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**Research Article**

# EVALUATION OF INVITRO ANTICANCER ACTIVITY OF SELECTED MANGROVE PLANT EXTRACTS AGAINST MCF7 CELL LINE

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**ABSTRACT**

Since many years, plants were known to posses anticancer activities against different cancer cell lines. In the present paper, we report a study based on anticancer properties of five mangrove plants *Avicennia marina, Avicennia officinalis, Calophyllum inophyllum, Bruguiera gymnorrhiza, and Aegiceras corniculatum.* The leaves of these plants were collected, shade dried and extracted with methanol as a solvent. Anti cancer activity was assayed with standard MTT colorimetric procedure against MCF-7 cell line. From the analysis, it was found that Avicenia officinalis and Bruguiera gymnorrhiza showed nearly 50 % cell line inhibition at 67.26 and 99ug/ml tested dose, where as the other plant species did not display much anticancer activity. Further work is in progress to identify the active chemical constituents present in these plant extracts.



## INTRODUCTION

Since medieval times, plants have been the major source of medicines for the treatment of various diseases. Currently plants remain an integral part of the health care in different countries including developed countries. According to World Health Organization (WHO), 80% of the people living in rural areas depend on the medicinal plants as primary health care system (1-2). Medicinal plants possess an important position in the drug discovery and many modern drugs have their origin in traditional medicine of different cultures. Hence regardless of the advantages of the synthetic and combinatorial chemistry as well as molecular modeling, medicinal plants remain an important source of new drugs, new drug leads, and new chemical entities (3-4). It was reported that out of over 800 new chemical entities introduced between 1981 and 2002 nearly the half were natural products and semi- synthetic natural products(5).

Cancer is a multistep disease developed by environmental, physical, chemical, metabolic and genetic factors. Cancer is a large group of diseases, all of which have one thing in common i.e. cells growing out of control or fundamentally a disease of tissue growth regulation failure. In order for a normal cell to transform in to cancer cell, the genes which regulate cell growth and differentiation must be altered (6). The causes of cancer are diverse, complex and partially understood. Many things are known to increase the risk of cancer, including tobacco use, dietary factors, certain infections, exposure to pollutants (7).

Conventional treatment of cancer includes interventions such as psychological support, surgery, radiotherapy, and chemotherapy.(19) Currently, the most commonly use cancer chemotherapy includes many alkylating agents, antimetabolites, antitumor antibiotics, platinum analogs, and natural anticancer agents. However due to increasing rate of mortality associated with caner and adverse or toxic side effects of cancer chemotherapy and radiation therapy, has greatly facilitated efforts in the search for more effective and novel secondary metabolites as anticancer lead molecules for some time. The plant extracts have traditionally been screened for such discoveries. Herbal medicine constitutes a major substitute for cancer prevention and treatment in anomalous countries around the globe. The effect of plant extracts as anti cancer was widely studied due to their low toxicity and side effects. Due to the aforementioned concerns, such studies investigating medicinal plants have been steadily held with interests. Currently, the number of plants reported to possess anti cancer properties are more than 3000 (8-9).

The discovery of new plant derived anticancer agents is a long term process and it contains several steps. Steps involved in the

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discovery of new anti cancer agents from plants include cytotoxicity screening of plant extracts, bioactivity guided isolation of active compounds with anti cancer properties, invitro testing, toxicity assessment of anticancer compounds and ultimately in vivo testing (10).

