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Anticancer activity of hydroalcoholic extract of *Enhalus acoroides*

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***Abstract*---**Cancer is a major public health concern in both developed and developing countries. Anticancer activity refers to the ability of natural and synthetic, biological and chemical agents to revert, stop, or halt carcinogenic progression. To treat the ailment, several synthetic pharmaceuticals are utilised, but they have negative effects, thus researchers are investigating into plant-derived chemotherapeutic treatments. *Enhalus acoroides,* a member of the Hydrocharitaceae family, is found along the southeast coast of India and the tropical western Pacific. Hydroalcoholic extract of *Enhalus acoroides* inhibits the proliferation of Liver cancer and Breast cancer. The cell viability was analysed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay, which shows that Hydroalcoholic extract of *Enhalus acoroides* significantly reduced the growth with IC_{50} value for HepG2 as 112.20µg/ml and MCF-7 as 101.60µg/ml. Hence the results of current study conclude that Hydroalcoholic extract of *Enhalus acoroides* is a potent anticancer

drug that can be utilized for the treatment of Hepatocellular cancer and Breast cancer.

Keywords---*Enhalus acoroides,* Liver cancer, Breast cancer, MTT Assay, HepG2, MCF-7.

Introduction

Medicinal plants have found major therapeutic benefits as a result of modern civilization and have become an integral part of human life, and they may be able to alleviate the suffering caused by a variety of illnesses. Pharmaceutical industries are now concentrating their efforts on developing medications based on medicinal plants (Md. Sanower Hossain et al., 2014). Medicinal plants are characterized as folk medicine since they are used in food and medicine (Taiwo Bamigboye J et al., 2017). Cancer is a vast category of diseases that can begin in almost any organ or tissue of the body and spread to other organs when abnormal cells proliferate uncontrollably, invade contiguous regions of the body, and relocate to other organs (Endang Linirin Widiastuti et al., 2019). Chemotherapy, radiation, and surgery are common cancer therapies that have adverse effects; indeed, many patients prefer natural remedies because they consider they are less hazardous (A.O.W. Kaya et al., 2017). Many human ailments can be healed with nutraceuticals and powerful therapeutics found in marine plants. Oceans are habitat to around 80% of the world's diverse plant and animal species. Seagrass contains 57 different species all around the world. *Enhalus acoroides* is a monotypic marine genus in the family Hydrocharitaceae that may be found in the coast of Indian ocean as well as the tropical regions of the Western Pacific. The leaves of *Enhalus acoroides* contain both sterol and fatty acid components. Since it contains active secondary metabolites like as phenolics, flavonoids, and tannins, the crude extract of *E.acoroides* scavenges free radicals, validating its usage in traditional remedies (Amudha P et al., 2018). Hepatocellular carcinoma (HCC) is a type of carcinoma that is the third leading cause of cancer-related death worldwide. Chronic inflammation and fibrosis in the liver are closely attributed to Hepatocellular carcinoma occurrences (R.Ramesh et al., 2019). Breast cancer is the most frequent disease among women, after skin cancer. More than one million women are predicted to develop a bosom tumour each year, with only a small proportion of individuals dying as a result of the cancer. In women aged 55 to 64, breast cancer is the most usually detected malignancy (Nazarali & Narod, 2014).

Materials and Methods

Sample collection

Enhalus acoroides was collected as a whole plant in the shallow coastal regions of Devipattinam, near Ramanathapuram District, Tamil Nadu, India. The plant sample was authenticated in the Plant Anatomy Research Centre by Dr. P. Jeyaraman, Ph.D., Director, Retd Professor, Presidency College. Fresh leaves were cleaned in water and dried in the shade at room temperature. An electric blender was used to pulverise the dry leaves.

Extraction

An electric blender was used to pulverise the dried leaves. The finely crushed powder was transferred to a conical flask and dissolved completely in 60% of Hydro-alcohol. The extract was filtered and dried using a rotatory evaporator and stored in refrigerator at 2-8°C. Standard phytochemical analysis was performed to examine the presence of phytocompounds in the extract. The assay was performed in The Harman Institute of Science Education and Research in Thanjavur.

***In-vitro* anticancer activity**
Cell line and culture

The Human breast cancer cell line (MCF-7) and Hepatocellular carcinoma (HepG2) were provided by the National Centre for Cell Science (NCCS) in Pune, and then grown in Eagles Minimum Essential Medium (EMEM) with 10% foetal bovine serum (FBS). The cells were incubated in Minimal Essential Media (MEM) supplemented with 10% Fetal Bovine Serum (FBS), penicillin (100 U/ml), and streptomycin (100µg/ml) in a humidified environment of 5% CO_2 at 37°C. The maintained cultures were subcultured once a week and the culture medium were renewed twice a week.

