Asian Journal of Pharmaceutical and Health Sciences
www.ajphs.com

Evaluation of *in vitro* Antitumour Activities of Microwave Assisted Extract and Fractions of *Pseudarthria viscida* Leaves

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ARTICLE HISTORY

Received: 03.07.2017

Accepted: 02.09.2017

Available online: 30.09.2017

Keywords:

Pseudarthria viscida, L929, HCT 15
Cyclophosphamide

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ABSTRACT

Leaf powder of *Pseudarthria viscida was* subjected to microwave assisted extraction with ethanol. After preliminary phytochemical investigation ethanolic extract and different fractions obtained from the leaves of *Pseudarthria viscida* were evaluated for its *in vitro* anti tumour activity against different cell lines. *In vitro* cytotoxicity of the extract in various cancer cell lines like L929, HCT 15, MCF7, SIHA and HeLA showed significant cytotoxicity with IC_{50} values 17.17, 160, 18.72, 55, 60 µg/ml respectively by MTT assay. The ethanolic extract showed significant activity as compared to standard Cyclophosphamide.

INTRODUCTION

Chemotherapy is the method of choice now adopted by the medicinal field for the cure of cancer. But due to the tremendous side effects caused by the chemicals now all are concentrating on the plants for deriving the phytochemicals which are having the cytotoxic activities.[1]. About 60% of antitumour drugs are of natural origin [2].

Pseudarthria viscida is a medicinal plant belonging to the family Fabaceae present widely in India. It is also known as Salaparni which is already reported to have various medicinal activities like antibacterial, antifungal, antitumour etc [3]. In this present study we selected leaves which were extracted using microwave energy for time saving and were fractionated by column chromatography. The extract and the fractions were subjected to preliminary phytochemical screening and *in vitro* antitumour evaluation.

MATERIALS AND METHODS

Collection of plant material

Pseudarthria viscida (L.) Wight and Arn. (Fabaceae) leaves were collected from Kizhattoor, Malappuram district and identified and authenticated by A K Pradeep, Calicut University (Voucher no. 73212). The leaves were shade dried and powdered using a grinder. *Pseudarthria viscida* was selected based on the ethno-medical importance and the leaves of this plant were subjected to the experimentation procedure as follows.

Chemicals

All the solvents, chemicals and reagents used in the present research work were of analytical grade. The solvents used for extraction and isolation were petroleum ether, chloroform and ethanol.

Preparation of extract by microwave irradiation

The leaves of *Pseudarthria viscida* Linn. were dried under shade and then powdered with a mechanical grinder. The powder was passed through sieve No. 42 and stored in an airtight container for further use. The dried powder material was extracted in ethanol using microwave energy. 200mg of the dried plant material taken into 50ml glass beaker with solvent ethanol 30ml closed with a glass lid and was placed in the extraction cabinet. Power (850 watt) was applied. After complete irradiation, the sample was filtered in a test tube. The extract was filtered through whatman 1 filter paper and evaporated to dryness by rotary evaporator and was used for the cytotoxic and antitumour studies.

In vitro Antitumour Evaluation by MTT Assay

In vitro antitumour evaluation of the plant leaf ethanolic extract was done by MTT assay using L929, HCT 15, MCF 7,

SIHA and HeLA cell lines. Percentage viability, percentage inhibition and the IC_{50} values were also calculated in all the cases.

Procedure

The cancer cell lines purchased from NCCS Pune were maintained in Dulbecco's modified eagles media (HIMEDIA) supplemented with 10% FBS (Invitrogen) and grown to confluency at 37°C in 5% carbon dioxide (NBS, EPPENDORF, GERMANY) in a humidified atmosphere in a carbon dioxide incubator. The cells were trypsinized (500µl of 0.025% trypsin in Phosphate buffer saline or 0.5mM EDTA solution (Himedia)) for 2 minutes and passaged to T flasks under complete aseptic conditions. The samples were added to grown cells at a concentration 10µg, 50µg and 100 µg from a stock of 10mg/ml and incubated for 24 hours. The percentage difference in viability was determined by standard MTT assay after 24 hours of incubation.

The cell culture suspension was washed with 1x PBS and then added 30 µl of MTT solution to the culture (MTT -5mg/ml dissolved in PBS). It was then incubated at 37^0C for 3h. MTT was removed by washing with 1x PBS and 200µl of dimethyl sulphoxide was added to the culture. Incubation was done at room temperature for 30 minutes until the cell lysed and colour was obtained. The solution was transferred to centrifuge tubes and centrifuged at top speed for 2 minutes to precipitate cell debris. Optical density was read at 540 nm using DMSO as blank [4, 5].

$$\text{Percentage viability} = \frac{\text{OD of control-OD of test}}{\text{OD of control}} \times 100$$

Where OD is the optical density

From the percentage viability, the percentage inhibition was calculated and also calculated the IC_{50} value.

