International Journal of Pharmacy
Journal Homepage: http://www.pharmascholars.com

Original Article **CODEN: IJPNL6**

PHYTOCHEMICAL ANALYSIS AND ANTIPROLIFERATIVE STUDIES OF VARIOUS EXTRACTS OF *MOLLUGO CERVIANA*

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Received on: 13-05-2016; Revised on: 05-06-2016; Accepted on: 25-06-2016

ABSTRACT

In the present work, phytochemical analysis and antiproliferative activities of various extracts of *Mollugo cerviana* were evaluated by trypan blue dye exclusion and MTT assay models. The extracts produce potent cytotoxic effect against Human cancer cell lines viz. Human cervical cancer (HeLa), lung cancer (A549), breast cancer (MCF7) and liver cancer (HepG2) cell lines. Hexane and water extracts showed moderate activity whereas ethanol, chloroform and ethyl acetate extracts showed potent activity against the tested cell lines. At the same time all the tested extracts showed 3-4 fold high IC_{50} values for the normal human dermal fibroblast cell line. The results revealed that the selective cytotoxicity of the plant extracts towards the cancer cells. Qualitative phytochemical analysis of these extracts revealed the presence of alkaloids, flavonoids, glycosides, polyphenolics and saponins. Antiproliferative and cytotoxic activity of the extracts may be due to the rich amount of these phytochemicals. Hence, the plant could be considered as a very good anticancer drug with renowned therapeutic potential.

Key Words: *Mollugo cerviana*, cytotoxicity, Trypan blue dye exclusion assay, MTT assay, phytochemicals.

INTRODUCTION

Cancer, the top killer in the world, has threatened human for centuries of years. Yet western medical treatments for cancer, including surgery, radiotherapy, chemotherapy and immunotherapy are either hard to achieve complete remission or produce severe adverse effects and they are increasingly tolerated by cancers over time. In order to counteract such problems, some attentions have shifted to the discovery of new anti-cancer strategy, such as the use of natural medicine, in the hope of producing anti-tumor effect without too many serious side effects or developing tolerance in cancer cell [1,2]. Medicinal plant research has been and continues to be considered a fruitful approach in the search for new drugs [3,4]. Bioactive compounds are by-products of the species primary metabolism; plants are therefore regarded as an invaluable repository of unusual chemical compounds, since these secondary metabolites are like chemical fingerprints of individual species. There are several reasons to believe that many drugs are yet to be found within the plant kingdom. Many medicinal plants have unique effects on physiology and can reduce the side effects of cancer treatments, while at the same time increasing their effectiveness [5]. Many herbs have long been known to affect the immune system, but only recently have scientists considered them as possible biological response modifiers and adjunct cancer therapies. Such medicinal plants and their phytochemicals often prompt the body's cells to secrete cytokines, which then enhance the immune response.

Mollugo cervina (Tamil: Parpataakam) belonging to the family Molluginaceae. It is an erect, slender, branched shrub found growing gregariously in sandy soil and in plains of India. *Mollugo cerviana* and other *Mollugo* species are used in Indian traditional system of medicine. It has long been used

by tribes and native medical practitioners to treat much kind of diseases such as rheumatism, arthritis, inflammation, tumor and liver diseases. It is used as stomachic, aperient and antiseptic. Flower and tender shoots are diaphoretic and febrifuge. Root boiled in oil for application in gout and rheumatism. It promotes flow of lochial discharges and is used as a cure for gonorrhea. Preliminary phytochemical screening and antimicrobial activity of entire plants of *M. cerviana* was studied and the findings suggested that the plant is used as an antimicrobial agent [6]. The ethanolic extracts of aerial shoots and leaves of *M. cerviana* were studied for antimicrobial activity against bacteria and fungi. The plant extract exhibited showed activity against the tested bacteria and no activity against fungi [7]. Hepatoprotective activity of *M. cerviana* was assessed against carbon tetrachloride induced hepatotoxicity in rats. The plant extract exhibit protective effect on liver cells and the authors concluded that the activity may be due to the presence of flavonoids [8]. Apart from the literature review, based on the ethnobotanical details obtained from siddha literatures, native medical practitioners and tribes, the plant *Mollugo cerviana* was selected for the present study.

Objective of the study:

The main objective of the present study is to evaluate the phytochemical analysis and antiproliferative activity of various solvent extracts of *M. cerviana*.

MATERIALS AND METHODS

Plant material and Extraction

Entire plants of *M. cerviana* were collected from Tiruchengode, Tamilnadu. The plant was authenticated by Dr. G.V.S. Murthy, Joint Director, Botanical Survey of India, Coimbatore, Tamilnadu, India. A voucher specimen is preserved in our laboratory for future reference (Voucher No.: P. Chem. MC 006). The plant material was shade dried, pulverized and extracted (500 g) with the solvents of increasing order of polarity viz. hexane, chloroform, ethyl acetate and ethanol using soxhlet extractor for 72 h. The residue obtained after extraction with ethanol was extracted with water by cold maceration process for 72 h. The prepared extracts were filtered and concentrated to dryness under reduced pressure and controlled temperature in a rotary evaporator and yield was calculated. The extracts were stored in a refrigerator until further use.

