Anticancer Activity of Whole Plant Extracts of Sesuvium Portulacastrum on Estrogen Receptor Positive Breast Cancer Cell Line

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Abstract: Breast cancer is the most common type of cancer in women worldwide. Present study aimed to investigate the anticancer potential of whole plant extracts of Sesuvium portulacastrum (S. portulacastrum) on estrogen receptor (ER) - positive human breast adenocarcinoma cells (MCF – 7). Different solvent extracts of *S. portulacastrum* were prepared using ethanol, methanol, acetone, hexane and diethyl ether. The anticancer activity of the extracts was evaluated against MCF – 7 by 3-(4,5-Dimethylthiazol-2-Yl)-2,5-Diphenyltetrazolium Bromide (MTT) assay. Propidium iodide staining method was used to observe the apoptosis-associated morphological changes under the microscope. Among all the extracts diethyl ether extract showed the highest activity with IC_{50} value of 521.50 ± 14.2 µg/mL. Further the bioactive compound responsible for the cytotoxic effect should be isolated and purified from the crude Diethyl ether extract.

Keywords* – *Sesuvium portulacastrum, MCF – 7, Plant extract, Anticancer activity, Propidium iodide, Apoptosis

I. INTRODUCTION

Breast cancer initially starts with DNA Damage and is followed by alterations in signaling pathways [1]. It is caused by the development of malignant cells in the breast. The malignant cells originate in the lining of the mammary gland or ducts of the breast (ductal epithelium). Breast cancer is the most common cancer in women, but it can also appear in men. In addition to unhealthy lifestyle, advancing age and family history of breast cancer can increase the women chances of getting cancer. It has high cure rates when detected early and treated according to best practice [2].

The anticancer drugs presently being used are highly toxic and expensive [3]. There is a need to identify the novel drug that is less toxic and less expensive. Historically plants and plant extracts have been used to cure different ailments, Taxol which is commercially being used to treat cancer, is a terpinoid and it comes from the bark of pacific yew tree. The other example of a well-known plant based drug is aspirin that is used to effectively treat aches and fevers. Aspirin contains salicylic acid as an active ingredient; salicylic acid is a phenol and is extracted from the bark of willow tree [4]. Usually the plants that grow in extreme climatic conditions will produce many secondary metabolites to protect themselves from the adverse effects. One such plant that has ability to survive under different abiotic stress conditions like salinity, drought, and heavy metal accumulation is *S. portulacastrum* [5].

S. portulacastrum, a mangrove associate, also known as sea purslane belongs to the Aizoaceae family. It is a fast growing, herbaceous, perennial, dichotomous, edible halophyte which grows naturally in the Mediterranean, subtropical, coastal and warmer areas [6]. It is a native to five continents Asia, Africa, Australia, South America and North America. In India it grows on the coastal areas of eastern and western regions [7]. *S. portulacastrum* is used as a remedy for fever, kidney diseases and is also a best-known antidote for stings of venomous fish [8]. The present study aims to determine the anticancer potential of edible medicinal plant, *S. portulacastrum* against MCF - 7.

II. MATERIALS AND METHODS

Materials

Methanol, acetone, hexane diethyl ether and Dimethyl Sulfoxide (DMSO) were obtained from Fisher Scientific (Pittsburg, USA). RPMI – 1640 medium, trypsin-EDTA, fetal bovine serum, sodium chloride and potassium chloride

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were purchased from Himedia Laboratories (Mumbai, India). Disodium hydrogen phosphate dihydrate ($Na_2HPO_4.2H_2O$), Potassium dihydrogen phosphate (KH_2PO_4) was obtained from Sisco Research Laboratories (SRL, Maharashtra, India). MTT, propidium iodide and streptomycin sulfate were purchased from Sigma Aldrich (St. Louis, USA). Penicillin G and was purchased from PAN – Biotech GmbH (Aidenbach, Germany).