Based on the results *Pseudarthria viscida* ethanolic extract was subjected to fractionation by column chromatography using the mobile phase petroleum ether, chloroform and ethanol in different ratios. The ten fractions PV1 to PV10 from *Pseudarthria viscida* were collected. These fractions were screened for their antitumour activity by MTT assay method.

The active fraction PV6 was again subjected to column chromatography using the mobile phases petroleum ether, chloroform and ethanol in different ratios. Four fractions from PV6 that is PV6a, PV6b, PV6c and were collected and evaluated for antitumour activity by MTT method. Based on the results, PV6a was selected for further study.

STATISTICALANALYSIS

All the biological assays results were checked and the P values were found to be < 0.05, were considered as statistically significant.

RESULTS

In vitro antitumour evaluation by MTT assay

1. Antitumour evaluation of ethanolic extract

The anticancer researchers have emphasized the great pharmacological importance of the plant extracts [6]. Cell viability assay was conducted to assess the *in vitro* anticancer activity. *Pseudarthria viscida* ethanol extract obtained by microwave assisted extraction was subjected to MTT assay. MTT assay is the method used for the quantification of both cell viability and proliferation in cell population using 96-well plate format. The biosafety of the plant extracts also can be carried out by this *in vitro* evaluation. In our study we used this method for the evaluation of cell viability and in turn the cell inhibitory action. Different cell lines used were L929, HCT 15, MCF 7, SIHA and HeLa. The ethanolic extract of the plant leaves showed a reduction in cell proliferation. The IC_{50} value obtained were 17.17, 160, 18.72, 55 and 60 respectively. The results were depicted in the table 1.

Based on the *in vitro* antitumour evaluation, *Pseudarthria viscida* ethanolic extract was undergone for further fractionation by column chromatography. Petroleum ether, Petroleum ether: chloroform (4:1, 3:2, 2:3, 1:4), chloroform, chloroform: ethanol (4:1, 3:2, 2:3, 1:4) and ethanol were the mobile phases used for fractionation.

Table 1 : The IC_{50} value of *Pseudarthria viscida* extract by MTT assay

CELL LINES	IC_{50} VALUE OF *Pseudarthria viscida* EXTRACT (µg/ml)
L929	17.17
HCT 15	160.00
MCF7	18.72
SIHA	55.00
HELA	60.00

Table 2 : The percentage inhibition by MTT assay of the fractions *(Pseudarthria viscida)* at 100µg/ml

Cell lines used	Percentage inhibition (%)									
	PV1	PV2	PV3	PV4	PV5	PV6	PV7	PV8	PV9	PV10
L929	36.62	48.56	36.66	62.32	26.33	75.23	56.33	47.25	36.35	58.65
HCT COLON 15	46.36	46.35	37.33	54.26	24.33	68.36	56.42	48.63	38.52	54.65
MCF7	37.42	52.23	39.65	48.33	35.66	78.36	57.45	36.33	48.56	55.23
SIHA	36.95	54.23	35.46	44.23	54.33	72.32	55.23	25.33	49.65	63.23
HeLa	38.65	26.32	56.32	23.22	58.33	68.36	48.56	38.49	54.26	62.25

Table 3 : The percentage inhibition by MTT assay of the fractions from PV6 at 100µg/ml

Different cell lines	Percentage inhibition (%)			
	PV6a	PV6b	PV6c	PV6d
L929	82.16	55.36	32.65	35.62
HCT COLON 15	81.34	48.39	44.26	34.26
MCF7	76.35	44.12	68.35	25.32
SIHA	79.23	46.32	45.26	32.33
HeLa	80.21	38.65	49.56	36.32

2. Antitumour evaluation of ethanolic fractions

By fractionation of the ethanolic extract of *Pseudarthria viscida*, ten different fractions namely PV1 to PV10 were obtained. All the fractions were subjected to *in vitro* antitumour evaluation against the cancer cell lines L929, HCT 15, MCF 7, SIHA and HeLA.

Among the first set of fractions PV6 showed 75.23% inhibition against L929 cell lines, 68.36% inhibition against HCT COLON 15 cell line, 78.36% inhibition against MCF7 cell line, 72.32% inhibition against SIHA cell line and 68.36% inhibition against HeLa cell lines respectively. The concentration used was 100µg/ml. The better fraction PV6 was again fractionated to get PV6a, PV6b, PV6c and PV6d. Table 3 and Figure 1 showed the result of percentage inhibition of *Pseudarthria viscida* fractions (PV6a, PV6b, PV6c and PV6d) obtained from PV6. Among these second set of fractions, the fraction PV6a showed better percentage inhibitory action, 82.16% inhibition against L929 cell line, 81.34% inhibition against HCT COLON 15 cell lines, 76.35% inhibition against MCF 7 cell lines, 79.23% inhibition against SIHA cell lines and 80.21% inhibition against HeLa cell lines respectively.

