Cytotoxic analysis of leaves of *Alangium salvifolium* (l.f.) Wangerin.

P.PRATHYUSHA
Assistant Professor
Department of Botany,
Nirmala College for Women,Coimbatore-18

Abstract

Alangium salvifolium is a well-known traditionally used medicinal plant in India and it is also one of the most versatile medicinal plant having a wide spectrum of biological activities like antidiabetic, antiulcer, analgesic, anti-inflammatory, antimicrobial, antioxidant, anti-arthritic, diuretic, antifertility, anthelmintic, antiepileptic and antifungal. *A. salvifolium* is a tall thorny tree and the genus contains 17 species of small trees, shrubs and lianas. *Alangium* name has been derived from the Malayalam word *Alangi*. Almost all the parts (root, bark, leaves, seeds and fruits) are known to have important therapeutic uses and are extensively used for different purposes in the indigenous herbal medicines. The present paper focus on the Cytotoxic activity of *A. salvifolium*.

Key words : Antiulcer, analgesic, anti-inflammatory, antimicrobial, antioxidant, anti-arthritic, diuretic, antifertility, anthelmintic, antiepileptic and antifungal.

Introduction

Alangium salvifolium Wang. belongs to the family *Alangiaceae* and is commonly known as sage leaved *Alangium*. *A. salvifolium* has been used traditionally for treatment of various ailments. Almost every part of *A. salvifolium* including roots, leaves, stem and bark are used in the Ayurveda and Siddha systems of medicines for the treatment of various diseases. In modern scientific literatures, the plant has been reported to have potential efficacy against diabetes, peptic ulcer, arthritis, inflammation and anthelminthic activities [1]. The synonyms of *A. salvifolium* are *A. decapetalum* Lam, *A. lamarckii* Thw, *A. latifolium* Miq.ex C.B. Clarke, *A. mohillae* Tul., *A. salvifolium* subsp. *Decapetalum* (Lam.) Wangerin, *A. sundanum* var. *Miqueliana* Kurz., *A. tomentosum* Lam., *Grewia salviifolia* L.f, *Karangolummohillae* (Tul.) Kuntze and *Karangolum salvifolium* (L.f.) Kuntze [2].

Plants have a long history of use in the treatment of cancer. In many instances, however, the "cancer" is undefined, or reference is made to conditions such as "hard swellings", abscesses, calluses, corns, warts, polyps, or tumors, to name a few. These symptoms would generally apply to skin, "tangible", or visible conditions, and may indeed sometimes correspond to a cancerous condition. Many of the claims for efficacy in the treatment of cancer, however, should be viewed with some skepticism because cancer, as a specific disease entity, is likely to be poorly defined in terms of folklore and traditional medicine. *A. salvifolium* is reported to contain various biologically active phytochemicals such as alangine, ankorine, tubulosine, alangicine, salsoline. The present paper highlights the cytotoxic activity of leaves of *A. salvifolium*.

Meterials and methods (Tian, *et al*., 2003)

The assessment of the anticancer activity of the studied plants the following human cancer cell lines were used: Vero (monkey kidney cell lines) and HEP-2 (human epithelial carcinoma cell line). Cells were grown in RPMI – 1640 medium at 37^0C under 5% CO_2 in a humidified incubator. Cells were harvested, counted ($3x10^4$ cells/ml), and transferred into a 96-well plate, and incubated for 24 hrs prior to the addition of test compounds in DMSO followed by dilution with RPMI-1640 medium to give final concentration at 50,25,12.5,6.25,3.125,1.0 and 0.1 mg/ml. Stock solutions of samples were prepared. Samples at 10µl and cell lines at 90 µl were incubated for 72 hrs. MTT solution at 5mg/ml was dissolved in 1ml of Phosphate Buffer Solution (PBS), and 10µl of it was added to each of the 96 wells. The wells were wrapped with aluminum foil and incubated at 37^0C for 4 hrs. The solution in each well containing media, unbound MTT and dead cells were removed by suction and 150µl of DMSO was added to each well. Then the plates were shaken and optical density was recorded using a micro plate reader at 540nm. Distilled water was used as positive control and DMSO as solvent control. Controls and samples were assayed in duplicate for each concentration and replicated three times for each cell line. The cytotoxicity was obtained by comparing the absorbance between the samples and the control. The values were then used to iteratively calculate the concentration of plant extracts required to cause a 50% reduction (IC_{50}) in growth (cell number) for each cell lines.

