



IN VITRO CYTOTOXICITY OF ETHANOLIC LEAF EXTRACT OF *ALTERNANTHERA SESSILIS* (L.) R.BR. EX DC AND *ALTERNANTHERA PHILOXEROIDES* (MART.) GRISEB AGAINST HUMAN OSTEOSARCOMA CELL LINE MG-63

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ABSTRACT

Crude ethanolic leaf extracts of *Alternanthera sessilis* and *Alternanthera philoxeroides* were examined for their anticancer activity. To determine anticancer activity, different concentrations of crude extracts were tested on MG-63, a human osteosarcoma cell-line by 3-(4, 5 dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay. *Alternanthera philoxeroides* extract showed a significant antiproliferative activity than *Alternanthera sessilis* and a dose dependent effect was observed. Maximum inhibition of 67.37% was observed for *Alternanthera philoxeroides* and 47.7% for *Alternanthera sessilis* at 300µg/ml. The results indicate the possible potential use of these plants in cancer therapy.

KEYWORDS: *Alternanthera sessilis*, *Alternanthera philoxeroides*, MG-63, MTT assay.

INTRODUCTION

Cancer is a major health problem and cause of mortality that increase annually. In the year 2000, there were 10.4 million new cancer cases and it is expected that this number will be doubled in 2030. Osteosarcoma (OS) is the most common, non-hematopoietic, primary malignant tumor of bone which occurs predominantly in adolescents and young adults.^[1] The osteosarcoma is a type of cancer that starts in the bones and not a common cancer. Osteosarcoma is a primary malignant tumor of bone that is characterized by the production of osteoid or immature bone by the malignant cells. It is a high grade neoplasm with rapid growth and early metastasis.^[2]

Nature is a best friend of our pharmacy field. Natural drugs are effective in action without side effects.^[3] Plants have a long history of use in the treatment of cancer and it is significant that over 60% of currently used anti-cancer agents are come from natural sources.^[4] Hidayatiet al.^[5] reported that cancer therapies that utilizing the natural products such as plants is a relatively new to prevent cancer but it is a promising strategy where it suppress, delay, reverse, or retard the process of carcinogenesis. This kind of therapies also known as cancer chemoprevention which applies specific natural compound to inhibit or reverse carcinogenesis and help to suppress the development of cancer from premalignant lesions.^[6] Thus, the discovery of new natural and

synthetic products for osteosarcoma treatment is of great urgency.

Alternanthera sessilis belongs to Amaranthaceae family. It is popularly known as "Water Amaranth". It is a perennial herb. The leaves and young shoots are used as vegetable. The plant is reported to possess antimicrobial, wound healing^[7], cytotoxic^[8], antioxidant^[9], antipyretic^[10], hematinic^[11], hepatoprotective^[12] and anti-inflammatory properties.^[13] Previous phytochemical investigations revealed that the plant contains lupeol, stigmaterol, β -sitosterol, handianol, campesterol, α and β -spinasterol, 24-methylenecycloartanol, cycloeucaleanol, 5 α -stigmasta-7-enol.^[14]

Alternanthera philoxeroides (Amaranthaceae) is an aquatic plant. The plant is known in English as the alligator weed. From the aerial parts of the plant, the antitumor compounds, alternanthin B and N-trans-feruloyl-3,5-dimethoxytyramine has been isolated.^[15] Pentacyclic triterpene saponins (philoxeroidesides A-D) isolated from the plant exhibited cytotoxic activities against SK-N-SH and HL60 cell lines.^[16] Oleanolic acid 3-O- β -D-glucuronopyranoside, isolated from n-butyl extract of the plant showed significant inhibitory effect against HeLa and L929 cell lines.^[17] This investigation evaluates the *in vitro* cytotoxicity of ethanolic leaf extract of *Alternanthera sessilis* and *Alternanthera*

philoxeroides against human osteosarcoma cell lines (MG-63).

MATERIALS AND METHODS

Collection of plant material

The plants *Alternanthera sessilis* and *Alternanthera philoxeroides* were collected from Coimbatore district and were identified by Botanist, Arignar Anna government arts college, Mussiri. The leaves were separated and shade dried. These leaves were then powdered and stored in air tight container at room temperature until further use.

Preparation of plant extract

10g of air dried powder of *Alternanthera sessilis* and *Alternanthera philoxeroides* were taken in 100mL of ethanol. Plugged with cotton wool and then kept on a rotary shaker at 190-220rpm for 24hours. After 24hours the supernatant were collected and the solvent were evaporated to make the final volume, one-fourth of the original volume and stored at 4°C in air tight container. A dose of crude material was prepared at concentrations of 18.75 µg, 37.5 µg, 75 µg, 150 µg, 300 µg/ml of ethanolic extracts of *Alternanthera sessilis* and *Alternanthera philoxeroides* and they were tested on human osteosarcoma cell lines (MG-63) for anti-cancer activity.