Free radical scavenging properties of different plant extracts are also of great importance because natural compounds with free radical scavenging activity can protect from damages due to free and reactive oxygen species in biological systems (11). Mangroves are intertidal productive forests that grow in estuarine swamps with high salinity, high temperature, low nutrients, and high radiation. Habitat of mangrove plant is commonly known as mangrove swamps, tidal forests, tidal swamp forests or mangals (12). These vascular halophytic plants constitute a vital component of marine flora and have significant ecological and socio- economic values. For centuries, mangroves have been traditionally used for food, feed and medicine purpose in different parts of the world. They are capable of growth in high environmental stress as they have unique properties to combat stress. Exposure to these stress situations results in the production of reactive oxygen species in these plants. In order to reduce the adverse effects of these reactive oxygen species, the mangrove plants produce various enzymes and various defense compounds including poly phenolic compounds (13-18). Hence, 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. Until now, more than 200 bioactive metabolites have been isolated from true mangroves of tropical and sub tropical populations (19). According to their chemical structures, most of the isolated compounds are triterpinoids and traces of hydrocarbon, sterols, triterpene alcohols and high amount of carbohydrates, lipids, and proteins whch having a wide range of therapeutic possibilities (20). India has a rich and prestigious heritage of mangrove forest and mangrove oriented medicines among the south Asian countries. Recent research evidences suggest Indian mangrove plant species have anti bacterial and anti cancer activities (). The potential of mangrove plants as a source of new bio active principles is still unexplored. Further there have been no detailed invitro studies on anticancer properties of leaves of mangrove plants from Corangi reserve forest, East Godavari district, Andhra Pradesh, India. Hence the current study is aimed on the evaluation of anticancer activity of four selected mangrove species of Corangi reserve forest.

***Plant Material***

In our present study, the fresh leaves of *Avicennia marina, Avicennia officinalis, Calophyllum inophyllum, Bruguiera gymnorrhiza and Aegiceras corniculatum* were collected from Corangi reserve forest, Kakinada, East Godavari district, Andhra Pradesh, India. Geographical location of Corangi reserve forest is between $16^{o}39^{I}$N longitude – $17^{o}$N longitude and $82^{o}14^{I}$E latitude – $82^{o}23^{I}$E latitude. The collected leaves were rinsed thoroughly with tap water in the laboratory to remove extraneous contaminants and kept in shade at room temperature in a well – ventilated place for about two weeks to dry. The dried leaves were ground to a coarse powder and subjected to solvent extraction.

***Solvent Extraction***

Methanol was used as solvent to prepare the crude leaf extracts. The material was first soaked for 12 hrs in 500 ml of methanol and then subjected to extraction by refluxing for 6 to 8 hrs below the boiling point of the solvent. The extract was concentrated by evaporating at a reduced pressure using rotary evaporator. The concentrated extract was further dried at $37^{o}$C for 3 to 4 days in order to facilitate complete evaporation of the solvent.

***Cell Lines and Culture Conditions***

MCF7 cell lines were purchased from the national centre for cell science (NCCS), Pune, India. The cell lines were cultured in Dulbecco Modified Eagle's Medium (DMEM). Culture medium was supplemented with 10% fetal bovine serum, antibiotic and antimycotic solution in conditions of 5% $CO_2$ and 95% air at $37^{o}$C.

***Invitro Cytotoxic Studies***

Common basic steps that are present in invitro cytotoxic activity include: 1) Isolation of cells, 2) Incubation of cell lines with drug for appropriate period of time, 3) Assessment of cell survival and 4) Interpretation of 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.

Cyto toxicity of sample on tumor cell was measured by Micro culture tetrazolium assay (MTT assay). (24) The MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) tetrazolium reduction assay was the first homogeneous cell viability assay developed for a 96 well format that was suitable for high throughput screening (HTS). The MTT substrate is prepared in physiologically balanced solution, added to cells in culture, usually at a final concentration of 0.2- 0.5 mg/ml, and incubated for appropriate period of time. The quantity of formazon is measured by recording changes in absorbance at 570nm using a plate reading spectrophotometer.

Viable cells with active metabolism convert MTT in to purple coloured formazon product with an absorption maximum near 570nm. When cells die, they lose the ability to convert MTT in to formazon, thus colour formation serves as a useful and convenient marker of only viable cells. The exact cellular mechanism of MTT reduction in to formazon is not well understood, but likely involves reaction with NADH or similar reducing molecules that transfer electrons to MTT.