Results and discussion

The effect of extract on the cell response of the Hep 2 (Human epidermoid cell line) by using the MTT assay. Fig.1 shows the *In vitro* cytotoxicity activity of compounds (10-50µg concentrations) against selected cancer cells. The experimental results demonstrate that the extract has the ability to inhibited cell proliferation in a dose dependent manner. From the Fig. 1 the IC_{50} values of extract against Hep 2 (Human epidermoid cell line) were calculated and it is found to be 23 ± 1.0 µg/ml concentrations. Further the execution of cytotoxicity in the cells was significantly higher than in the untreated control. Since the unique properties extract may plays an imperative role in the promising effect on cell proliferation, in this depiction, we have explored the cytotoxic effect of extract with different concentrations. In our present findings, extract possibly would bring to bear cytotoxic effect on selected cancer cell lines at minimal concentrations and insignificant toxicity in normal cells. In the current study suggests that extract cytotoxicity may be related to enhance membrane-mediated apoptosis.

Cell morphology analysis

Morphological changes in Hep 2 cells after treatment with extract with various concentrations for 24 h. Phase-contrast micrographs reveal that the extract has the ability induces increased cell shrinkage, membrane blebbing and forms floating cells, compared to the control cells in a dose-dependent manner. Cytological investigations elucidate the antiproliferative effect routed through membrane blebbing, membrane instability and disturbing the cytoskeleton of the cells by the extract. In (Fig. 2 b, c and d) clearly shows that the membrane blebbing and formation of floating cells in the treated cancer cells. Moreover it could found that the isolated compounds showed significant cytotoxicity and anti-proliferative effects on selected human cancer cells.

Fluorescence microscopy analysis of nuclear fragmentation
(Acridine orange /Ethidium bromide (AO/EtBr) staining Method)

In order to elucidates the apoptotic activity of synthesized compounds apoptotic staining fluorescence microscopic analysis carried out. Fluorescence microscopy images of Hep 2 cells in the

absence of compound (Control) and in the presence of compound are shown in **Fig.3.** Fig. (a) shows that the untreated Hep 2 cancer cells (control) did not show any significant adverse effect compared to the extract treated cancer cells. Whereas the treated (Fig.b, c and d) showed a concentration dependant apoptotic activity. It can be observed that with the addition of extract to the cancer cells, the green colour of cells are converted into orange/red colour cells which is due to induced apoptosis and the nuclear condensation effect on the cells. On the basis of the overall cell morphology and the cell membrane integrity, apoptotic cells were distinguished from one another using fluorescence microscopy. Here we can differentiate the apoptotic cells induced by the extract through activation apoptotic signals in the treated cells, confirms that the extract has an ability to inhibit the cells via apoptotic mechanism.

Conclusion

Most modern medicines currently available for treating cancers are very expensive, toxic and less effective in treating the disease. Thus, one must investigate further in detail the agents derived from this plant, for the prevention and treatment of cancer disease. More clinical trials are also needed to validate the usefulness of these agents either alone or in combination with existing therapy.

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Table 1. Cytotoxic activity of leaves of *A. salvifolium* (MTT Assay)

Sample (µg/ml)	**Hep 2 (Human epidermoid cell line)** IC_{50} **value**
Sample	23 ± 1.0
DOX	14 ± 0.5

Standard Used: Doxorubicin

Statistics: All the in vitro experiments were done in triplicate, and the experiments were repeated at least thrice. The statistical software SPSS version 17.0 was used for the analysis. *P* value <0.01 was considered significant.

