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IN VITRO CYTOTOXIC ACTIVITY OF ROOT METHANOL EXTRACT OF *PSEUDARTHRIA VISCIDA (L.)* WIGHT AND ARN. AGAINST HELA, HEP G2 AND L929 CELL LINES

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ABSTRACT

Pseudarthria viscida (L.) Wight and Arn. (Leguminosae) commonly called "Moovila", is a perennial diffuse under shrub with high medicinal value. It is an essential component of many famous Ayurvedic formulations like Dashamoola, Mahanarayana taila and Dhantara taila. The roots are astringent, thermogenic, digestive, anthelmintic, antiinflammatory, antifungal, antidiarrhoel, antioxidant, aphrodisiac, nervine, febrifuge, cardio and rejuvenating tonic. The main aim of the proposed work is the phytochemical screening and evaluation of *in vitro* cytotoxic activity of root methanol extract of *Pseudarthria viscida* against HeLa (cervical cancer cell line), Hep G2 (hepatic carcinoma cells) and L929 normal fibroblast cell lines. The preliminary phytochemical study of root methanol extract was done for the detection of phytoconstituents, using standard chemical tests. For *in vitro* cytotoxic analysis, different concentration of crude root extracts (10µg/ml, 50 µg/ml and 100 µg/ml) were incubated in the cell culture suspension. The cytotoxic effect was determined by MTT cell viability assay and the effects were expressed as IC_{50}. Methotrexate was used as standard drug. The results indicated the presence of important secondary metabolites such as glycosides, flavonoids, alkaloids, terpenoids and tannins which could be the reason for cytotoxic activity of the plant. Crude methanol extract showed potential cytotoxic activity against different cancer cell lines with IC_{50} values of 40 µg/ml against HeLa cell line and 60 µg/ml against HeP G2 cell line. They all exhibited IC_{50} exceeding 100 µg/ml for L929 normal cell line. The present study thus confirmed that methanol root extract of *Pseudarthria viscida* (L.) possessed good cytotoxic activity.

KEYWORDS: *Pseudarthria viscida*, Cytotoxic analysis, MTT cell viability assay, Methotrexate.

INTRODUCTION

Cancer is a group of diseases caused by loss of cell cycle control. It is associated with abnormal uncontrolled cell growth. Cancer is caused by both external factors (tobacco, chemicals radiation and infectious organisms) and internal factors (inherited mutation, hormones, immune conditions and mutations that occur from metabolism). It is a significant worldwide health problem generally due to the lack of widespread and comprehensive early detection methods, the associated poor prognosis of patients diagnosed in later stages of the disease and its increasing incidence on a global scale.[1]

The use of natural products as anticancer agents has a long history that began with folk medicine and has been incorporated into traditional and allopathic medicine through the years. Several drugs currently used in chemotherapy were isolated from plant species or derived from a natural prototype.[2] Active constitutes of *Catharanthus roseus, Angelica gigas, Podophyllum peltatum, Taxus brevifolia, Podophyllum emodii, Ocrosia elliptica* and *Campototheca acuminate* have been used in the treatment of advanced stages of various malignancies. There are various medicinal plants reported to have anticancer activity in Ayurvedic system of medicine.[3] One of such medicinally important plant is *Pseudarthria viscida.*

Pseudarthria viscida (L.) Wight and Arn. is a perennial under shrub, belonging to the family Leguminosae, distributed throughout India especially found in river basins and in hills up to above 900m.[4] The roots are with astringent, thermogenic, digestive, anthelmintic, antiinflammatory, antifungal, antidiarrhoel, antioxidant, aphrodisiac, nervine, febrifuge, cardio and rejuvenating properties.[5,6,7] They are useful in vitiated conditions of cough, bronchitis, asthma, tuberculosis, helminthiasis, cardiopathy, fever, hemorrhoids, gout, diabetes, hyperthermia and general debility. Major chemical compounds reported to be present in the roots are 1, 5 dicaffeoyl quinic acid, oleic acid, tetradecanoic acid, rutin, quercetin, gallic acid, ferulic acid and caffeic

acid.[8] The present investigation was undertaken to evaluate the preliminary phytochemical analysis and *in vitro* cytotoxic activity of root methanol extract of *Pseudarthria viscida*.

