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Research Article

IN-VITRO CYTOTOXICITY ACTIVITY OF *MALAXIS RHEEDII* SW METHANOL EXTRACT AGAINST *HELA* CELL LINE AND MCF-7 CELL LINE

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ABSTRACT

Objective: Cancer is a group of diseases caused by loss of cell cycle control. Cancer is associated with abnormal, uncontrolled cell growth. The study was aimed to evaluation of the anticancer activity of the *Malaxis rheedii* Sw. on the *HeLa* cell line and MCF-7 cell line.

Methods: The whole plant parts of the *M. rheedii* methanolic extract were tested for its inhibitory effect on *HeLa* cell line and MCF-7 cell line. The cytotoxicity of *M. rheedii* on *HeLa* cell and MCF-7 cell line were evaluated by the 3-(4,5-dimethythiazol-2-yl)-2,5- diphenyltetrazolium bromide (MTT) assay.

Results: *M. rheedii* methanolic extract has a significant cytotoxicity effect on MCF-7 cell line in a concentration range between 18.75 and 300 µg/ml using MTT assay, and the study also showed that inhibitory action on *HeLa* cell line in a concentration range between 18.75 and 300 µg/ml using MTT assay. Methanol extract of the whole plant part of *M. rheedii* was found to be 7.3%, 16.6%, 25.4%, 36.3%, and 47.1% toxic in *HeLa* cell line and 7.9%, 13.9%, 26%, 48.4%, and 66.3% toxic in MCF-7 cell line. Inhibitory concentration 50 (IC_{50}) value of *M. rheedii* on MCF-7 cell was 167.76 µg/ml, and IC_{50} value of *M. rheedii* on *HeLa* cell was not found by MTT assay.

Conclusion: From the performed assay, the methanol extract of these drug shows greater activity on MCF-7 cell line and little activity on *HeLa* cell line and that mean *M. rheedii* can be used as an anticancer activity.

Keywords: Cytotoxicity activity, 3-(4,5-dimethythiazol-2-yl)-2,5- diphenyltetrazolium bromide assay, *Malaxis rheedii* Sw., *HeLa* cell line, MCF-7 cell line.

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INTRODUCTION

Cancer is a disease that has always been a major threat and has been characterized by proliferation of abnormal cells [1]. Currently, chemotherapy and radiotherapy treatments were followed for the treatment of various cancers but are found to be having limited survivability and possess various side effects [2]. Medicinal plants represent a vast potential source for anticancer compounds and support the immune system, thus improving body resistance to the disease and its treatments [3]. Plants have long history used in the treatment of cancer [4]. In Ayurveda, a traditional Indian medical practice using plant drugs has been successful from very early times in using these natural drugs and preventing or suppressing various tumors with different lines of treatment [5]. In recent years, the use of traditional medicine information on plant research has again received considerable interest, and worldwide, efforts are on discover new anticancer agent from plants [6,7]. The National Cancer Institute has screened about 35,000 higher plant species for activity against cancer [8]. *Malaxis rheedii* Sw. under the family Orchidaceae is a rare, terrestrial, endangered medicinal herb that generally grows to a height of 15 cm long and comes under the genus *Malaxis* [9]. *M. rheedii* Sw. is used for used externally for snake poison by Kattunayakans [10]. In Ayurveda, "Ashtawarga," a group of eight drugs, is used for preparation of tonic such as "Chyavanprasam – Ayurvedic tonic" and consists of four orchid species, of which, *M. rheedii* is also among them [11]. *M. rheedii* has great potential as an antimicrobial agent against selected pathogenic microorganisms due to the presence of selected alkaloid and flavonoid compounds [12]. The aim of our study was to evaluate the potential anticancerogenic effect of the methanol extract of *M. rheedii* Sw. on the *HeLa* cell line and MCF-7 cell line.

METHODS

Plant materials

Wild plant species were collected from Malappuram, Kerala, India. The plant was authenticated by the Taxonomist, Department of Botany, Kongunadu Arts and Science College, Coimbatore, Tamil Nadu, India. The specimen voucher is maintained in the Institute.

Preparation of plant extracts

The methanolic extracts of *M. rheedii* were dissolved in dimethyl sulfoxide (DMSO) and made into stock solution.