MTT assay

The MTT reduction assay [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium] was used to analyze the cytotoxic assay (Mario Ferrari et al., 1990). Single cell suspensions were prepared using trypsin-ethylenediaminetetraacetic acid (EDTA) after the monolayer cells were removed. A hemocytometer was used to count the live cells, and the cell suspension was diluted with medium containing 5% FBS to produce a final density of 1x105 cells/ml. 96-well plates with a plating density of 10,000 cells per well were implanted with one hundred microlitres of cell suspension per well and incubated for cell adhesion at 37° C, 5% CO_2, 95% of air, and 100% relative humidity. Aliquots of 100 µl of various concentrations of extracts (12.5, 25, 50, 100, 200, and 400g/ml) dissolved in DMSO (1%) were added to appropriate wells containing 100 l of medium, resulting in the required final sample concentrations after 48 hours at 37°C, 5% CO2, 95% air, and 100% relative humidity. After 48 hours of incubation, 20 µl of 0.5 percent 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl—tetrazolium bromide (MTT) phosphate-buffered saline solution was added to each well (5 mg/ml) and incubated for 4 hours at 37°C. The MTT metabolic product is then dissolved in 100µl of 0.1 % of DMSO in each well. The plate is shaken for 5 minutes at 150 rpm. The absorbance at 570nm assess which cells were viable. Measurements were conducted, and the concentration required for inhibition (IC50) was graphically determined. Microscopic observations are recorded using an inverted phase contrast tissue culture microscope (Olympus CKX41 with Optika Pro5 CCD camera) after 24 hours of treatment on entire plate. Cell morphological changes, rounding or shrinking of cells, and vacuolization in the cytoplasm are signs of cytotoxicity. The medium without samples was used as a control, and all concentrations were performed in triplicates. The anticancer activity of *Enhalus*

acoroides extract on the HepG2 and MCF-7 cell line were expressed by % of Cytotoxicity using the following formulas:

% Cytotoxicity = 100 - [Abs (sample) /Abs (control)] x100.

% Cell Viability = [Abs (sample) /Abs (control)] x100.

Results and Discussion

In-vitro* anticancer activity of Hydroalcoholic extract of *Enhalus acoroides

Table 1
Effect of different concentration of *Enhalus acoroides* extract on viability and cytotoxicity against HepG2 cell line determined by MTT assay

S.No	Concentration (µg/ml)	Absorbance (O.D)	Cell Viability (%)	Cell growth inhibition (%)
1	12.5	0.724	87.24	12.76
2	25	0.652	78.51	21.49
3	50	0.533	64.17	35.83
4	100	0.386	46.53	53.47
5	200	0.225	27.15	72.84
6	Standard (5µg/ml)	0.206	24.86	75.14
7	Cell control	0.830	100	0
IC_{50} Value				112.20µg/ml

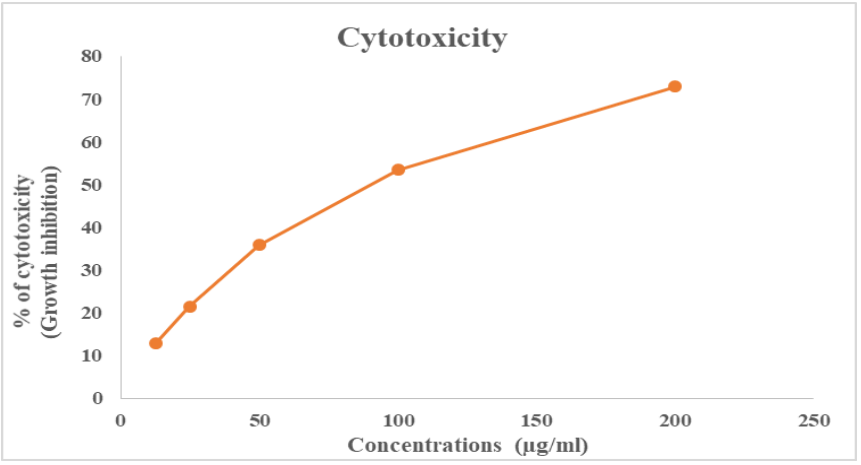


Figure 1: Percentage of cell growth inhibition of *Enhalus acoroides* extract on HepG2 cell line by MTT assay

Table 2
Effect of different concentration of *Enhalus acoroides* extract on viability and cytotoxicity against MCF-7 cell line determined by MTT assay