MATERIALS AND METHODS

Plant materials were collected from the Botanical garden of University College, Thiruvananthapuram, Kerala. Collected plant material (root) was cleaned, separated, weighed and dried under shade followed by oven drying. The dried samples were powdered. Fifty gram of dried root powder was extracted in methanol in a soxhlet apparatus for 8h continuously. The extract were concentrated, weighed and made up to a known volume and was used for the detection of phytochemicals and *in vitro* cytotoxic analysis.

Preliminary phytochemical studies

The preliminary phytochemical studies of root metanol extract of *Pseudarthria viscida* was done for the detection of phytoconstituents such as alkaloid, glycosides, terpenoids, steroids, tannin, flavonoids, saponins and coumarins, using standard chemical tests.[9]

Alkaloids (Dragendroff's test)

The plant extract was evaporated to dryness and the residue was heated on a boiling water with 10 ml of 2 % sulphuric acid for 2 minute. After cooling, the mixture was filtered and treated with a few drops of Dragendorff's reagent. Formation of orange brown precipitate showed the presence of alkaloid.

Glycosides (Keller - Kiliani test)

To the solution of the extract in glacial acetic acid, few drops of ferric chloride and concentrated sulphuric acid are added, and observed for a reddish brown ring at the junction of two layers.

Flavonoids (Shinoda test)

Four ml of extract solution was treated with 1.5 ml of 50 % methanol solution. The solution was warmed and metal magnesium was added. To this solution, 5-6 drops of concentrated hydrochloric acid was added and red colour was observed for flavonoids.

Steroids (Liebermann Burchard Reaction)

Four ml of extract was treated with 0.5 ml of acetic anhydride and 0.5 ml of chloroform. Then concentrated sulphuric acid was added slowly. The formation of green bluish colour indicated the presence of steroids.

Terpenoids (Salkowski Reaction)

The extract was dissolved in 2 ml chloroform and followed by 3 ml of concentrated sulphuric acid was poured along the side walls to form a layer. Formation of reddish brown inter phase indicated the presence of terpenoids.

Saponins

Five ml of extract was heated with 5 ml of distilled water and the froathing indicated the presence of saponins.

Tannins

The extract was added with 2 drops of 2 % ferric chloride solution and the presence of dirty green indicated the presence of tannins.

Coumarins

The extract was added with sodium hydroxide and then dissolved in alcoholic sodium hydroxide. Then the concentrated hydrochloric acid was poured through the sides of the test tube. Appearance and the disappearance of yellow colour indicated the presence of coumarins.

In vitro cytotoxic analysis

HeLa cervical cell line, Hep G2 cells hepatic carcinoma and L929 normal fibroblast cell lines were purchased from NCCS Pune, were maintained in Dulbecco's modified eagle's media and grown to confluency at 37°C and 5 % CO_2 in a humidified atmosphere in a CO_2 incubator. The cells were trypsinized (500 µl of 0.025% Trypsin in PBS (Phosphate Buffered Saline)/ EDTA (Ethylene diamine tetra acetic acid) solution) for 2 minutes and passed to T flasks in complete aseptic conditions and incubated. Crude Methanol extract from *Pseudarthria viscida* was added in the concentration of 10µg, 50 µg and 100 µg were taken from a stock concentration of 100 mg / ml and was added, then incubated for 24 hours. The anticancer effect was determined by MTT cell viability assay.[10]

MTT cell viability assay

MTT assay is a colorimetric assay that measures the reduction of yellow 3-(4, 5dimethythiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) by mitochondrial succinate dehydrogenase. The MTT enters the cells and passes into the mitochondria where it is reduced to an insoluble, coloured (dark purple) formazan product. The cells are then solubilised with isopropanol and thereby released formazan. Since reduction of MTT can only occur in metabolically active cells the level of activity is a measure of the viability of the cells.