Human cell lines

The human breast adenocarcinoma cancer (MCF-7) and human cervical cancer (*HeLa*) cell lines were obtained from the National Centre for Cell Sciences, Pune. MCF-7 and *HeLa* cells were cultured in minimum essential media (MEM) with earle salt without glutamine medium supplement with 10% heated fetal bovine serum, 1% of 2 mM L-glutamine, 50 IU/ml penicillin, 50 µg/ml streptomycin, 1% non-essential amino acid, and maintained at 37°C in a 5% CO_2 atmosphere with 95% humidity. According to their growth profiles, the optimal plating densities of breast adenocarcinoma cancer cell line was determined 3×10^3 cells/well to ensure exponential growth throughout the experimental period and to ensure a linear relationship between absorbance at 492 nm and cell number which was analyzed by 3-(4,5-dimethythiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.

MTT assay

The cytotoxic effect of methanolic extract of *M. rheedii* Sw. (Orchidaceae) was evaluated by MTT assay using human breast adenocarcinoma cancer (MCF-7) and human cervical cancer (*HeLa*) cell lines. This MTT assay was performed according to a slight modification of the procedure reported by Mosman, 1983. Cells were cultured in MEM supplemented with glutamine (0.6 g/L), gentamicin (25 mg/ml), 10% fetal calf serum at 37°C, and in humidified 5% CO_2. For experiments, cells were plated in 96-well plate (105 cells/well for adherent cells or 0.3×10^6 cells/well for suspended cells in 100 µL of medium). After 24 hrs, the extracts (0.01, 0.1, 1, 10, and 100 µg/ml) dissolved in DMSO (1%) was added to each well and incubated for 96 hrs. The control

Fig. 1: *Malaxis rheedii* Sw.

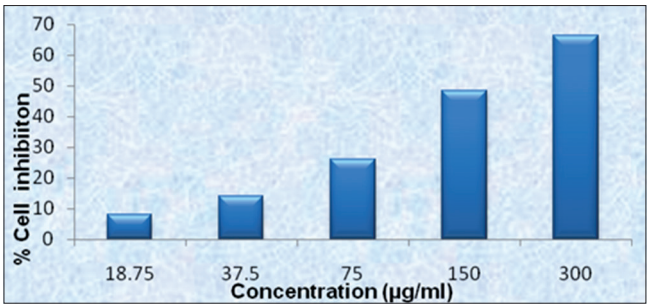

Fig. 2: *In-vitro* cytotoxic activity of *Malaxis rheedii* methanol extract in MCF-7 cancer cell line

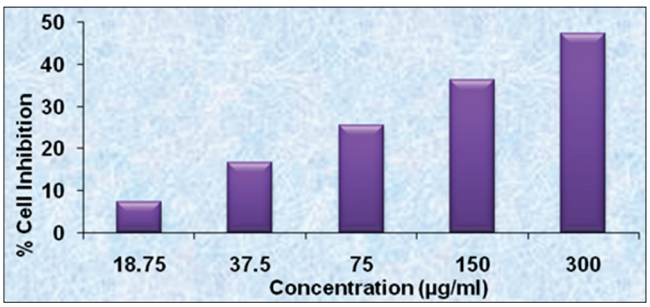

Fig. 3: *In-vitro* cytotoxic activity of *Malaxis rheedii* methanol extract in *HeLa* cancer cell line

groups received the same amount of DMSO. Doxorubicin (0.01, 0.1, 1, 10, and 100 µg/ml) was used as positive control. Growth of tumoral cells was quantified by ability of living cells to reduce the yellow dye MTT to a blue formazan product. At the end of 96 hrs incubation, the medium in each well was replaced by fresh medium containing 0.5 mg/ml of MTT. 4 hrs later, the formazan product of MTT reduction was dissolved in DMSO and absorbance was measured at 550 nm. Drug effect was quantified as the percentage of control absorbance of reduced dye at 550 nm. Percentage inhibitions [100 − (absorbance of test wells/absorbance of control wells) × 100] were calculated and plotted against the concentrations used to calculate the inhibitory concentration 50 (IC_{50}).

RESULTS

In this study, the *in-vitro* confirmation of their toxicity on human cervical cancer cell line (*Hela*) and breast cancer cell line (MCF-7) were studied using MTT assay. The cytotoxicity study was carried out