Collection of Plant Material

S. Portulacastrum was collected from the forest area of Tirumala hills, Andhra Pradesh. Plant was washed under tap water and dried in the shade to avoid thermal degradation. Dried plant was then grounded to powder and stored at a cool and dry place in dark airtight containers to avoid oxidation.

Extraction

Solvents, ethanol, methanol, acetone, hexane and diethyl ether were used to extract the crude bioactive compounds from the plant using an orbital shaker. Initially, the plant powder was diluted with above solvents with a solvent to sample ratio of 10:1(w/v) and kept at room temperature for 24 hrs. Then sample solvent mixture was kept in orbital shaker for a period of 48 hrs. During this period the bioactive compounds get extracted into the solvent system. The solvent is then filtered and concentrated under reduced pressure at 40°C in rotaevaporator. The concentrated extracts were dried and stored at room temperature for further analysis.

Cell culture

Human breast cancer cells (MCF -7) were procured from NCCS, Pune. Cells were cultured in tissue culture flasks in RPMI – 1640 medium supplemented with 10% fetal bovine serum, penicillin (100IU/mL), streptomycin sulfate (100g/mL) at 37°C in a humidified incubator with 5% CO_2.

MTT Assay

The anticancer activity of *S. portulacastrum* extracts was determined by MTT assay according to the method described by Kedari et al [9] with slight modification. Initially, the cells were detached and made into a single suspension by treating them with 0.25% Trypsin and 0.02% EDTA in Hank's balanced salt solution. Viable cells were counted using trypan blue exclusion in a haemocytometer and diluted with the medium.

MCF – 7 cells were seeded in 96 well plate at a density of 1 - 2 x 10^4 cells per well and plate was incubated at 37°C in a 5% CO_2 atmosphere. The following day media was removed and the cells were treated with standard, doxorubicin (0.5 – 1.5 µg/mL) and plant extracts (100-500 µg/mL) at different concentrations. Initially the extracts were dissolved in 1:1 ratio of ethanol-ethyl acetate mixture and are further diluted with medium to get the required concentrations. The final mixture used for treating the cells contained not more than 0.5% of the ethanol-ethyl acetate mixture. Negative controls were treated with the same percentage of ethanol-ethyl acetate mixture.

The treated plate was incubated at 37°C in a humidified incubator with 5% CO_2 for 24 hr. After the incubation period, the media was removed, 100 µl of 0.1 mg/mL MTT reagent was added and incubated at 37°C in a humidified incubator with 5% CO_2 for 1 hr or till purple MTT formazan crystals were visible under the microscope. The purple formazan crystals were dissolved by adding 100 µl DMSO and spectrophotometric readings were taken at 540 nm.

The cytotoxic activity of plant extract was calculated as percentage of cell growth inhibition by using following formula

$$\% \text{ Growth inhibition } (\%) = [(A_{Control} - A_{Sample})/A_{Control}] \times 100$$

Where $A_{control}$ indicates the absorbance of control containing 0.5% Ethanol-ethyl acetate mixture. A_{Sample} is the absorbance of treated cells at different concentration. IC_{50} values (the concentration of sample required for inhibition of 50% of cell growth) were obtained from the regression line.

Cellular morphology analysis using phase contrast inverted microscope

Cellular morphological changes were observed under phase contrast inverted microscope. After trypsinization, the cells were seeded in 12 well plate at a cell density of 1 x 10^5 cells per well. Following day, cells were treated with ethanol, methanol, acetone and diethyl ether extracts at their respective IC_{50} concentrations. As hexane extract did not show much activity in the tested concentration, cells were treated with a concentration of 700 µg/mL hexane extract. After 18 hrs, cells were washed with phosphate buffered saline and cell morphology was captured using phase contrast microscope at 100X magnification.