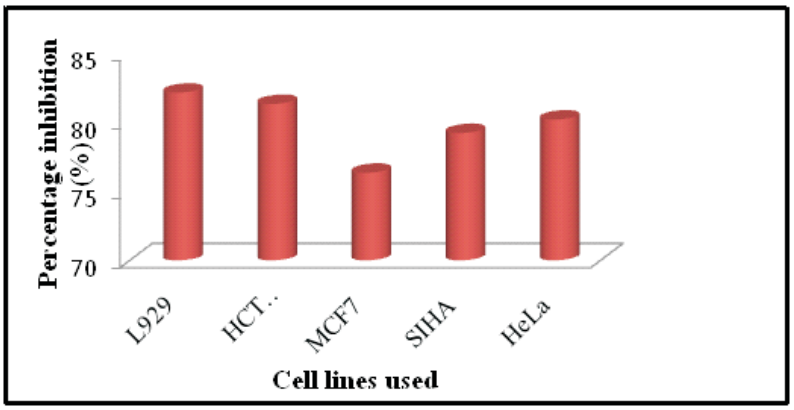
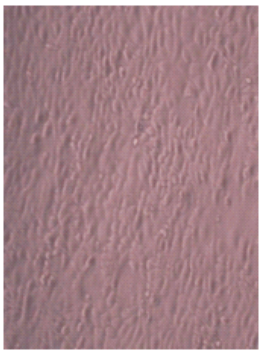
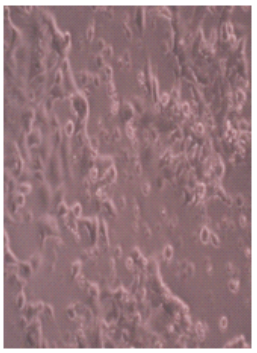


Fig 1 : The percentage inhibition by MTT assay of the fraction PV6a at 100µg/ml



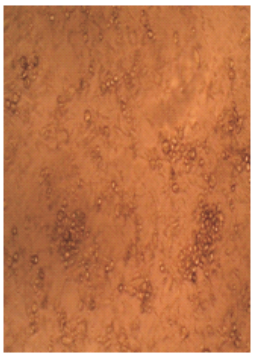
(a) SIHA Control



(b) SIHA with PV6

Fig. 2 : SIHA control and the cells treated with the fractions obtained from *Pseudarthria viscida* (MTT assay)

(a) HeLA Control



(b) HELA with PV6

Fig. 3 : HeLA control and the cells treated with the fractions obtained from *Pseudarthria viscida* (MTT assay)

DISCUSSION

Now a day a number of people are affected by cancer. It is a challengeable problem. A number of herbs are having the medicinal properties. As per the World Health Organization (WHO) report, 80% of the populations of developing countries depend traditional medicines especially plant drugs [7]. Use of herbal drugs has increased dramatically in the last two decades [8]. Most of the plant secondary metabolites are having anticancer activity. As per the literature review and as per our study reveal *Pseudarthria viscida* is having antitumour activity. The systematic screening of the ethanolic extract of *Pseudarthria viscida* leaf was found to be cell dependent. The maximum activity was found against the cell lines L929 and MCF7 with IC_{50} values 17.17 and 18.72 µg/ml respectively. But in case of fractions obtained from *Pseudarthria viscida* ethanolic extract, other than PV6, all other fractions showed somewhat similar inhibitory actions against all the selected cell lines. But in case of PV6a fraction obtained from PV6 showed comparatively better action than other fractions like PV6b, PV6c and PV6d. So the active metabolite may be present in that particular fraction (PV6a) which is to be isolated. We plan to do more pharmacological evaluation including the *in vivo* studies and also to tackle the problem of blood cancer. These types of fabaceae family plants could be act as a source for new lead molecules in designing of new derivatives to combat cancer.

CONCLUSION

Vast number of plant products is reported to have anticancer activity which emphasizes the importance of traditional medicinal plants as an unavoidable resource for providing anticancer compounds. Based on these facts we initiated our study. The results obtained in our study confirm the *in vitro* anticancer activity of the ethanol extract of *Pseudarthria viscida* leaves. Our study has systematically screened the anticancer effect of *Pseudarthria viscida* leaf ethanolic extract. In future this plant based study may give valuable suggestions for the development of new anticancer agents. The further studies are in process and to be extended a lot to confirm its safe usage.

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