S.No	Concentration (µg/ml)	Absorbance (O.D)	Cell Viability (%)	Cell growth inhibition (%)
1	12.5	0.798	93.39	6.61
2	25	0.721	84.32	15.68
3	50	0.534	62.51	37.49
4	100	0.281	32.92	67.08
5	200	0.195	22.80	77.20
6	Standard (5µg/ml)	0.215	25.14	74.86
7	Cell Control	0.855	100	0
Half Inhibition Concentration (IC_{50})				101.60µg/ml

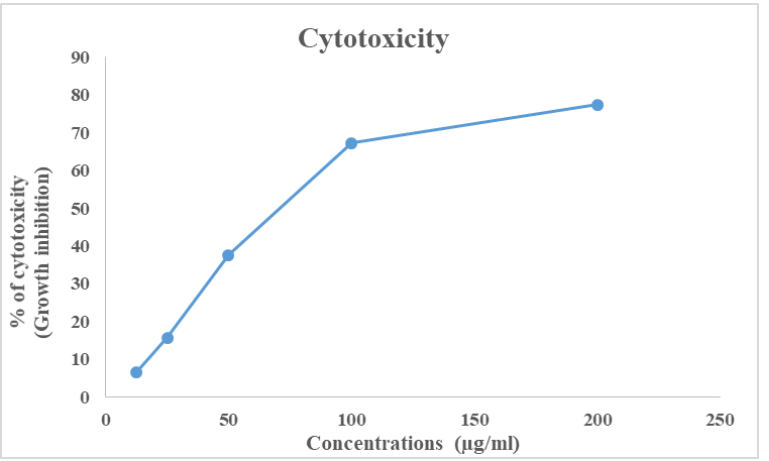
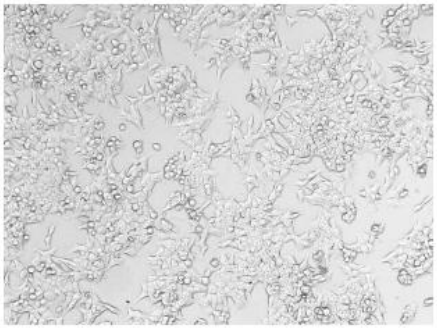


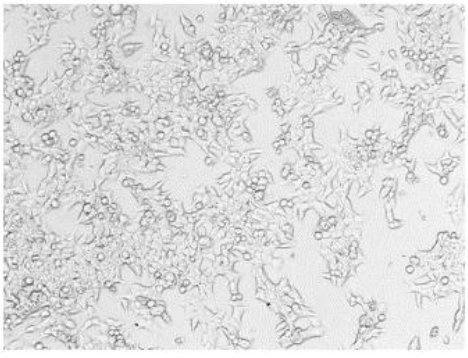
Figure 2: Percentage of cell growth inhibition of *Enhalus acoroides* extract on MCF-7 cell line by MTT assay