Cellular morphology analysis by propidium iodide staining

Propidium iodide staining was done to observe the morphological changes associated with apoptosis in the cells treated with plant extracts by the method of Rima T et al [10] with few modifications. After trypsinization, the cells were seeded in 12 well plate at a cell density of 1 x 10^5 cells per well. Following day, cells were treated with ethanol, methanol, acetone and diethyl ether extracts at their respective IC_{50} concentrations. In case of hexane extract 700 µg/mL concentration was used. After 18 hrs, cells were washed with cold phosphate buffered saline (PBS) and fixed in absolute ethanol at 4°C for 30 min. After the incubation period, ethanol was removed, PBS was added and the cells were incubated at 37°C in a humidified incubator with 5% CO_2 for 10 min. Later 100 µl of 20 µg/mL Propidium iodide was added and photomicrographs were taken under the florescent microscope at 200X magnification.

III. RESULTS AND DISCUSSION

MTT Assay

MTT is a cell viability assay used to evaluate the cytotoxicity of different drugs. It is a water soluble tetrazolium salt that is converted to a purple formazan by succinate dehydrogenase inside the mitochondria of viable cells. The formazan product formed is impermeable to intact cell membranes of live cells. The intensity of purple color formed is directly proportional to cellular metabolic activity [11].

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Antiancancer ability of *S. portulacastrum* was evaluated by MTT assay. Plant extracts inhibited the proliferation of MCF-7 in a concentration dependent manner as shown in figure 1. Diethyl ether showed the highest anti proliferation activity when compared to all the extracts against MCF-7 with an IC_{50} value of 521.50 ± 14.2 µg/mL. After Diethyl ether, acetone is the next extract that showed the potent activity with an IC_{50} value of 530 ± 19.11 µg/mL. There is no significant difference between anti cancer activity of Diethyl ether and acetone, which is evident from their nearby IC_{50} values. After these two extracts ethanol (IC_{50} – 549.73 ± 14.67) and methanol (IC_{50} – 741.46 ± 42.66) showed the anticancer activity. Hexane extract did not show any anti cancer activity in the tested concentrations. The anticancer activity of the standard, Doxorubicin (IC_{50} – 0.66 ± 0.04) is significantly higher than that of plant extracts. To the best of our knowledge, this is first report on the anticancer activity of *S. portulacastrum* extracts on estrogen receptor - positive human breast adenocarcinoma cell line, MCF-7. The variation in solvent-based cytotoxic activity can be attributed to the extraction of bioactive compounds differently in different solvents based on their polarity. The compound that is active against MCF - 7 could have extracted more into the diethyl ether solvent. On the other hand the yield of diethyl ether extract of *S. portulacastrum* is very low compared to other extracts [12]. This showed that the extraction yield is not the important factor for achieving high anticancer activity as reported in Alzeer et al [13].

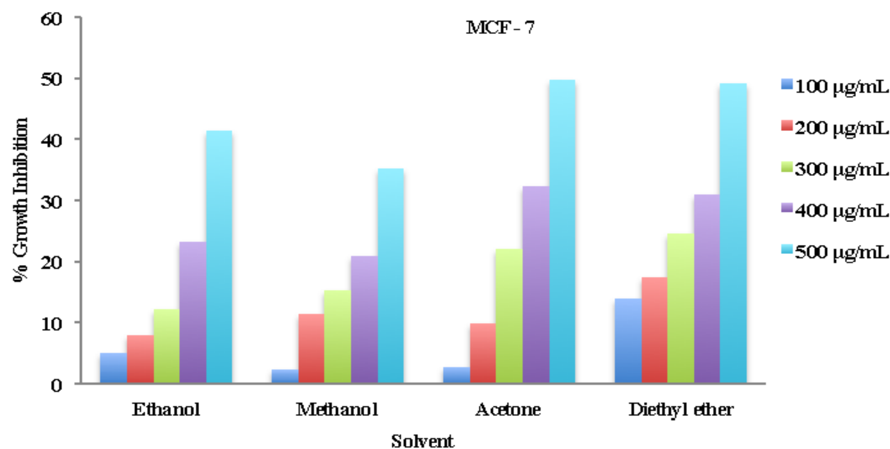

Figure. 1: Cytotoxic effect of *S. portulacastrum* extracts at different concentration in MCF - 7 Cells

Cellular morphology analysis using phase contrast inverted microscope

Phase contrast microscope was used to observe the cellular morphological changes after treatment with plant extracts. Figure 2 shows the morphological changes in MCF - 7 cells after treatment with the plant extracts. Treated Cells lost their normal morphology and exhibited significant morphological changes like reduction in cells volume and shrinkage of cell. Also Increased number of floating or dead cells was observed before PBS washing.