Preliminary phytochemical screening

Prepared plant extracts of *M. cerviana* were analyzed for the presence of various phytochemical constituents employing standard procedures [9]. Conventional protocol for detecting the presence of steroids, alkaloids, tannins, flavonoids, glycosides, etc., was used.

***In vitro* anticancer activity**

Tumor cells and inoculation

Normal Human Dermal Fibroblast cells (NHDF), Human Cervical Cancer cells (HeLa), Human Liver Cancer cells (HepG2), Human Lung cancer cells (A549) and Human Breast Cancer cell lines (MCF7) were obtained from National Centre of Cell Sciences (Pune, India). The cultures were maintained in Dulbecco's modified eagles medium (DMEM) containing 10 % inactivated calf serum and were grown in $25cm^2$ tissue culture flasks (Tarson Products Ltd, Kolkatta, India) until confluent and used for cytotoxic assays. EAC cells were obtained from Amala Cancer Research Centre (Trissur, Kerala, India). The cells were maintained *in vivo* in Swiss albino mice by intraperitoneal transplantation. Tumor cells aspirated from the peritoneal cavity of mice were washed with normal saline and were used for further studies.

Preparation of test samples

For cytotoxicity assays, various extracts of *M. cerviana* were dissolved in dimethyl sulfoxide (DMSO) and the volume made up to 10 ml to obtain a 1000 µg/ml stock solution. Serial two-fold dilutions were made using DMSO to get lower concentration.

Short term cytotoxic activity

Short term cytotoxicity study of various extract of *M. cerviana* was determined by using trypan blue dye exclusion method [10]. EAC cells were cultured in peritoneal cavity of healthy albino mice by injecting a suspension of tumor cells (1×10^6 cells/ml) intraperitoneally. The cells were aspirated aseptically from the peritoneal cavity of the mice on day 15 and washed with normal saline and centrifuged for 15 min at 1500 rpm in a cooling centrifuge. The pellet was resuspended with normal saline and the process was repeated until to get a clear supernatant. Finally the cells were suspended in a known quantity of normal saline and the cell count was adjusted to 1×10^6 cells/ml. Then, 0.1 ml of this cell suspension was distributed in to Eppendrof tubes and exposed to 0.1 ml of various concentrations of plant extracts (500 – 31.25 µg/ml) and incubated at 37 ºC for 3 h. After 3 h, the trypan blue dye exclusion test was performed to determine the percentage cytotoxicity and the IC_{50} value was calculated.

Antiproliferative studies on human cancer cell lines

Stock cells of normal and human cancer cells were

cultured in RPMI-1640 and DMEM supplemented with 10 % calf serum, penicillin (100 IU/ml) and streptomycin (100 µg/ml) in a humidified atmosphere of 5 % CO_2 at 37 ºC until confluent. The cells were dissociated with 0.2 % trypsin and 0.02% EDTA in PBS. The cytotoxic assay was carried out by adding 0.1 ml of cell suspension containing 10,000 cells to each well of a 96-well microtitre plate (Tarson, Kolkatta, India) and fresh medium containing various concentrations of extracts were added at 24 h after seeding. Control cells were incubated without the extracts and with DMSO. The microtitre plates were incubated at 37 ºC in a humidified atmosphere with 5 % CO_2 for a period of 72 h. The percentage cytotoxicity was determined by the standard MTT assay method and IC_{50} value was calculated [11].

RESULTS AND DISCUSSION

Various solvent extracts of *M. cerviana* was prepared and the percentage yield was calculated and the results are presented in Table 1. Among the extracts, maximum yield was obtained by using ethanol and water. Prepared extracts were analyzed for the presence of various phytochemical constituents and the results were presented in table 2.

Preliminary phytochemical screening of the plant extracts showed the presence of various phytochemical constituents. Terpenoids, phytosterols, alkaloids were present in hexane and chloroform extracts. Ethyl acetate extract contains terpenoids, amino acids, flavonoids and saponins whereas phytosterols, carbohydrates, glycosides, alkaloids, terpenoids, tannins, saponins, proteins and amino acids were present in ethanol extract. Aqueous extract consist of many polar constituents like carbohydrates, glycosides, tannins, flavonoids and saponins. Gums and mucilage were absent in all the prepared extracts. The results clearly showed that the presence of each phytoconstituents depends upon the solubility of phytochemical constituents in the particular solvents. Many of the constituents were extracted by ethanol.

