International Journal of Bioassays
ISSN: 2278-778X
CODEN: IJBNHY
OPEN ACCESS

Original Research Article

ANTICANCER ACTIVITY OF *ACANTHUS ILLICIFOLIUS* Linn. FROM CHETTUVA MANGROVES, KERALA, INDIA

Smitha RB*, Madhusoodanan PV and Prakashkumar R
Malabar Botanical Garden, Kozhikode-673614, Kerala, India.

Received for publication: October 01, 2014; **Accepted**: October 17, 2014

Abstract: Mangroves are salt-tolerant plants of tropical and subtropical intertidal regions of the world. In spite of the commercial properties, mangroves are used in folklore medicine. The main objective of the present study is to identify the anti-cancer activity of *Acanthus illicifolis* (Acanthacea), used in folklore medicine for the treatment of several diseases. The present study was aimed at using leaf and root extract as anticancer agent. The ethyl acetate extract was treated under various concentrations in MCF-7 and PA-1 cell lines. The results showed that at 100µg/mL, the leaf extracts showed 99.8±0.2 and 100±0 percentage of inhibition on MCF-7 cell lines and 99.9±0.2 and 100±1 on PA-1 cell lines. The CC 50 values clearly indicated the anticancer activity of *Acanthus illicifolius*.

Key Words: *Acanthus illicifolis* L., Ethyl acetate, Cytotoxicity, MCF-7, PA-1

INTRODUCTION

Man has been using herbs and plant products for combating diseases since times immemorial. Indian systems of medicine have a deep root in our cultural heritage and cater to the medicare of large sections of our population. Natural products have been used as a major tool for discovery of drugs of pharmaceutical importance. In recent years, antimicrobials derived from the plants have been receiving increasing attention, as the synthetic antibiotics have shown ineffectiveness against several pathogenic organisms, due to increasing drug resistance.

Natural products and related drugs are used to treat 87% of all categorized human diseases including bacterial infection, cancer and immunological disorders (1). About 25% of prescribed drugs in the world originated from plants and over 3000 species plants have been reported to have anticancer properties (2). About 80% of the populations in developing countries rely on traditional plant based medicines for their primary health care needs.

Mangroves are biochemically unique, producing a wide array of novel natural products. Substances in mangroves have long been used in folk medicine to treat diseases. Although the chemical constituents of most mangrove plants still have not been studied extensively, investigations have led so far to the discovery of several novel compounds with prospective medicinal value for the discovery of new chemotherapeutic agents (3). Until now, more than 200 bioactive metabolites have been isolated from true mangroves of tropical and subtropical populations (4). According to their chemical structures, most of the isolated compounds are triterpenoids (betulic acid 0.3%, taraxerol 0.06% and taraxerone 0.05%) and traces of hydrocarbon, Sterols (β-sitosterol & stigmasterol), triterpene alcohols, iridoid glycosides and high amount of carbohydrates, lipids and proteins (5). India has a rich and prestigious heritage of mangrove forest and mangrove oriented medicines among the South Asian countries. Recent research evidences suggest that Indian mangrove plant species have antibacterial activity (6-8). Traditional records and ecological diversity indicate that Indian plants represent an exciting resource for possible lead structures in drug design.

Common basic steps of in-vitro cytotoxic screening include: (a) isolation of cells, (b) incubation of cells with drugs, (c) assessment of cell survival and (d) interpretation of the result. Colorimetric assay (MTT) is mainly useful in the determination of cellular proliferation, viability and activation. The need for sensitive, quantitative, reliable and automated methods led to the development of standard assays. Cell proliferation and viability assays are of particular importance for routine applications. These techniques are considered to be fast and economical for the evolution of anticancer compounds (9).

Acanthus illicifoilis L. is a common mangrove plant and is used in several countries to treat different diseases. Previous studies (10) have confirmed that some of the species produce compounds that can exert some pharmacological, antimicrobial and anti - inflammatory activities. Potential anticancer study of *Acanthus illicifolis* on ethyl acetate gave a good result against HELA and KB cell lines (11). Hence the aim of the present study is to evaluate the anticancer activity by *in vitro* method on Michigan Cancer Foundation-7 (MCF-7) cell line and PA-1 cell line.