Cells were seeded in to individual 96- well plates and incubated under the above conditions. After a day of incubation, cells were treated with various concentrations of the four selected mangrove plant extracts ranging from 10ug to 200ug/ml. In order to obtain IC50 values, absorbance was measured at

570nm in an ELISA multiplate reader. The percentage of inhibition of growth was calculated using the formula
% of Cell viability = 100- {100x ($A_c$-$A_t$)/$A_c$}
Where, $A_t$ = Absorbance value of test compound
$A_c$ = Absorbance value of control

## RESULTS

The Cytotoxic activity of the five extracts of *Avicennia marina*, *Avicennia officinalis*, *Calophyllum inophyllum*, *Bruguiera gymnorrhiza* and *Aegiceras corniculatum* were evaluated with invitro cytotoxic assay against MCF-7 cell line. Percentage of cell viability was measured by MTT assay using different concentrations of the extract such as 10ug/ml, 25ug/ml, 50ug/ml, 100ug/ml and 200ug/ml. A plant extract is usually regarded as interesting for invitro cytotoxic activity if IC50 is below 100ug/ml. As the concentration of extract in culture increased, the cell viability was decreased significantly as shown in fig 1-3. After 24 Hrs of extract treatment all the cells exhibited significant decrease in cell viability even at lower concentrations. The Cytotoxic effect of selected mangrove plant extracts were showed in fig-4

The cytotoxic effects of all extracts against MCF-7 cell line were shown in table -1. Among the tested plant extracts, the methanolic extracts of *Avicennia officinalis* and *Bruguiera gymnorrhiza* showed a moderate anti proliferative activity with IC50 values of 67.26ug/ml and 99ug/ml respectively. Other plant extracts showed less anticancer activity against MCF-7 cell line at all the concentrations tested. The shape of dose response curve indicates a significant inhibition of cell growth in a dose dependent manner.

