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Phytochemical Screening And In Vitro Evaluation Of Anticancer Activity Of Ethanol Extract Of Leaves Of Paederia Foetida

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Abstract

Paederia foetida is a native Bangladeshi plant, used for the treatments of various diseases in folklore medicine. In this study, the anticancer potential of Paederia foetida extract was evaluated by using a series of well established MTT assay systems. The present study aimed at the preliminary phytochemical investigation and in Vitro Evaluation of skin melanoma cancer cell line (B16F10) inhibitory Activity of ethanol extract of Paederia Foetida.

The activity was performed using ethanolic extract of leaves of Paederia Foetida at various concentrations (100µg, 200µg, 400µg, 800µg, 1000µg,). In this study five flurouracil was used as a standard drug. Paederia foetida extract anticancer activity in a dose dependent manner. In the phytochemical investigation , flavonoids, saponin, triterpines, steroids and tannins are present The results were noted in terms of highest percentage inhibition of skin cancer cell line due to presence of some responsible phytoconstitutes in the extracts.

Keywords: Paederia Foetida., Phytochemical, B16F10 skin cancer, 5- flurouracil, MTT assay

Introduction

Cytotoxicity testing using cell cultures is a rapid, standardized, sensitive, and inexpensive means to determine whether a material contains significant quantities of biologically harmful extractable . There are several *in-vitro* toxicity tests where different cell lines are used, each test using different indicators [1].

A number of alternative tests that have been proposed for rapid screening are freshwater rotifer (*Branchionus calyciflorus*), brine shrimp (*Artemia salina*), lettuce (*Luctuca saliva*), and mysid shrimp (*Mysidop sishahia*). These tests are useful in situations where their rapidity and relatively low cost make it practical to screen large numbers of samples for preliminary indications of toxicity [2].

A large number of members of this family are ethnomedicinally important and showed a wide variety of biological activities. *Paederia foetida* belongs to *Rubiaceae* family, is a climbing, herbaceous, hairy or quite smooth, slender vine distributed. Leaves are considered as a good remedy for diarrhea and dysentery and used as household remedy during convalescence from acute illness.

Leaves are also used as a poultice to relieve distension of the abdomen due to flatulence and also used to treat herpes and earache. Decoction of leaves is considered as diuretic.[3]

Paederia foetida has been used extensively in traditional medicine. It is claimed to promote sexual vigour, increases the quantity of semen and body strength and produces a youthful glow [4].

The present study was intended for the phytochemical screening and its anticancer activity by using B16 F10 skin cancer cell line on ethanol extract of leaves of *Paederia foetida.*

Materials And Methods

Chemicals MTT , TCA (Trichloro acetic acid) were obtained from Sigma Chemical Co., USA. Five flurouracil was obtained from SD Fine Chem. Ltd., Biosar, India. Phosphate buffer was obtained from research lab, Mumbai, India. DMEM with high glucose (Cat No-11965-092), FBS (Gibco, Invitrogen) Cat No -10270106 Antibiotic – Antimycotic 100X solution (Thermo fisher Scientific)-Cat No-15240062.

Plant material

Paederia foetida herbs were collected from the Deharadun, Himachal pradesh, in May 2021 and authenticated through approved botanist and a voucher specimen was deposited in the Laboratory.

Extraction:

The shade-dried leaves were coarsely powdered and extracted with mixture of ethanol: water (7:3 ratios) by a Soxhlet apparatus. The solvent was completely removed by rotary evaporator and obtained gummy exudates. This crude extract was used for further investigation for potential anticancer properties [5].

Phytochemical screening

Phytochemical screening of the extract was performed using the following reagents and chemicals: Alkaloids with Dragendorffs reagent, flavonoids with the use of Mg and HCl; tannins with ferric chloride and potassium dichromate solutions and saponins with ability to produce suds. Gum was tested using Molish reagents and concentrated sulphuric acid [6].

Procedure:

Experimental procedure:

Cells were incubated at a concentration of 1 × 104cells/ml in culture medium for 24 h at 37°C and 5% CO2.Cells were seeded at a concentration (70µl) 104cells/well in 100 µl culture medium and 100µL sample of red dye Sample in (10, 20, 40, 80, 100 µL/ml) into micro plates respectively (tissue culture grade, and 96 wells). Control wells were incubated with DMSO (0.2% in PBS) and cell line. All samples were incubated in triplicate. Controls were maintained to determine the control cell survival and the percentage of live cells after culture. Cell cultures were incubated for 24 h at 37°C and 5% CO2 in CO2 incubator (Thermo scientific BB150) After incubation, the medium was completely removed and Added 20 µl of MTT reagent (5mg/min PBS). After addition of MTT, cells incubated for 4 hrs at 37oC in CO2incubator. Observed the wells for formazan crystal formation under microscope. The yellowish MTT was reduced to dark coloured formazan by viable cells only. After removing the medium completely. Added 200µl of DMSO (kept for 10 min) and incubate at 370C (wrapped with aluminium foil).Triplicate samples were analyzed by measuring the absorbance of each sample by a Elisa microplate reader (Benesphera E21) at a wavelength of 570 nm [7,8].

Results and Discussion:

Phytochemical screening of the extracts indicated the presence of flavonoids, saponin, gum and tannins (Table 1).

Table 1: Phytochemical screening of *Paederia foetida* extract.