Cell line

The human osteosarcoma cell line (MG-63) was obtained from National Centre for Cell Science (NCCS), Pune and grown in Eagle's Minimum Essential Medium containing 10% fetal bovine serum (FBS). The cells were maintained at 37°C, 5% CO₂, 95% air and 100% relative humidity. Maintenance cultures were passaged weekly, and the culture medium was changed twice a week.

Determination of cell viability by MTT Assay

Principle

The ability of the cells to survive a toxic insult has been the basis of most Cytotoxicity assays. This assay is based on the assumption that dead cells or their products do not reduce tetrazolium. The assay depends both on the number of cells present and on the mitochondrial activity per cell. The principle involved is the cleavage of tetrazolium salt 3-(4,5 dimethyl thiazole-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) into a blue coloured product (formazan) by mitochondrial enzyme succinate dehydrogenase. The number of cells was found to be proportional to the extent of formazan production by the cells used.^[18]

Cell treatment procedure

The monolayer cells were detached with trypsin-ethylenediaminetetraacetic acid (EDTA) to make single cell suspensions and viable cells were counted using a hemocytometer and diluted with medium containing 5% FBS to give final density of 1x10⁵ cells/ml. One hundred microlitres per well of cell suspension were seeded into 96-well plates at plating density of 10,000 cells/well and

incubated to allow for cell attachment at 37°C, 5% CO₂, 95% air and 100% relative humidity. After 24 h the cells were treated with serial concentrations of the test samples. They were initially dissolved in neat dimethylsulfoxide (DMSO) and an aliquot of the sample solution was diluted to twice the desired final maximum test concentration with serum free medium. Additional four serial dilutions were made to provide a total of five sample concentrations. Aliquots of 100 µl of these different sample dilutions were added to the appropriate wells already containing 100 µl of medium, resulting in the required final sample concentrations. Following sample addition, the plates were incubated for an additional 48 h at 37°C, 5% CO₂, 95% air and 100% relative humidity. The medium containing without samples were served as control and triplicate was maintained for all concentrations.^[18]

After 48 h of incubation, 15µl of MTT (5mg/ml) in phosphate buffered saline (PBS) was added to each well and incubated at 37°C for 4h. The medium with MTT was then flicked off and the formed formazan crystals were solubilized in 100µl of DMSO and then measured the absorbance at 570 nm using micro plate reader. The % cell inhibition was determined using the following formula.

$$\% \text{ Cell Inhibition} = 100 - \frac{\text{Abs (sample)}}{\text{Abs (control)}} \times 100$$
^[19]

RESULT AND DISCUSSION

MTT assay is based on the reduction of yellow tetrazolium MTT to a purple formazan dye by mitochondrial succinate dehydrogenase.^[20] As per MTT assay the ethanolic leaf extract of *A.sessilis* and *A.philoxeroides* showed considerable activity on MG-63 cell line. Nonlinear regression graph was plotted between % Cell inhibition and Log concentration and IC₅₀ was determined using Graph Pad Prism software. The results are tabulated in table 1.

The ethanolic leaf extract of *A.philoxeroides* showed % inhibition concentration of 37.5µg, 75 µg, 150 µg, 300 µg to an extent of 2.21, 2.72, 11.86 and 67.3 respectively. The IC₅₀ value for *A.philoxeroides* is 249.2. The regression value is 0.9975. The inhibition concentration of *A.sessilis* 37.5µg, 75µg, 150µg, 300µg was found to be an extent of 0.58, 0.36, 5.81, 15.2 and 47.71 respectively. The IC₅₀ value for *A.sessilis* is 314 and its regression value is 0.9969.