The cell culture suspension with crude methanol extract from *Pseudarthria viscida* at concentration of 10µg/ml, 50µg/ml and 100µg/ml from a stock of 100mg/ml, was washed with 1x PBS and then added with 200µl MTT solution to the culture (MTT -5mg/volume dissolved in PBS), followed by incubation for 3 hours at 37^{o}C. Media was removed, washed with 1x PBS and 200µl of DMSO was added. Incubated at room temperature for 30 minutes until the cell got lysed and colour was obtained. The solution was transferred to centrifuge tubes and centrifuged at top speed for 2 minutes to precipitate cell debris.[10] OD was read at 540 nm using DMSO as blank. Percentage viability was calculated using the formula.

Percentage viability = {OD of test/OD of control} x100

RESULTS

Preliminary phytochemical studies

Conventional protocols detected the presence of important secondary metabolites such as glycosides, flavonoids, alkaloids, terpenoids and tannins which could be the reason for cytotoxic activity of the plant.

In vitro cytotoxic analysis

In the present study, crude methanol extract of *Pseudarthria visida* showed potential cytotoxic activity against different cancer cell lines like HeLa, HeP G2 and L929 cell lines. Methotrexate was used as standard drug. Cells which are pretreated with various concentrations of root extracts (10µg/ml, 50 µg/ml and 100 µg/ml) showed decrease in percentage of cancer cells viability such as 68.48%, 43.23% and 15.11% in HeLa cell lines and 65.03%, 53.16% and 48.29% in HeP G2 cell lines for sample concentration 10µg/ml, 50 µg/ml and 100 µg/ml respectively and the results were significant(Table 1, Fig. 1).

The cytotoxic effects were expressed as IC_{50}. The IC_{50} values against HeLa cell line is 40 µg/ml and 60 µg/ml against HeP G2 cell line. They all exhibited IC_{50} exceeding 100 µg/ml for L929 normal cell line. IC_{50} value of crude root extract showed high cytotoxicity effect compared to standard drug methotrexate (Table 2). All the experiments were repeated thrice.

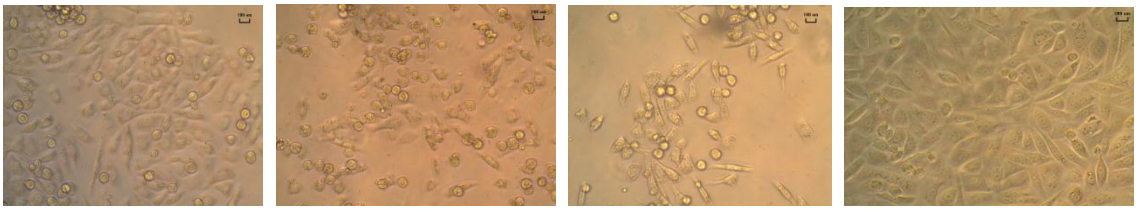
Table 1: Cytotoxic effect of the standard drug methotrexate and the crude root methanol extract of *Pseudarthria viscida.*

Compound name	Conc. (µg/ml)	% of viability		
		HeLa	HeP G2	L929
Standard drug (Methotrexate)	10	63.23	55.01	86.34
	50	42.38	36.26	64.56
	100	16.38	13.34	56.40
Crude methanol root extract of *P.viscida*	10	68.48	65.03	91.23
	50	43.23	53.16	82.70
	100	15.11	48.29	70.47

Table 2: IC_{50} values of methotrexate, crude methanol extract by MTT assay.

