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In Vitro Anticancer Activity of Ethanolic Leaves Extract of *Avicennia marina* against Different Mammalian Cell Lines

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Abstract: Cancer is a crippling illness brought on by unchecked growth of cells. The anticancer potential of plants against HepG2, PC-3, and HT-29 was examined in this study. The MTT assay was used to assess the cytotoxicity of leaf extracts of *Avicennia marina*. The ethanolic leaves extract of *Avicennia marina* exhibited a moderate level of anticancer activity, and the IC_{50} value was noted. The most potent anticancer activity was observed with the ethanolic extract of *Avicennia marina* with IC_{50} values of 45.50µg/ml, 58.25µg/ml and 65.75µg/ml on HepG2, PC-3 and HT-29cells, respectively. The powerful plant extracts' high levels of phenols and flavonoids, which may be crucial to their anticancer properties, were discovered by phytochemical analyses. In order to isolate and characterize the active ingredients for lead optimization studies, additional researches are needed.

Keywords: Anticancer, cytotoxicity, *Avicennia marina*, HepG2, PC-3, HT-29, MTT assay

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Introduction

Mangroves represent a taxonomically varied group of salt-tolerant plant species that inhabit the intertidal regions where land meets the sea in tropical and subtropical areas (Ravishankar *et al*., 2004). These plants are highly specialized and capable of thriving under challenging conditions, such as elevated salinity, high temperatures, strong winds, anaerobic soils, and extreme tidal fluctuations (Mazda *et al*., 2005). Their survival in these harsh environments is attributed to their advanced morphological and physiological adaptations. Over time, various mangrove species have been utilized by local communities for purposes such as wood harvesting and traditional medicine (Kathiresan and Ramanathan, 1997).

The white mangrove, *Avicennia marina*, belongs to the Avicenniaceae family and is a multi-branched tree ranging from 3 to 11 meters in height. It is equipped with a robust underground root system featuring pencil roots, also known as

pneumatophores, which can extend up to 90 mm. This species has garnered interest due to its chemical composition, particularly its phenolic compounds, which serve as secondary metabolites involved in specialized physiological roles (Bharathi *et al*., 2011; Dhayanithi *et al*., 2012; Borojeni *et al*., 2013). Previous studies have highlighted the tannin-rich bark of *A. marina* for its role in stabilizing estuarine banks in saline conditions (Bandaranayake, 2002). In this study, specific *in vitro* experiments were conducted to evaluate the pharmacological activities of *A. marina*, including nitric oxide scavenging, total antioxidant activity, protein denaturation inhibition, and metal ion chelating abilities.

Cancer is one of the leading causes of mortality worldwide, causing immense physical and economic suffering. Chemotherapy is a common treatment modality; however, it often comes with severe side effects and toxicity. Researchers are continuously exploring innovative strategies to manage and treat various cancers (Modha and Modha, 2007). Notably, over 60% of anticancer drugs currently available originate from natural sources, with many pharmaceutical drugs being derived from natural products (Cragg *et al*., 1997). Consequently, phytochemicals in medicinal plants and herbal formulations have shown potential in cancer prevention and therapy (Modha and Modha, 2007). The increasing preference for herbal treatments in cancer therapy is likely due to the perception that they are safer and offer advantages over conventional allopathic treatments. Given the adverse effects associated with traditional therapies, there is an ongoing demand for new herbal cancer treatments (Aquil *et al*., 2006).

Apoptosis, or programmed cell death, is a critical mechanism for maintaining cellular homeostasis in living organisms. It involves monitoring cellular integrity and, when necessary, initiating self-destruction via cascade-like processes to preserve the organism's overall integrity. Morphological features of apoptosis include cell shrinkage, nuclear condensation, membrane blebbing, and the formation of apoptotic bodies. These morphological changes are accompanied by biochemical events such as proteolytic cleavage of intracellular proteins and DNA fragmentation. This study aimed to investigate the antiproliferative effects of the ethanolic leaf extract of *Avicennia marina* against various cancer cell lines.

Materials and Methods

Collection and Identification of plant material: The leaves of *Avicennia marina* were collected from Pichavaram Mangroves in Tamil Nadu, India. Our lab has the voucher specimen after the plant was verified by the Director of the Plant Anatomy and Research Center in Chennai, India.

Preparation of plant materials and extract: The leaves underwent meticulous cleaning, drying in the shade, and powdering. A closed, airtight plastic container was used to store the powdered material at a low temperature. Through maceration (3×24 h) at room temperature, the 50 g of powdered plant material was extracted using 300 ml of the solvent, ethanol. After being concentrated at 45°C in a rotary vacuum evaporator, the collected solvent was dried with a freeze dryer. All extracts were prepared by dissolving them in dimethyl sulfoxide (DMSO), along with acyclovir, which was obtained from commercially available tablets. The final concentration of DMSO in the cell culture medium was maintained at 0.1% v/v.