MATERIALS AND METHODS

Material used

Acanthus illicifolius L. (Acanthaceae) was collected from Chettuva, Thrissur District of Kerala, India (Lat.10° 31' 00N. Long. 76° 13' 15E) (Figure 1). The

***Corresponding Author:**
Dr. Smitha RB,
Scientist,
Malabar Botanical Garden,
Kozhikode – 673614, Kerala, India.

cell lines used were MCF-7 and PA-1 responsible for causing breast cancer and prostrate cancer.

Figure 1: Habit of *Acanthus illicifolius*

Extraction by Soxhlet apparatus

The leaf and roots of *Acanthus illicifolius* were washed in tap water, dried in shade and powdered in mechanical grinder and stored in airtight bottle for future use. The shade dried parts of the plant (Leaf and root) were coarsely powdered (50 g) and extracted with Ethyl acetate (300 mL) for 48 hours in soxhlet apparatus. After evaporation of the solvent under reduced pressure, the respective crude extract was obtained and stored at 4°C for testing anticancer activity.

Cytotoxicity assay (MTT assay)

MCF-7 and PA-1 purchased from NCCS, Pune were cultured in DMEM (Dulbecco's Modified Eagle's Medium) medium supplemented with 10% fetal calf serum, 100 U/ml penicillin and 100*µg/ml* Streptomycin. Cultures were maintained at 37°C in humidified 5% CO_2 atmosphere. When the cells were about to cover 80% of the flask area, they were disrupted and seeded on 24 well plates. $3x10^4$ viable cells from each cell line were seeded in a 24-well plate and incubated for 24 - 48h.When cells reached >80% confluence, the medium was replaced and cells were incubated with crude extracts at 1, 5, 10, 25, 50 and 100*µg* dissolved in dimethyl sulphoxide (DMSO) at a maximum concentration of 0.05%. After 72 h incubation, 10*µl* of 3-(4,5 dimethylthiozol-2-yl)- 2,5 diphenyl tetrazolium bromide solution (MTT, Sigma) solution (5mg/ml) was added to each well and incubated at 37° C for 4h. The medium was removed and formazan, was dissolved in acidified isopropanol (0.4N HCl). The amount of MTT-formazan is directly proportional to the number of living cells and was determined by measuring the optical density (OD) at 570nm using a bio-assay reader (biorad, USA). The concentration of the crude extract that killed 50% of the cells (CC50) was calculated. The results were analysed and photographs were taken using an Epifluorescent microscope at 400X magnifications. All determinations were performed in triplicate.

Statistical analysis

The data from these experiments were pooled and the mean ± SD values were calculated. Differences between cell lines were tested by two way ANOVA. Dunnett's test was used to determine statistical difference between the control cells and *Acanthus ilicifolius* extract treated MCF-7 and PA-1 cells. All p-values <0.05 were considered to be significant.

RESULTS

Percentage of inhibition using MCF-7

The ethyl acetate extract of leaf and root samples were checked for the percentage of inhibition using different concentrations of the extracts such as 1µg/mL, 10 µg/mL, 25µg/mL, 50 µg/mL and 100µg/mL. The percentage of inhibition showed 8.3±0.1 for 1µg/mL extract, 14.5±0.2 for 10 µg/mL, 51.6±0.1 for 25 µg/mL, 87.3±0.1 for 50 µg/mL and 99.8±0.2 for 100 µg/mL while using the leaf extract on MCF-7 cell line. But the percentage of inhibition was 8.2±0.1 for 1µg/mL extract, 10.5±0.2 for 10 µg/mL,34.3±0.2 for 25 µg/mL, 86.5±0.2 for 50 µg/mL and 99.9±0.2 for 100 µg/mL while using the root extract on MCF-7 cell line. The results are shown in Table No. 2 and it clearly suggests that the leaf and root extracts have much significant anticancer activity while using the two cell lines.