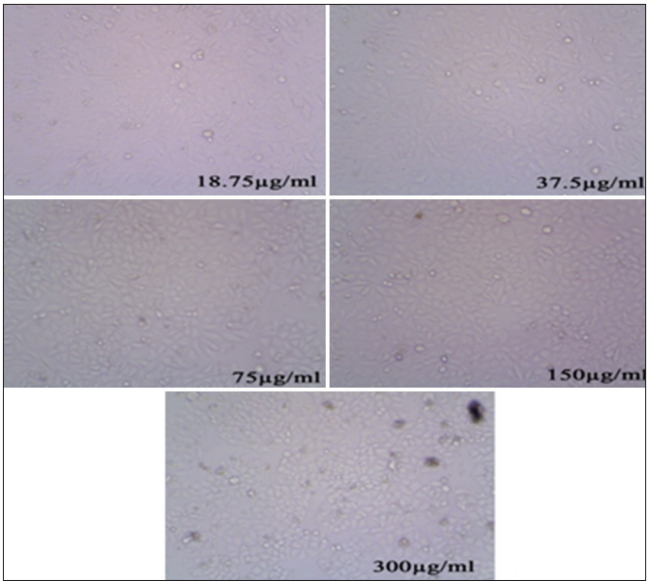

Plate 1: Anticancer activity on *HeLa* cell line in methanolic extract of *Malaxis rheedii*

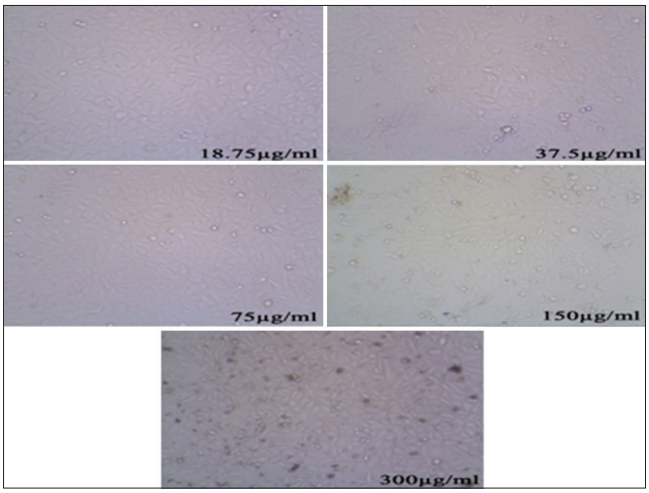

Plate 2: Anticancer activity on *HeLa* cell line in methanolic extract of *Malaxis rheedii*

for plant methanolic extract of the whole plant part of *M. rheedii*. The methanol extract was screened for its cytotoxicity against two human cancer cell lines at different concentrations to determine the IC_{50} by MTT assay. Cytotoxicity of methanol extract of the whole plant part of *M. rheedii* against *HeLa* cell was found to be 7.3%, 16.6%, 25.4%, 36.3%, and 47.1% toxic at a concentration of 18.75, 37.5, 75, 150, and 300 µg/ml; and cytotoxicity of methanol extract of *M. rheedii* against MCF-7 cell was found to be 18.75, 37.5, 75, 150, and 300 µg/ml toxic at a concentration of 7.9%, 13.9%, 26%, 48.4%, and 66.3%, respectively. IC_{50} value of 167.76 µg/ml was obtained for breast cancer cell line (MCF-7). Cytotoxicity of methanol extract of the whole plant part of *M. rheedii* toward MCF-7 was found to suppress the cell proliferation, and it showed good cytotoxicity than *HeLa* cell (Tables 1 and 2). The percentage growth inhibition was found to be increasing with increasing concentration of test compounds and that show in Figs. 1-3 and Plates 1 and 2.

CONCLUSION

In this investigation, this medicinal plant possesses good anticancer activities. The results obtained from the *in-vitro* studies performed

Table 1: *In-vitro* cytotoxic activity of *M. rheedii* methanol extract in *HeLa* cancer cell line

S. No.	Concentration (µg/ml)	% Cell inhibition
1	18.75	7.306667
2	37.5	16.69333
3	75	25.49333
4	150	36.32
5	300	47.14667

M. rheedii: *Malaxis rheedii*

Table 2: *In-vitro* cytotoxic activity of *M. rheedii* methanol extract in MCF-7 cancer cell line

S. No.	Concentration (µg/ml)	% Cell inhibition	IC_{50} value (µg/ml)
1	18.75	7.998129	167.76
2	37.5	13.93826	
3	75	26.00561	
4	150	48.40973	
5	300	66.32367	

IC_{50}: Inhibitory concentration 50%, *M. rheedii*: *Malaxis rheedii*

using the MCF-7 cell lines reveals that the methanol extract of *M. rheedii* has good anticancer activity than *HeLa* cell line. More efforts are needed to explore potent anticancer plants from the mother earth and save humans around the world from cancer. The results of this study have helped to find supporting evidence for the ethnomedicines that have been utilized by peoples for millennia.

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