Phytochemical Analysis: The leaves of *Avicennia marina* were subjected to successive extraction using a Soxhlet apparatus, and the obtained extracts were initially analyzed for their phytochemical properties. The extracts were vacuum-dried until all solvent had evaporated. Chemical tests were conducted to detect various phytoconstituents, such as proteins, amino acids, alkaloids, carbohydrates, phenolics, flavonoids, saponins, mucilage, and resins, following standard protocols (Harborne, 1973; Trease and Evans, 1983; Sofowora, 1993).

Tumour cell lines: HepG2, PC-3, and HT-29 were

among the cell lines of various tissue origin that were employed. MEM (Minimum Essential Media) supplemented with sodium bicarbonate, EDTA, and FCS (fetal calf serum) was used to cultivate the cells. They were then incubated at 37°C in a humidified environment with 5% CO_2. Every two days, the culture medium was switched. To better simulate the effects of plant extracts on human cancer cells, all cell lines used were human. Typically, cells were cultivated in 75 cm^2 tissue culture (T-75) flasks with 10 ml of the suitable medium at 37˚C in a humidified environment with 5% CO_2 and 95% air. Every week, cells were passage, and every two weeks, the medium was changed.

MTT assay (*Mossman, 1983*): *In vitro*, MTT ([3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide]) assays were used to quantify antiproliferative effects. Following treatment, 20 µl of a 5 mg/ml MTT solution was added to test the living cells. Wells with untreated cells served as controls, and the reduced MTT was lastly measured at 545 nm. Depending on the number of cells and the length of treatment (72 h, 5000 cells/w, and 24 h, 25000 cells/w, respectively), antiproliferative and cytotoxic effects were detected. Stock solutions of the extracts were prepared in dimethyl sulfoxide (DMSO), ensuring that the final DMSO concentration in the medium did not exceed 0.3%, as this concentration did not significantly affect cell growth. Extract concentrations ranging from 20 to 200 µg/ml were tested to determine their effects.

The medium's maximum DMSO concentration (0.3%) had no discernible impact on the growth of the cells. These extracts were chosen for additional *in vitro* testing (cytotoxicity and dose-response curve) because they showed strong activity (growth inhibition > 50%). Using a checkerboard approach, the interactions between doxorubicin and acridones were investigated. Acridines were tested in a series of 2-fold dilutions, along with doxorubicin at 2-fold dilutions. The cell growth rate was determined with MTT staining drug interactions were evaluated according to the following system (fractional inhibitory index = FIX):

FIX < 0.5	Synergism	1 < FIX < 2	Indifferent effect
FIX = 0.51-1	Additive effect	FIX > 2	Antagonism

Results and Discussion

The presence of alkaloids, flavonoids, phytosterols, tannins, and phenols was found in the ethanolic extracts of *A. marina* after phytochemical screening (Table 1). The medicinal properties of the plant extract of *A. marina* were taken into consideration when selecting it for this work. Earlier research involved phytochemical analysis of naturally occurring ethanolic extracts of *Avicennia* spp. Alkaloids, cardiac glycosides, terpenoids, saponins, tannins, flavonoids, and steroids were found to be present in the crude extract during phytochemical screening; however, reducing sugars, carbonyl (aldehyde), and phlobatannins were found to be absent (Makinde *et al*., 2007).

Phenols, flavonoids, quinones, coumarins, tannins and their glycosides, alkaloids, essential oils, and other secondary metabolites are all present in these naturally growing plants; Sofowora (1993) has highlighted the significance of these compounds as microbial agents against the pathogen. The ethanolic extract in the current study was found to contain the highest concentration of the various metabolites found in *A. marina*. Tannins, alkaloids, flavonoids, and phenolic compounds were found in the phytochemical analysis of the extracts of *H. indicum* and *C. procumbens*, according to Boominathan and Ramamurthy (2009). Proline-rich proteins have been shown to form irreversible complexes with tannins.

The presence of flavonoids, for example, indicates that the plant has a variety of biological effects, such as anti-inflammatory, anti-allergic, anti-microbial, antioxidant, and anti-cancer activity (Kunle and Egharevba, 2009). It also suggests that the plant might have diuretic properties (Jayyir *et al*., 2002). The presence of

Table 1: Qualitative Phytochemical screening on extracts of *Avicennia marina*

S. No.	Name of Test	Test applied / Reagent used	Ethanol
1	Alkaloids	A] Mayer's B] Wagner's C] Hagner's D] Dragndorff's test	+++ +++ +++ ++
2	Flavonoids	HCl and magnesium turnings	+++
3	Carbohydrate	Molisch's test	++
4	Tannins and Phenols	A] 10% Lead acetate B] $Fecl_3$	+++ +++
5	Test for Steroids	A] Salkowski's Test B] Libermann-Burchard's Test	++ ++
6	Gums and Mucilages	Alcoholic Precipitation	-
7	Fixed oil and Fats	Spot test	+
8	Saponins	Foam test	++
9	Phytosterols	LB test	+
10	Volatile oils	Hydro distillation method	+
11	Protein & free amino acids.	A] Biuret test B] Ninhydrin test C] Xanthoprotein test	+++ +++ +++

tannins shows that the plant is astringent as documented and suggests that it might have antiviral and anti-bacterial activities and can relief in wound healing and burns (Haslem, 1989). Because some of them are cardioactive and used to treat heart conditions, saponins and glycosides are also very significant classes of secondary metabolites (Oloyode, 2005). Additionally, some researchers have looked into the possibility that certain saponins have immune-modulatory and anti-cancer effects (Evans, 2002). The pharmaceutical and cosmetic industries use volatile oils for a variety of reasons, including as an active ingredient in respiratory tract infections and as a raw material for emollients.