Percentage of inhibition using PA-1

The ethyl acetate extract of leaf and root samples were checked for the percentage of inhibition using different concentrations of the extracts such as 1µg/mL, 10 µg/mL, 25 µg/mL, 50 µg/mL and 100 µg/mL. The percentage of inhibition showed 9.1±0.4 for 1µg/mL extract, 47.7±0.12 for 10 µg/mL, 79.4±0.5 for 25 µg/mL, 99.8±0.2 for 50 µg/mL and 100±0 for 100 µg/mL while using the leaf extract on PA-1 cell line. But the percentage of inhibition was 19.5±0.3 for 1µg/mL extract, 31.2±0.2 for 10 µg/mL,62.5±0.2 for 25 µg/mL, 98.4±0.2 for 50 µg/mL and 100±0.1 for 100 µg/mL while using the root extract on PA-1 cell line. The results are shown in Table No. 6 and it clearly suggests that the leaf and root extracts at the ratio of 50 µg/mL was enough for controlling the cancerous activity of the cells.

Cytotoxicity of MCF-7 and PA-1

The ethyl acetate extracts of leaf and root of *Acanthus illicifolius* were tested for their anticancer activity on MCF-7 and PA-1 by the MTT assay (comet assay). The ethyl acetate extract was the most statistically significant inhibitor on MCF-7 and PA-1 cell

line. The extract at 100 µg ml^{-1} from leaf and root of the plant exhibited the highest anticancer activity on MCF-7 and PA-1 (Table 3). Ethyl acetate extract from root of *Acanthus illicifolius* have shown the most potent cytotoxicity on MCF-7 and PA-1 (99.9 ± 0.2 and 100 ± 0.1), followed by ethyl acetate extract of leaf (99.8 ± 0.2 and 100 ± 0.1). The ethyl acetate extract from root on MCF-7 and PA-1 gave CC50 values of 99.9 µg/mL and 100 µg/mL (Fig. 2. C & F) and from leaf on MCF-7 and PA-1 gave CC50 values of 99.8 µg/mL and 100 µg/mL at 24 h respectively (Fig. 2. B & E). The results are compared with untreated MCF-7 and PA-1 cell lines (Fig. 2A & D).

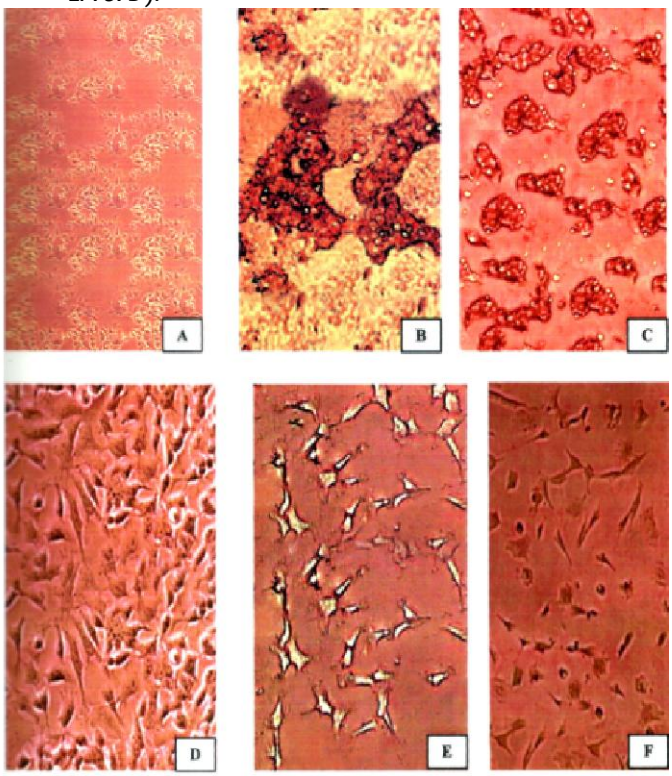

Fig. 2: Figure showing the MTT assay of *Acanthus illicifolius* L. against MCF-7 and PA-1 cell lines. Figures A-C represents MTT assay of MCF-7 and D-E represents PA-1. (A). Untreated MCF-7 cell lines (B). CC 50 of leaf extract (C). CC50 of root extract (D). Untreated PA-1 cell lines (E). CC 50 of leaf extract and (F). CC 50 of root extracts.