Chemical constituent	Test	EtOH extract of leaves
Alkaloids	Dragendroff's test	-

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Chemical constituent	Test	EtOH extract of leaves
	Wagner's test	-
	Hager's test	-
	Mayer's test	-
Carbohydrates	Molisch's test	--
	Barfoed's test	--
	Benedict's test	--
Flavonoids	Shinoda's test	++
	Lead acetate solution.	++
	NaOH test	++
	5% Ferric chloride test	++
Saponin	Foam test	++
Triterpines	Liebermann Burchard test	++
Steroids	Salkowaski test	++
	Liebermann Burchard test	++
	Sulphur test	++
Tannins	5% Ferric chloride test	++
	10% lead acetate	++
	Acetic acid	++
	Pot. Permagnate	++
	Dil. Iodine	+

Phytochemical screening of the extracts indicated the presence of flavonoids, saponin, triterpines, steroids and tannins

The MTT Cell Proliferation and Viability Assay is a safe, sensitive, in vitro assay for the measurement of cell proliferation or, when metabolic events lead to apoptosis or necrosis, a reduction in cell viability. Cells are cultured in flat-bottomed, 96-well tissue culture plates. The cells are treated as per experimental design and incubation times are optimized for each cell type and system. The tetrazolium compound MTT (3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide) is added to the wells and the cells are incubated. MTT is reduced by metabolically active cells to insoluble purple formazan dye crystals. Detergent is then added to the wells, solubilizing the crystals so the absorbance can be read using a spectrophotometer. Samples are read directly in the wells. The optimal wavelength for absorbance is 570 nm, but any filter that absorbs between 550 and 600 nm may be used. The data is analyzed by plotting cell number versus absorbance, allowing quantitation of changes in cell proliferation. The rate of tetrazolium reduction is proportional to the rate of cell proliferation.

Table 2: Anticancer activity of ethanol extract of leaves of *Paederia foetida against B16 F10 skin cancer cell line.*

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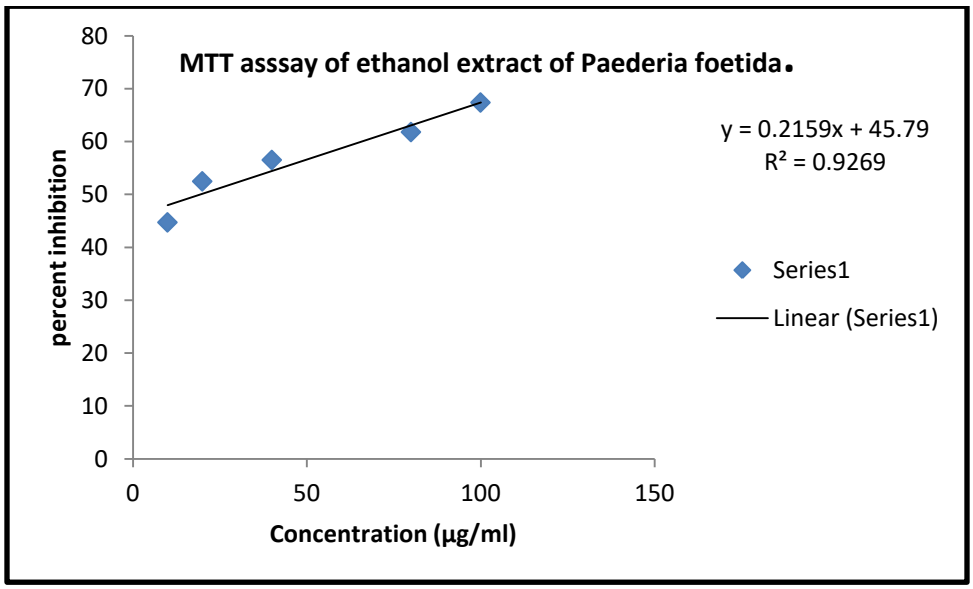
Sr. no	Sample	Concentration (µg/ml)	OD	% inhibition	IC 50 (µg/ml))
1	Control		0.322		
2	Std. 5 FU	10 **µg/ml**	0.099	69.25	1.09
		20 **µg/ml**	0.081	74.84	
		40 **µg/ml**	0.079	75.46	
		80 **µg/ml**	0.071	77.95	
		100 **µg/ml**	0.064	80.12	
3	**Ethanol extract of leaves of *Paederia foetida.***	100**µg/ml**	0.178	44.72	19.58
		200 **µg/ml**	0.153	52.48	
		400 **µg/ml**	0.140	56.52	
		800**µg/ml**	0.123	61.80	
		1000 **µg/ml**	0.105	67.39	



The plant *Paederia foetida* contains various phytoconstituents triterpenoid saponins such as ursolic acid, epifriedelinol and friedelin [36].

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At the different concentration (10 µg/ml to 100 µg/ml) of Ethanol extract of leaves of *Paederia foetida.*, it was observed that the ethanol extract showed good activity against skin cancer cell line when you compared to standard drug 5 FU. According to IC50 value, Ethanol extract of leaves of *Paederia foetida* showed less concentration which

required for the killing of 50 percent cancer cell. So it indicates that it showed promising anticancer activity.

The present study demonstrated the effectiveness of *Paederia foetida* leaves extract as a potent anticancer activity in various *in vitro* anticancer assays by using various cancer cell lines.

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