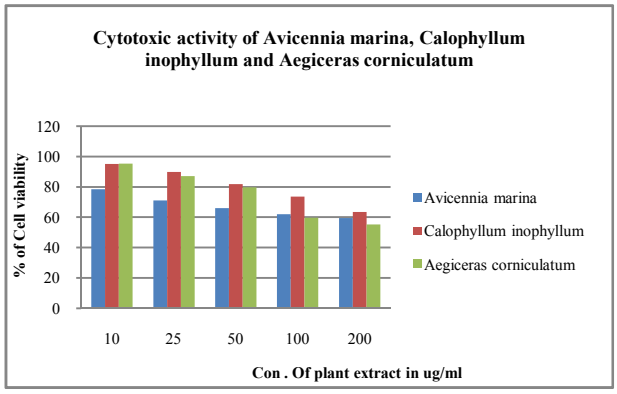


**Fig1** Cytotoxic activity of *Avicennia marina*, *Calophyllum inophyllum* and *Aegiceras corniculatum*

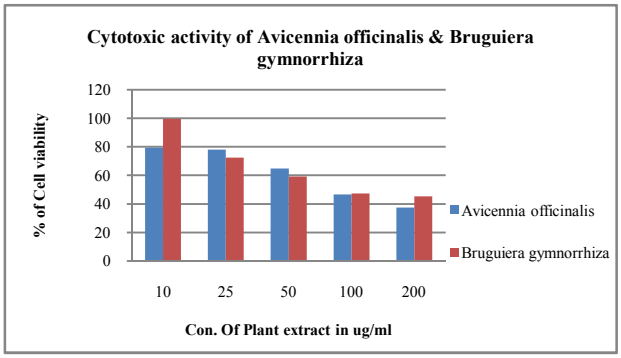


**Fig2** Cytotoxic activity of *Avicennia officinalis* and *Bruguiera gymnorrhiza*

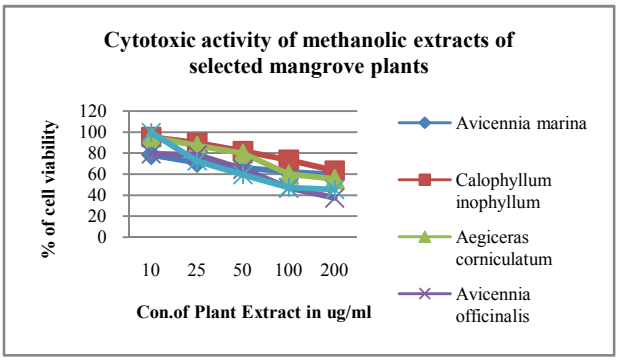


**Fig 3** Graphical representation of cytotoxic activity of selected mangrove plants.

**Table1** Cytotoxic activity of plant extracts (IC50) on MCF7 Cell line

| S.NO | Plant Extract | IC 50 Value |
|---|---|---|
| 1 | *Avicennia marina* | 200 |
| 2 | *Avicennia officinalis* | 67.26 |
| 3 | *Calophyllum inophyllum* | 200 |
| 4 | *Bruguiera gymnorrhiza* | 99 |
| 5 | *Aegiceras corniculatum* | 200 |

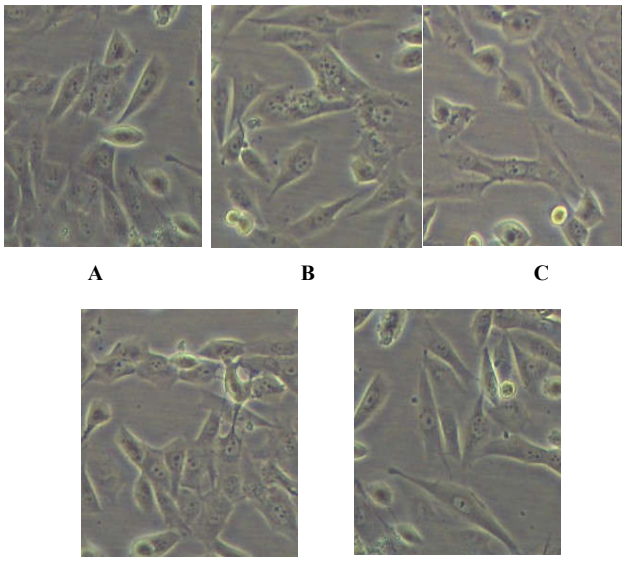


**Fig-4** Cytotoxic effect of selected plant extracts .(A)- Avicennia marina, (B)- Avicennia officinalis, (C)- Callophyllum inophyllum, (D)- Bruguiera gymnorrhiza, (E)- Aegiceras corniculatum.

## DISCUSSION

For the present study, the crude extracts of *Avicennia marina*, *Avicennia officinalis*, *Calophyllum inophyllum*, *Bruguiera gymnorrhiza* and *Aegiceras corniculatum* were collected from methanol solvent. The methanol solvent is further used to evaluate the anticancer activity by MTT assay. Natural products especially from plants have been used for the treatment of various diseases for thousands of years. Countries such as Egypt, China, India and Greece have practiced use of plants as medicines from ancient times and impressive number of modern drugs have been developed from them. Natural compounds, capable of inhibiting cell proliferation including apoptosis or modulating signal transduction are currently used for the treatment of cancer. The use of multiple chemo preventive agents or agents with multiple targets on cancer cells are considered to be more effective in cancer treatment.

Breast cancer is the most common malignancy among the women.

MCF7 cell line has become a prominent model system for the study of breast cancer as it relates to the susceptibility of the cells to apoptosis. Despite the fact that many tumors initially respond to chemotherapy, breast cancer cells can be subsequently survive and gain resistance to the treatment.

In the present study, the effects of methanol extracts of *Bruguiera gymnorrhiza* and *Avicennia officinalis* had high anti cancer activity as evidenced from the MTT assay in concentration dependent manner. The methanol extract of *Avicennia officinalis* and *Bruguiera gymnorrhiza* showed notable cell death against the MCF7 cancer cell line. Previous studies have highlighted the importance of anticancer effects of plants in bioactive compounds like Flavonoids, phenols, phyto sterols, and also fatty esters such as n- dodecanoic acid, stigma sterol and vit-E from plant derivatives which has been confirmed in the present study.

## CONCLUSION

The present investigation provided important information that methanol extracts of *Avicennia officinalis* and *Bruguiera gymnorrhiza* plants are considered to have potent cytotoxic activity against MCF7 cell line among the selected plants. The outcome of the present study encourages to carry out further studies to be extended for other cell lines and invivo cytotoxicity investigation and required to identify anticancer activity.

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