Table 1: The percentage of inhibition using the ethyl acetate extracts of leaf and root on MCF-7

Extracts		1 µg/mL	10 µg/mL	25 µg/mL	50 µg/mL	100 µg/mL
Leaf	Ethyl acetate	8.3±0.1	14.5±0.2	51.6±0.1	87.3±0.1	99.8±0.2
Root	Ethyl acetate	8.2±0.1	10.5±0.2	34.3±0.2	86.5±0.2	99.9±0.2

Table 2: The percentage of inhibition using the ethyl acetate extracts of leaf and root on PA-1

Extracts		1 µg/mL	10 µg/mL	25 µg/mL	50 µg/mL	100 µg/mL
Leaf	Ethyl acetate	9.1±0.4	47.7±0.12	79.4±0.5	99.8±0.2	100±0
Root	Ethyl acetate	19.5±0.3	31.2±0.2	62.5±0.2	98.4±0.2	100±0.1

DISCUSSION

Acanthus illicifolius L. is a valuable medicinal plant that is widespread in tropical Asia and Africa, through Malaya to Polynesia. *A. illicifolius* extracts have been used in various folk medicines as remedies against rheumatism, neuralgia, poison arrow wounds, coughs, asthma and bacterial infections with subsequent scientific supports to these claims (10). In the present study, we assessed the cytotoxic efficacy of ethyl acetate extract obtained from the leaf and roots of *A. illicifolius* using MTT assay (Comet assay). Data generated from this assay strongly suggest that the plant extracts are moderately cytotoxic to MCF-7 and PA-1 cells.

Ethyl acetate extract from root of *Acanthus illicifolius* have shown the most potent cytotoxicity and percentage of inhibition on MCF-7 and PA-1 cell lines. The results showed that 99.8±0.2 percentage of inhibition on MCF-7 cell lines while using the leaf extracts at 100 µg/mL, but 99.9±0.2 percentage of inhibition on root extracts at the same concentration (Table 1). In the case of PA-1 cell lines the extracts at 100 µg/mL showed 100 % of inhibition (100±0.0 and 100±0.1) on both leaf and root extracts (Table 2). The CC50 values were also calculated for both the cell lines. The extract obtained from root showed CC50 value of 99.9 µg/mL at 24 h on MCF-7 cell line and 100 µg/mL for PA-1 cell lines. Similarly the extract from leaf showed CC50 value of 99.8 µg/mL on MCF-7 and 100 µg/mL on PA-1 cell lines at 24 h (Table 3).

Table 3: Cytotoxicity of plant extracts (CC50±SD).

Cell lines	Ethyl acetate Extracts	
	Leaf	Root
MCF-7	24.22 ± 0.2 µg /ml	29.20 ± 0.1 µg /ml
PA-1	15.74 ± 0.1µg/ml	20.0 ± 0.1 µg /ml

The results obtained from the present study are correlated with some of the recent works. Khajure and Rathod 2011 (11) conducted *in vitro* comet assay for anticancer using Hela and KB Cell lines. They used the air dried whole plant parts of *Acanthus ilicifolius*. The found that ethyl acetate extract obtained from the whole plant of *Acanthus illicifolius* showed a good cytotoxic effect against Hela cells by *in vitro* method. Patra and Thatoi 2013 (12) also reported the ribose derrivatives of benzoxazoline extracted from *A. illicifoilis* is active against cancer.

Some of the recent studies also reveal the anti-cancerous property of other mangroves plant species. Batsa and Periyasamy 2013 (13) carried out a work to identify the anti-cancer activity of *Excoecaria agallocha*, a Mangrove Plant used in folklore medicine for the treatment of several diseases. They used the leaf extract of methanol and chloroform and found that the

cell viability was maximum at the lower concentration (3.906µg/ml) when compare to higher concentration. Sukhramani and Patel 2013 (3) also reported the anticancer activity of *Avicennia marina*, a true mangrove plant. The *in-vitro* anticancer activity of its leaf extract on various cancer cell lines (HL-60, HepG2, NCI-H23 & HEK-293T) were determined by MTT bioassay. With use of MTT dye, % cell viability and % inhibition of the hit compounds was evaluated and the data obtained from MTT bioassay screening revealed that methanolic and aqueous extract of *Avicennia marina* showed cytotoxicity against all the selected cell lines.