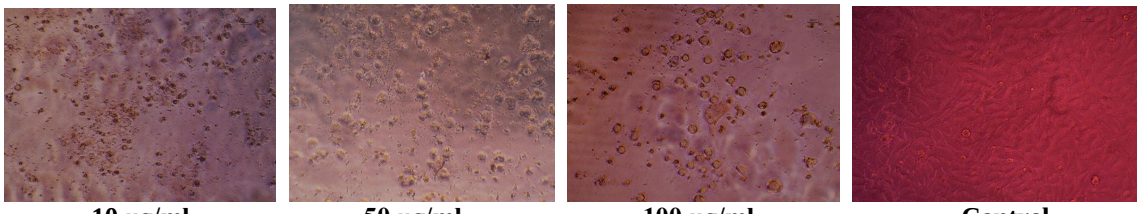
Compound name	IC_{50}(µg/ml)		
	HeLa	HePG2	L929
Standard drug (Methotrexate)	35	22	>100
Crude methanol extract of *P.Viscida*	40	60	>100

a. HeLa cell line



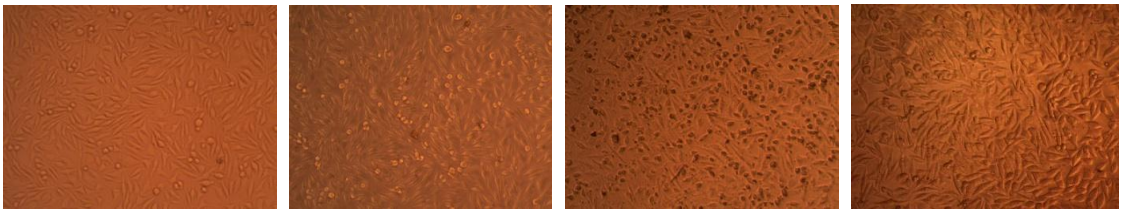
10 µg/ml **50 µg/ml** **100 µg/ml** **Control**

b. HeP G2 cell line



10 µg/ml **50 µg/ml** **100 µg/ml** **Control**

d. L929 cell line



10 µg/ml **50 µg/ml** **100 µg/ml** **Control**

Fig.1: Cytotoxic effect of crude methanol extract of *Pseudarthria viscida.*

DISCUSSION

Cancer is a class of diseases in which a cell or a group of cells represents uncontrolled growth, invasion, and metastasis. For the treatment of cancer, limited number of effective anticancer drugs are currently in use, even though they have higher cases of nausea, vomiting, diarrhea, skin rashes, and headache, etc. so that there is real need for new, side effect safe, cheap, and effective anti-cancer drugs to combat this dreaded disease. Natural products continue to be a major source of pharmaceuticals and for the discovery of new bioactive molecules.[11]

Approximately, 60% of the currently used anticancer drugs have been isolated from natural product from the plants. At this time, more than 3000 plants have been reported to possess anticancer properties worldwide. Extracts of these medicinal plants are believed to contain a wide array of polyphenolic compounds which might possess cancer preventive and therapeutic properties.[12] In the present study, preliminary phytochemical analysis indicated the presence of glycosides, flavonoids, alkaloids, terpenoids and tannins. Flavonoids and alkaloids have been found to possess antimutagenic and antimalignant effects.[13]

Major aims of the present study was to determine whether the crude root extract of the plant exerted an inhibitory effect on cancer cell proliferation and caused cell death. The result of the present study suggest that methanol root extract of *Pseudarthria viscida* posses the strongest cytotoxic effect on both HeLa and HeP G2 cell lines which was compared with L929 normal fibroblast cell lines. To be a good drug, the IC_{50} values of such agent should be sufficiently low to avoid any possible unspecific effects. The American National Cancer Institute assigns a significant cytotoxic effect of promising anticancer product exerts an IC_{50} value < 30 µg/ml.[14] In the present study, the IC_{50} values against HeLa cell line is 40µg/ml and 60 µg/ml against HeP G2 cell line. So root extract of *Pseudarthria viscida* used as promising anticancer agent. Similar type of Cytotoxic effect against HeLa and HePG2 cell lines were reported in *Sophora interrupta* by Vithya *et al*., 2012.[15]