All the prepared plant extracts were screened for their cytotoxic property against animal and human cancer cell lines. Hexane and aqueous extracts show moderate activity against EAC cell lines in trypan blue dye method. Ethanol, ethyl acetate and chloroform extracts exhibit good activity and the results are displayed in table 3.

In MTT assay, the percentage cytotoxicity progressively increased in a concentration dependent manner. The IC_{50} of various extracts of *M. cerviana* showed good activity against all the human cancer cell lines used. However the IC_{50} values against the normal human dermal fibroblast cells (NHDF) were found to be very high when compared to that of cancer cell lines. This indicates that the extracts possess selective cytotoxicity against the cancerous cell lines, but is safer towards the normal cells (Table 4).

The systemic literature collection, pertaining to this investigation indicates that the plant phenolics constitute one of the major groups of compounds acting as primary antioxidants or free radical scavengers. Flavonoids are the most diverse and widespread group of natural compounds and are likely to be the most important natural phenolics. These compounds possess a broad spectrum of chemical and biological activities including radical scavenging, antiangiogenic and antimutagenic [12,13].

Alkaloids, terpenoids and phytosterols like compounds are commonly found in both edible and medicinal plants and they have been reported to have various biological effects including cytotoxic and anticancer activity. The cytotoxic activities of these compounds are mainly due to their interaction with DNA, microtubules and cell cycle. Various extracts of *Mollugo cerviana* showed moderate to strong cytotoxic activities in various *in vitro* systems tested. Moreover the plant extracts also contain terpenoids, alkaloids, glycosides, saponins and tannins. The cytotoxic effect of various extracts of *M. cerviana* is may be due to the presence of various phytochemical compounds present.

CONCLUSION

In conclusion, the results obtained from short term cytotoxicity study and antiproliferative activity reveals that various extracts of *Mollugo cerviana* have significant cytotoxic activity against all the animal and human cancer cell lines studied. Further studies in our laboratory are in progress for the isolation and identification of phytochemical compounds and to ensure the medicinal properties of the plant *in vivo* correlate with its anticancer activity.

CONFLICT OF INTEREST

The authors declare no conflicts of interest with respect to the present paper.

Table 1. Extractive Yields of various Extracts of *Mollugo cerviana*

Name of the extract	*Mollugo cerviana*		
	Colour	**Nature**	**Yield (%)**
Hexane	Dark green	Solid mass	1.3
Chloroform	Green	Solid mass	3.6
Ethyl acetate	Greenish yellow	Solid mass	7.4
Ethanol	Brown	Semi solid mass	9.6
Water	Brown	Dry powder	10.4

Table 2. Preliminary phytochemical analysis of various extracts of *Mollugo cerviana*

Name of the Phyto-chemical	Name of the extract				
	Hexane	**Chloroform**	**Ethyl acetate**	**Ethanol**	**Water**
Carbohydrates	-	-	-	+	+
Glycosides	-	-	+	+	+
Alkaloids	-	+	-	+	-
Terpenoids	+	-	-	+	-
Phytosterols	+	-	-	+	-
Flavonoids	-	-	+	+	+
Phenolics	-	-	-	+	+
Tannins	-	-	-	+	+
Saponins	-	-	-	+	+
Proteins & amino acids	-	-	-	+	+
Fixed oils & fats	+	+	-	-	-
Gums & mucilages	-	-	-	-	-

(+) Present (-) Absent

Table 3. Short Term Cytotoxicity Studies of Various Extracts of *Mollugo cerviana* against EAC Cell Line by Trypan Blue Dye Exclusion Method

Name of the Extract	IC_{50} (µg/ml)*
Hexane	292.33
Chloroform	188.11
Ethyl acetate	154.31
Ethanol	124.62
Water	240.69

*Average of three determinations, three replicates

Table 4. *In Vitro* Cytotoxic Activity of Various Extracts of *Mollugo cerviana*

Name of the Extract	IC_{50} Value (µg/ml)*				
	A549	**HeLa**	**MCF7**	**HepG2**	**NHDF**
Hexane	306.7 ± 15.3	290.3 ± 4.04	274.7 ± 9.5	284± 10.7	545 ± 7.94
Chloroform	208.6 ± 6.4	212.3 ± 7.6	205.3 ± 6.43	194.7 ± 9.9	494.3 ± 6.1
Ethyl acetate	182.7 ± 8.02	175 ± 8.9	164.3 ± 7.4	173 ± 7.4	410.7 ± 19.4
Ethanol	151 ± 5.6	145.7 ± 5.5	153 ± 11.3	128 ± 6.56	404.7 ± 14.3
water	210.7 ± 16.04	212 ± 19.7	239.3 ± 15.9	237 ± 9.85	531 ± 12.01

*Average of three determinations, three replicates; IC_{50}, Drug concentration that produce 50% cell death following 72 h of drug exposure.

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