A. marina's anticancer properties were investigated using various mammalian cell lines. The MTT cytotoxicity assay was used to assess the anticancer activity of both the standard and the ethanolic extract of *A. marina*. The ethanolic extract demonstrated a good yielding capacity of phytocompound activity in the preliminary investigation. Table 2 presents the results of the current study of the ethanolic extract of *A. marina* in HepG2, PC-3, and HT-29 cell lines. The standard medication tamoxifen was also used in the study.

The 200 µg/ml concentration of *Avicennia marina* exhibited the most pronounced reduction in cell viability, indicating its significant antiproliferative potential. For the anticancer activity of the ethanolic extract of *A. marina* against HepG2, PC-3, and HT-29 cell lines, the IC_{50} values were 45.50 µg/ml, 58.25 µg/ml, and 65.75 µg/ml. For this investigation, tamoxifen served as a standard. Higher concentrations of the standard resulted in the lowest cell viability (17.5%) and the highest cell inhibition (82.5%). In the various concentrations of HepG2, PC-3, and HT-29 ethanolic extract, which varied from 20 to 200 µg/ml, the percentage of cell inhibition was recorded. The lowest cell inhibition (20.5%) was recorded in the lowest concentration, and the highest cell inhibition (84.5%) was noted in the higher concentration of ethanolic extract of *A. marina*.

Table 2: Survival analysis of cancer cells treated with extracts of *Avicennia marina*

Concentrations (µg ml-1)	PC-3		HT-29		HepG2	
	Cell viability (%)	Cell inhibition (%)	Cell viability (%)	Cell inhibition (%)	Cell viability (%)	Cell inhibition (%)
20	79.5	20.5	74.7	25.3	80.5	19.5
40	67.4	32.6	62.5	37.5	74.9	25.1
60	56.6	43.4	53.7	46.3	63.8	36.2
80	48.7	51.3	45.6	54.4	55.7	44.3
100	37.5	62.5	40.2	59.8	43.5	56.5
125	29.6	70.4	28.5	71.5	31.1	68.9
150	21.1	78.9	22.4	77.6	25.3	72.7
200	18.6	81.4	17.5	82.5	14.2	85.8
Vehicle control (DMSO)	100	0	100	0	100	0

Numerous natural compounds that have been extracted from various plant extracts have been shown to have anticancer properties. Around the world, research is being done to identify a lead compound that can prevent human cancer from developing. Nature has always played a significant role in achieving this objective. Flavonoids, terpenoids, and steroids are examples of plant-derived natural products that have drawn a lot of attention because of their various pharmacological characteristics, which include cytotoxic and chemopreventive effects (Abdullaev, 2001). According to Cragg and Newman (2005), they were the first substances to enter clinical use for the treatment of cancer.

*Withania somnifera*is a potential source of new molecules that can curtail cancer growth were studied by Dredge *et al*. (2003). *A. marina* has also been shown to inhibit the growth of human cancer cell lines comparable to that produced by tamoxifen. The leaves extract produced antiproliferative activity on HepG2, PC-3 and HT-29tumor cell lines. In this study *A. marina*plant extracts shown more than 85% inhibition against human cell lines whereas Jayaprakasam *et al*. (2003) reported that the inhibitory concentrations obtained were 25.1±0.91 against the colon cell line HCT-116. Additionally, this study reported that *A. marina* has growth-inhibitory importance against a variety of cancer cell lines, including HepG2, PC-3, and HT-29 tumor cell lines. Therefore, this study has shown that the leaf extract of *A. marina* has remarkable anticancer potential.

Conclusion

Due to their ability to contain multiple chemical entities, plants are now frequently used in research investigations. Numerous natural substances that have been isolated from various plant extracts have been shown to have anticancer properties. Around the world, research is being done to identify a lead compound that can prevent human cancer from developing. Nature has always played a significant role in achieving this objective. Additionally, this study demonstrated that *Avicennia marina* leaf extract's cytotoxic effects can be tested on cancer patients in clinical trials. According to the current study, *Avicennia marina* exhibited anticancer activity against the PC-3, HT-29, and HepG2 cell lines. The results of this study demonstrated the strong anti-cancer effects of *Avicennia marina* extract in cell lines. The phytocompounds' presence is most likely what causes the anti-cancer action.

Conflict of Interest

The authors declare no conflicts of interest.

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