From the aerial parts of the plant *A.philoxeroides*, the antitumor compounds, alternanthin B and N-*trans*-feruloyl-3, 5- dimethoxytyramine has been isolated.^[15] Pentacyclic triterpene saponins (philoxeroidesides A-D) isolated from the plant exhibited cytotoxic activities against SK-N-SH and HL60 cell lines.^[16] Oleanolic acid 3-O-β-D-glucuronopyranoside, isolated from n-butyl extract of the plant showed significant inhibitory effect against Hela and L929 cell lines.^[17]

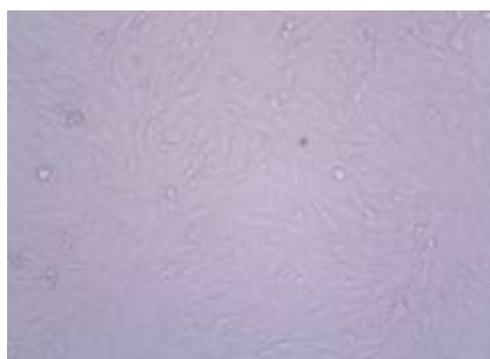
Phytochemical investigations revealed that the plant *A.sessilis* contains lupeol, stigmasterol, β -sitosterol, handianol, campesterol, α and β -spinasterol, 24-methylenecycloartanol, cycloeucalenol, 5 α -stigmasta-7-enol.^[14] So the phytochemicals alkaloids, flavonoids, phenols, found in plants may be the reason for cytotoxic activity on MG-63 cell line.

From the present study, it was observed that both the plant extracts showed moderate cytotoxic activity against MG-63 cell line. The cytotoxicity of extracts was found to be in dose dependent and non selective as reflected by uniform IC₅₀ values independent of cell line origin. Now overall study evaluated that the ethanolic leaf extract of *A.philoxeroides* showed better cytotoxic activity when compared with *A.sessilis*.

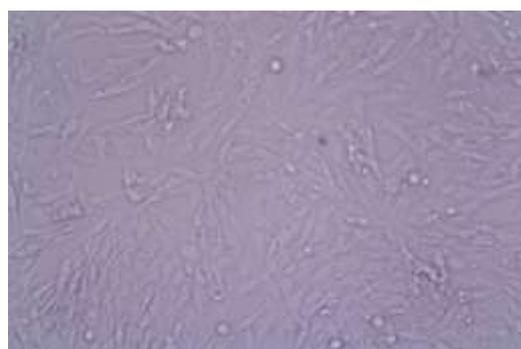
Alternanthera philoxeroides



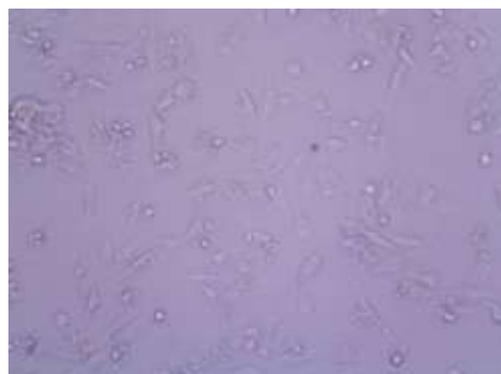
18.75µg.



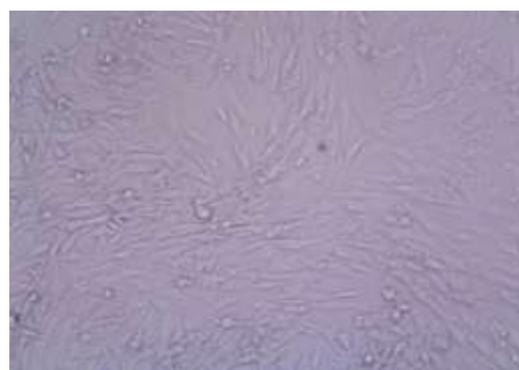
37.5µg.



75µg.



150µg.



300µg.

Alternanthera sessilis



18.75µg.



37.5µg.



75µg.



150µg.



300µg.



Control.

Conc(ug/ mL)	<i>Alternanthera philoxeroides</i> (%)inhi bition	<i>Alternanthera sessilis</i> (%)inhibi tion
37.5	2.209131	0.368189
75	2.724595	5.817378
150	11.85567	15.243
300	67.3785	47.7123
IC ₅₀ µg/mL	249.2	314
R ₂ µg/mL	0.9975	0.9969

CONCLUSION

The present study concluded that the ethanolic leaf extract of *Alternanthera philoxeroides* showed better cytotoxic activity when compared with *Alternanthera sessilis* against the experimental human osteosarcoma cell lines (MG-63). These plants might be a potential alternative agent for human osteosarcoma cell lines (MG-63). Hence, it is anticipated that *Alternanthera sessilis* and *Alternanthera philoxeroides* would be a useful pharmaceutical material to treat human osteosarcoma cancer. Future research should focus on the molecular mechanism of *Alternanthera sessilis* and *Alternanthera philoxeroides* for anticancer action. There is a need for further investigation of this plant in order to identify and isolate its active anticancer principle(s) to treat cancer. The results of the study will also need to be confirmed using *in vivo* models.

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