was effective then the ethanol extract.

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