Control

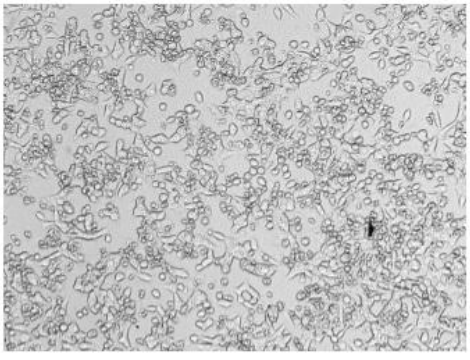


12.5µg/ml

25µg/ml



50 µg/ml



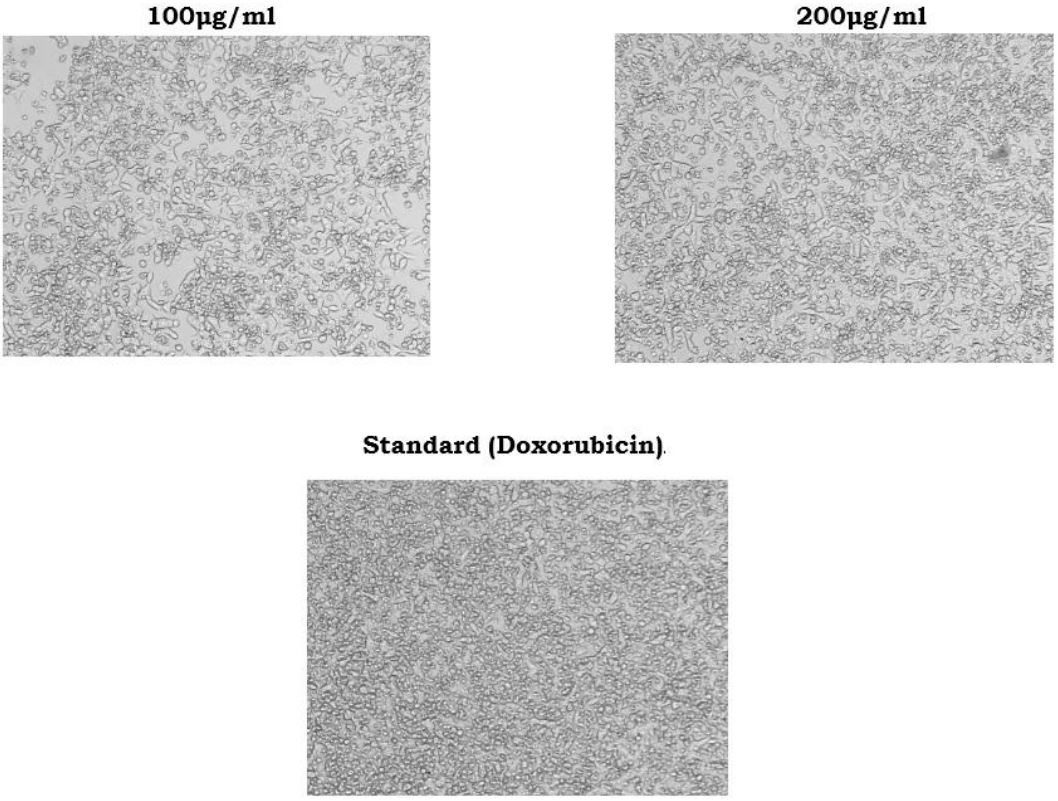


Figure 3: Photomicrograph of HepG2 cell line on different concentrations of *Enhalus acoroides* extract treatment