Heritiera fomes Buch. Ham., a dominant mangrove tree species occurring in Bhitarkanika mangrove forest, Odisha, has been known for its ethno medicinal uses for the treatment of gastrointestinal disorders, hepatic disorders, skin diseases, diabetes and goiter. Scientific evidence in support ethno medicinal uses of this plant is lacking. Hence Patra and Thatoi 2013 (12) conducted a study to determine the anticancer activity both *in vitro* and *in vivo*. They used the methanolic extract of both leaf and stem powder and the extracts showed anticancer properties with 40 % inhibition against B16 mouse melanoma (in vitro system) and Ehrlich Ascites Carcinoma (EAC) in Swiss albino mice (in vivo system). Bark extract of *Bruigueria sexangula* is active against two tumours Sarcoma 180 and Lewis Lung Carcinoma was reported by Kathiresan et al., 2005 (14). They reported the anticancer activity is due to tannins, an unidentified alkaloid tropine and its acetic acid ester (brugine) present in the extract.

CONCLUSION

Anticancer study demonstrated that the ethyl acetate extract of *Acanthus illicifolius* reduces cellular viability in MCF-7 and PA-1 cell lines. The ethyl acetate extracts of *Acanthus illicifolius* leaf and root were tested for their anticancer activity on MCF-7 and PA-1 by the comet assay. The ethyl acetate extract was the most statistically significant inhibitor on MCF-7 and PA-1 cell line. The extract at 100µg/mL from leaf and root of the plant exhibited the highest anticancer activity on MCF-7 and PA-1.

ACKNOWLEDGEMENTS

The authors are thankful to Unibiosys Laboratory, Cochin for helping in the cytotoxicity studies. The first author is thankful to DST, Government of India for the award of Fast Track Fellowship.

REFERENCES

1. Newman DJ and Cragg GM. Natural products as sources of new drugs over the last 25 years, National Proceedings Journal, 2007, 70, 461-477.
2. Graham JG, Quinn ML, Fabricant DS, Farnsworth NR. Plants used againstcancer - an extension of the work of Jonathan Hartwell, Journal of Ethnopharmacology, 2000, 73, 347–377.
3. Sukhramani PS, Patel PM, Biological screening of *Avicennia marina* for anticancer activity, Pelagia Research Library, 2013, 4, 125-130.
4. Wu J, Xiao Q, Xu J, Li M, Pan J, Yang M, Natural products from true mangrove flora: source, chemistry and bioactivities, National Proceedings and Reports, 2008, 25, 955-981.
5. Bandaranayake WM, Traditional and medicinal uses of mangroves, Mangroves and Salt Marshes, 1998, 2, 133-148.
6. Arivuselvan N, Silambarasan D, Govindan T, Kathiresan K, Antibacterial activity of mangrove leaf and bark extracts against human pathogens. Advances in Biological Research, 2011, 5, 251-254.
7. Abeysinghe PD, Antibacterial activity of some medicinal mangroves against antibiotic resistant pathogenic bacteria. Indian Journal of Pharmaceutical sciences, 2010, 72, 167-172.
8. Jaimini D, Sarkar C, Aaftab A, Shabnam, Jadhav BL, Evaluation of antibacterial properties of mangrove plant *Sonneratia apetala* Buch. Ham leaf. World Applied Sciences Journal, 2011, 14, 1683-1686.
9. Rubinstein LV, Comparison of *in vitro* anticancer-drug-screening data queerated with tetrazolium assay-screening assay versus a protein assay against a diverse paued of human tumor cell tissues. Journal of the National Cancer Institute, 2003. 82, 113-118.
10. Mastaller M, Mangroves: The Forgotten Forest between Land and Sea. Tropical Press, 1997. pp. 97.
11. Khajure PV, Rathod JL, Potential anticancer activity of *Acanthus illicifolius* extracted from the mangroves forest of Karwar, West coast of India. World Journal of Science and Technology, 2011, 1, 1–6.
12. Patra JK, Thatoi H, Anticancer activity and chromatography characterization of methanol extract of *Heritiera fomes* Buch. Ham., a mangrove plant from Bhitarkanika, India. Oriental Pharmacy and Experimental Medicine, 2013, 13, 133-142.
13. Batsa MA, Periyasamy K, Anticancer activity of *Excoecaria agallocha* leaf extract in cell line model. International Journal of Pharmacy and Biological Sciences, 2013, 3, 392-398.
14. Kathiresan K, Boopathy NS, Kavitha S, Coastal vegatation an underexplored source of anticancer drugs, Natural Product Radiance, 2005, 5, 115-119.

Source of support: DST, Government of India
Conflict of interest: None Declared