CONCLUSION

Crude root methanol extract of *Pseudarthria viscida* showed potential cytotoxic activity against different cancer cell lines with IC_{50} values of 40 µg/ml against HeLa cell line and 60 µg/ml against HeP G2 cell line. They all exhibited IC_{50} exceeding 100 µg/ml for L929 normal cell line. The present study thus confirmed that root extract of *Pseudarthria viscida* act as a promising anticancer agent. This will help the maximum utilization of the plant along with the identification and isolation of useful bioactive molecules responsible for this cytotoxic activity.

REFERENCES

1. Sumitra Chanda and Krunal Nagani. *In vitro* and *in vivo* methods for Anticancer activity Evaluation and some Indian medicinal plants possessing anticancer properties: An overview. J pharma. Phytochem, 2013; 2(2): 140-152.
2. Valko V, Fickova M, Pravdova E, Nagy M, Grancai D, Czigle S. Cytotoxicity of water extracts from leaves and branches of *Philadelphus coronaries* L. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub, 2006; 150(1): 71-73.
3. Sanjay P, Nirav G, Ashok S, Anand S. *In vitro* cytotoxicity of *Solanum nigrum* extract against HeLa cell line and Vero cell line. Inter j pharma pharmace scien, 2009; 1(1): 38-46.
4. Krithikar K R and Basu B D. Indian Medicinal Plants. Sudhindranath Basu, Bhuwaneshwari Asramam, Behadurganj., 1918; 761-1319.
5. Deepa M A, Narmatha B V, Basker S. Antifungal properties of *Pseudarthria viscida*. *Fitoterapia*, 2004; 75: 581-584.
6. Gincy M M, Sasikumar J M. Antioxidant activity of *Pseudarthria viscida*. *Int. J. Pharm. Sci*, 2007; 69(4): 581-582.
7. Warrier P K, Nambiar V P D, Ramankutty C. Indian medicinal plants: a compendium of 500 species. Orient Longman Ltd, Hyderabad., 1996; 4:-336.
8. Vijayabaskaran M, Venkatesaramurthy N, Babu G, Khatale P N. Antidiarrhoeal activity of *Pseudarthria viscida* roots. *Int. J. Pharma Tech*, 2010; 2: 307-313.
9. Kokate C K. Practical Pharmacognosy(1st Ed.) Vallabha Prakashan, New Delhi., 1986; 110-111.
10. Masters R W. Animal cell culture: Cytotoxicity and viability assays. 3rd ed., 2000; 207.
11. Schaufelberger D E, Koleck M P, Beutler JA, Vatakis A M, Alvarado A B, Andrews P, Marzo LV, Muschik G M, Roach J, Ross JT . The Large-Scale Isolation of Bryostatin 1 from Bugulaneritina following Current Good Manufacturing Practices. *J. Nat. Prod*, 1991; 54: 1265-1272.
12. Khakdan F andPiri K. Invitro cytotoxic activity of aqueous root extract of Althea kurdica against Endothelial human bone marrow cells (line k 562) and human lymphocytes.Bull.Env. Pharmacol.LIFE Sci, 2013; 2(6): 23-29.
13. Brown J P. A review of the genetic effect of naturally occurring flavonoids anthraquinones and related compounds. Mutat. Res, 1980; 75: 243-247.
14. Suffness M and Pezzuto J M. Assays related to cancer drug discovery (Eds. Hostettmann K). Methods in Plant Biochemistry: Assay for Bioactivity, Aademic Press, London., 1990; 71-133.
15. Vithya T, Dr. V. Kavimani, Alhasjajiju K, Rajkapoor B, Savitha B K. An *In vitro* evaluation of cytotoxic activity of *Sophora interrupta*. Inter J Pharma Bio Sci, 2012; 3(2): 420-425.