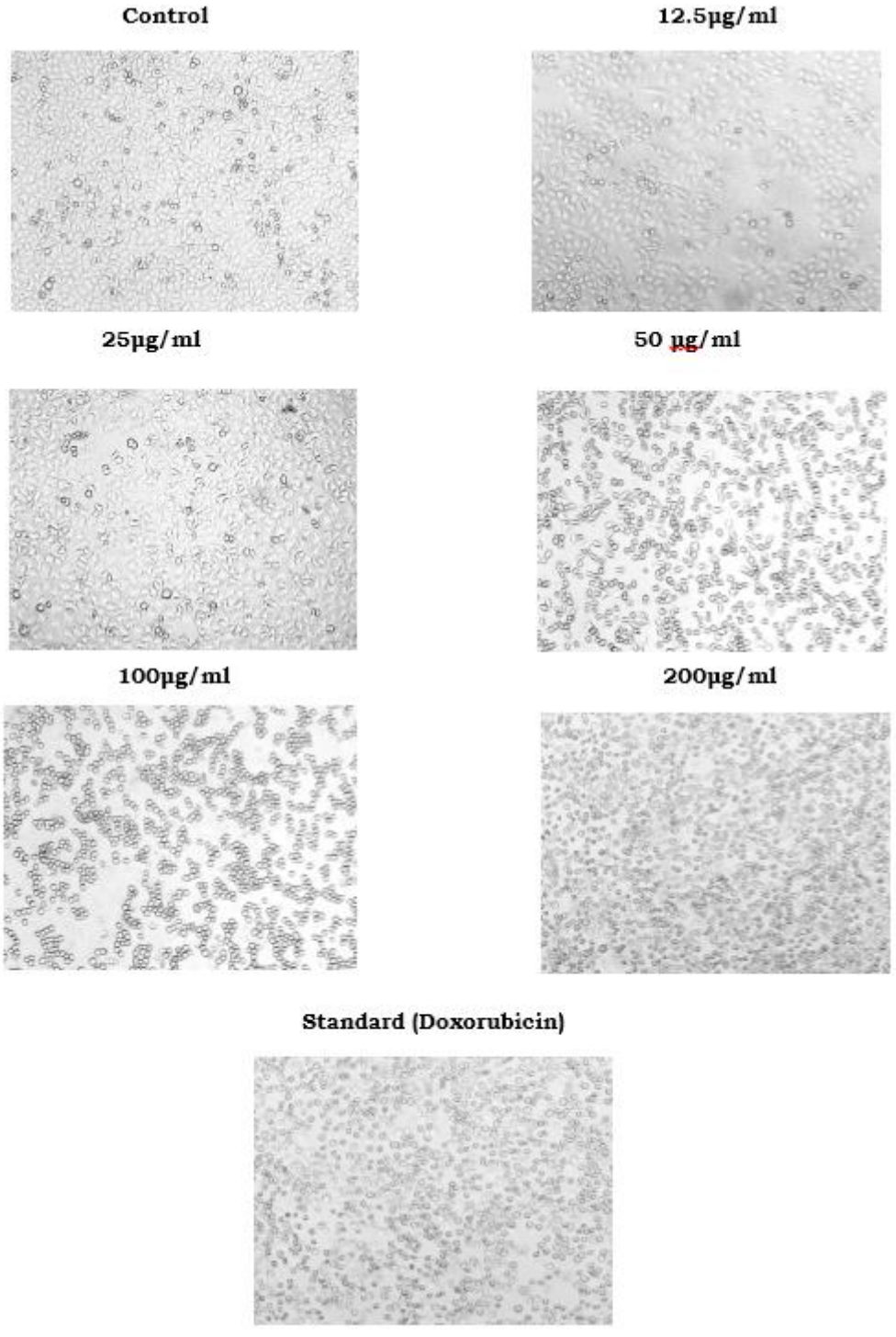


Figure 4: Photomicrograph of MCF-7 cell line on different concentrations of *Enhalus acoroides* extract treatment

Cancer cell lines have been extensively utilized in research and have revealed to be a useful tool in the genetic approach. The characterisation suggests that they are, effective model for studying the molecular systems involved in cancer. The cell model was utilised in the development and testing of current anticancer therapeutics as well as the development of future therapies (Daniela Ferreira., 2013). The cell growth inhibition of the *Enhalus acoroides* extract against HepG2 and MCF-7 cell line at different concentrations (12.5, 25, 50, 100 and 200 µg/ml) and standard as Doxorubicin (5µg/ml). The *Enhalus acoroides* extract was revealed to have promising anticancer potential against cell lines. The MTT assay revealed that increasing the concentration of the plant extract resulted in a considerable increase in cytotoxicity. The method for detecting viable cells uses succinate dehydrogenase to split the tetrazolium ring and convert MTT to an insoluble purple formazan, with the amount of formazan provided equal to the number of viable cells (Lee et al., 2004). The treated cells exhibit characteristics of apoptosis, including detachment from the culture plate, cytoplasmic condensation, cell shrinkage, condensation and accumulation of nuclear chromatin, and loss of contact with neighbouring cells (Monga et al., 2013). The IC_{50} values for *Enhalus acoroides* extract in HepG2 and MCF-7 cell lines were revealed to be 112.20 µg/ml and 101.60 µg/ml. The result of study depends on the concentration of extract, as increase in concentration the cell growth inhibition increases. The maximum growth inhibition was 72.84% at 200 µg/ml and standard (Doxorubicin) was 74.86% at 5µg/ml in HepG2 cell line. The maximum growth inhibition was 77.20% at 200 µg/ml while standard (Doxorubicin) was 74.86% at 5µg/ml in MCF-7 cell line. After treatment with Hydroalcoholic extract of *Enhalus acoroides*, the number of viable cells was considerably reduced at all dosages. The active compounds in methanol extract may have a variety of anticancer actions. The potent bioactive content functions as a signal transduction barrier. Growth factors are used in signal transduction, that begins with stimulation from outside the cell and is captured by the receptor. The proliferative signal will eventually pass to proteins in the cytoplasm by the receptor. Alkaloids also block oxidative processes that can lead to cancer initiation. This method is mediated by a reduction in the peroxidation enzymes Lipooksigenase (LOX) and Xanthine Oxidase Cyclooxygenase (COX), which delays the cell. Tannins inhibit the S phase or synthesis of the cell cycle. The cell will carry out DNA synthesis and chromosomal replication during the S phase. Saponins prevent Bcl-2 from forming. Bcl-2 is an anti-apoptotic protein that promotes cell division (Endang Linrin Widiastuti et al., 2019).

Conclusion

The present study reveals that extract of *Enhalus acoroides* has anticancer property in HepG2 and MCF-7 cell line. Hydroalcoholic extract of *Enhalus acoroides* possess various bioactive compounds responsible for various medical activities. Several anticancer medicines derived from marine species are reported to be effective. The enriched therapeutic phytochemical compounds such as phenols, flavonoids, and terpenoids may be responsible for anticancer activity of HEEA. Based on the dose of the extract after a 24-hour incubation period, the observations strongly suggest that the crude extract of *Enhalus acoroides* possess anticancer potential against Human breast cancer and Liver cancer cells. Further research will be proceeded to isolation and characterization of

compound, molecular mechanism of action of isolated compound and anti-cancer potential against human breast cancer cells and liver cancer cells in *in-vitro.*

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