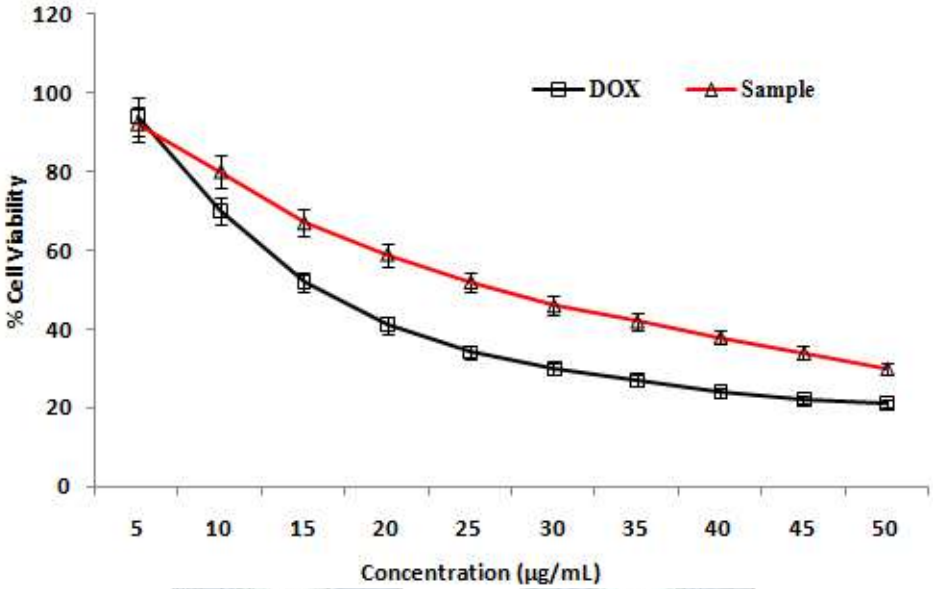

Figure 1. MTT assay

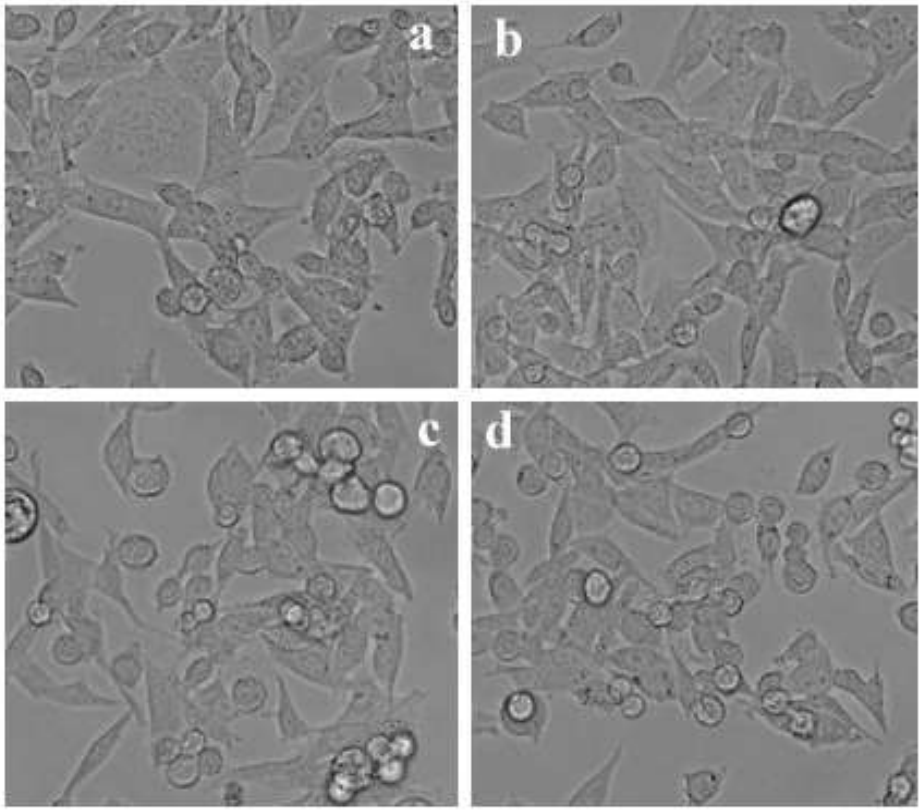

Figure 2. Morphological changes upon the treatment

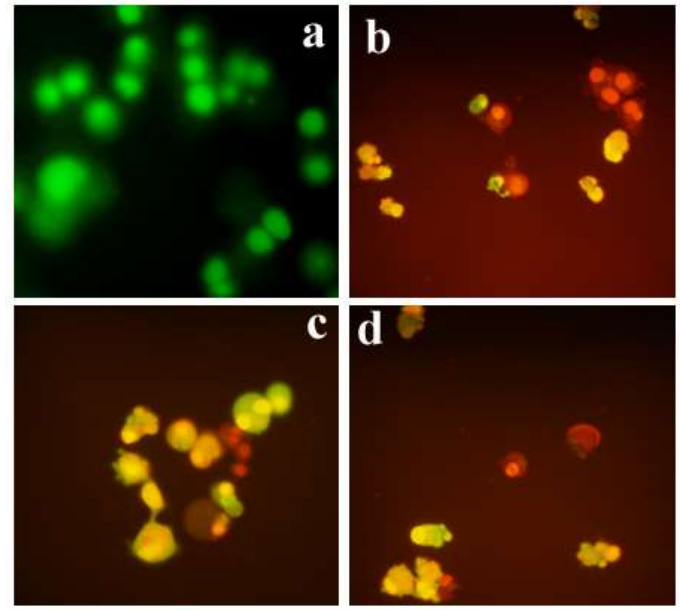

Figure 3. Apoptotic activity in Hep 2 by using AO/EtBr

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