Research Article

IN-VITRO CYTOTOXIC ACTIVITY STUDIES OF CLITORIA TERNATEA LINN FLOWER EXTRACTS

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ABSTRACT

This study is to evaluate the in vitro cytotoxic effect of petroleum ether and ethanolic flower extracts of Clitoria ternatea Linn by using trypan blue dye exclusion method. Both extracts exhibit significant cell cytotoxic activity. Phytochemical screening of petroleum ether extract reveals the presence of steroids, triterpinoids, tannins, saponins, while in ethanolic extract shows the presence of flavanoids.

Keywords: Clitoria ternatea, cytotoxic activity, trypan blue exclusion method, flower extract.

INTRODUCTION

Nature has been a source of medicinal agents since time immemorial. The plant kingdom harbors an in exhaustible source of active ingredients invaluable in the management of many intractable diseases.[1]

Clitoria ternatea is a leguminous tropical herb belongs to the family Fabaceae. It is a very common garden flower plant found all over India especially in southern India. The plant is considered useful for eye infections, skin diseases, urinary troubles, ulcer and has anti dotal properties[2]. The flowers reportedly contain flavanoid glycosides.[3,4]

Cancer is a disease characterized by a loss in normal control mechanisms that govern cells survival, proliferation and differenciation[5]. Cancer is a major public burden in both developed and developing countries. In United States where one in four deaths is due to cancer. Plants have long been used in the treatment of cancer[6] The anti cancer cells either kills cancer cells or modify their growth[7].

The present investigation was undertaken to find out the unexplored cytotoxic effect of flower extracts of Clitoria ternatea.

MATERIALS AND METHODS

The flowers of plant Clitoria ternatea Linn was collected from Mangalore. The taxonomical identity of the plant was confirmed by Dr Neoline J pinto, H.O.D, Department of Botany, ST Agnes College, Mangalore. A voucher specimen was deposited in Shreedevi college of pharmacy, Kenjar, Mangalore.

Preparation of extracts

The powdered plant material were air dried and subject to successive solvent extraction with solvents like petroleum ether (60-80°c), benzene, chloroform, acetone, ethanol and water[8]. The petroleum ether and ethanolic extract was prepared by distill off the solvent from the corresponding extracts and evaporate to dryness, stored in a desiccator.

In-vitro cytotoxicity studies

The petroleum ether extract and ethanolic extract were studied for short term in vitro cytotoxicity using Dalton's Lymphoma ascites cells.

10mg of the extract was taken in an eppeandorf vial of capacity 1ml and dilute to six different concentrations with its duplicate and control (50%) using DMSO as a solvent and mixed with the help of a vortexing machine. Aspirated tumor cells from peritoneal cavity of mice was obtained from Amala cancer research centre, Amala nagar Thrissur, Kerala. The procedure was approved by institutional animal ethics committee. The cell viability was checked by try pan blue dye (1%). The cell suspension (1x 10^6 cells in 0.1ml) was added to tubes containing various concentrations of the test compounds and the volume was made up to 1ml using phosphate buffered saline (PBS). Control tube contained only cell suspension. These assay mixtures were incubated for 3 hour at 37^0C. After incubation 0.1 ml trypan blue was added and number of dead cells determined by using haemocytometer[9]. Results were tabulated in table-1 (for petroleum ether extract) and table-2 (for ethanolic extract).

Statistical Analysis

Results are expressed as mean ± statistical significance was accessed using one-way analysis of variance (ANOVA) followed by Tukey–karmer multiple comparison tests. $p<0.05$ was considered significance.

Phytochemical screening of crude extracts

The phytochemical screening of crude petroleum ether and ethanolic flower extracts of Clitoria ternatea were carried out. It reveals the presence of following phytoconstituents like steroids, triterpenoids, saponins, tannins, and resins in petroleum ether extract and

flavanoids in ethanolic extract[10]. Results were shown in Table-3.

Table 1: cytotoxic activity of petroleum ether extract using DLA cell lines.

Concentration (µg/ml)	DLA (% cytotoxicity)	IC_{50}
10	8±0.73***'''	36 µg/ml
20	36.16±1.01***'''	
50	79±0.51***'''	
100	89 ± 0.98***'''	
200	95.16 ± 0.70***	
500	100 ± 0.00	
Control	00 ± 00	

All values are mean ± SEM n=6 ,. P<0.1 ..p<0.01 ...p<0.001 when compared to 250 µg/ml .p<0.1,..p<0.01 ,...p<0.001 when compared to 200 µg/ml.

Table 2: Cytotoxic activity of ethanolic extract using DLA cell lines.

Concentration (µg/ml)	DLA (% cytotoxicity)	IC_{50}
10	1.33 ± 0.33***'''	57 µg/ml
20	29.1 ± 0.40***'''	
50	48.66 ± 0.49***'''	
100	58.50 ± 0.84***'''	
200	62.83 ± 0.60***'''	
500	80 ± 0.51	
Control	00 ± 00	

All values are mean ± SEM n=6 ,. P<0.1 ..p<0.01 ...p<0.001 when compared to 250 µg/ml .p<0.1,..p<0.01 ,...p<0.001 when compared to 200 µg/ml.

Table 3: Phytochemical analysis of petroleum ether and ethanolic flower extracts of Clitoria Ternatea.

Phytoconstituents	Petroleum Ether flower extract	Ethanolic flower extract
Saponins	+	-
Tannins	+	-
Steroids	+	-
Flavonoids	-	+
Cardiac glycosides	-	-
Triterpenoids	+	-

+ positive; - Negative

RESULTS AND DISCUSSION

Estimation of cytotoxicity was done by trypan blue exclusion method. The various concentration of plant extracts used were 10, 50, 100, 200, 500 µg/ml and control (without extract). For both the extracts decrease in cell count was observed with increase in concentration of the extract. There was a dose dependent increase in cytotoxic activity for all the concentrations tested. For petroleum ether extract the concentration 10 µg/ml showed a reduction of 8 % and 100% reduction observed at 500µg/ml. In case of ethanolic extract at 10 µg/ml concentration 1.33 % reduction was observed and at 500µg/ml 80 % reduction in cell count was observed.

On the basis of above results it can be concluded that both the extract poses significant cell cytotoxic activity studied by invitro models. Further investigation is required to find active component of the extract and isolation of the components responsible for activity.

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