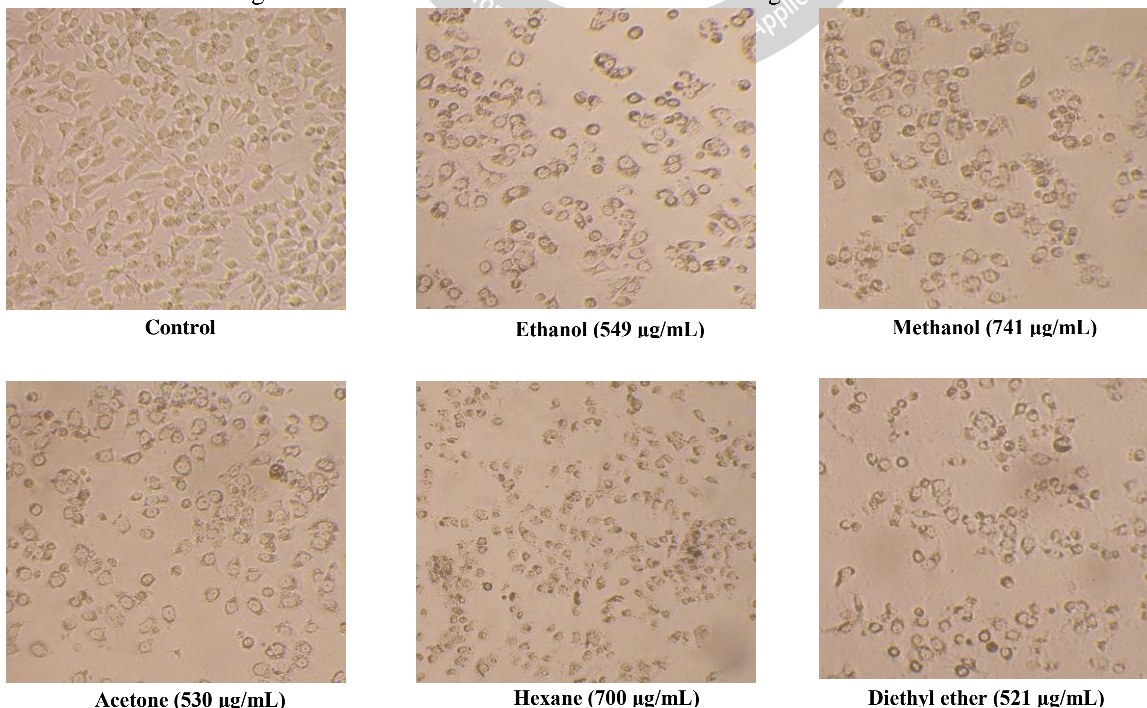

Figure. 2: Morphological changes in MCF - 7 cells exposed to IC_{50} concentrations of S. *portulacastrum* extracts

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Cellular morphology analysis by propidium iodide staining

The extracts of *S. portulacastrum* showed anticancer activity in MCF-7 Cells. To confirm the apoptotic effects of plant extracts, propidium iodide staining method was used to study the morphological changes in the cells under the florescent microscope. When cells are stained with propidium iodide we can observe some of the apoptotic related changes in the cells like plasma membrane blebbing, cell shrinkage, chromatin condensation, DNA fragmentation and formation of small apoptotic bodies [14].

Propidium iodide stained cells showed morphological changes associated with apoptosis after treatment with plant extracts (Figure 3). The arrows in the figure shows the morphological changes like fragmentation into apoptotic bodies, nuclear condensation and shrinkage of the cells that are some of the morphological characteristics of the cells, which are undergoing apoptosis.

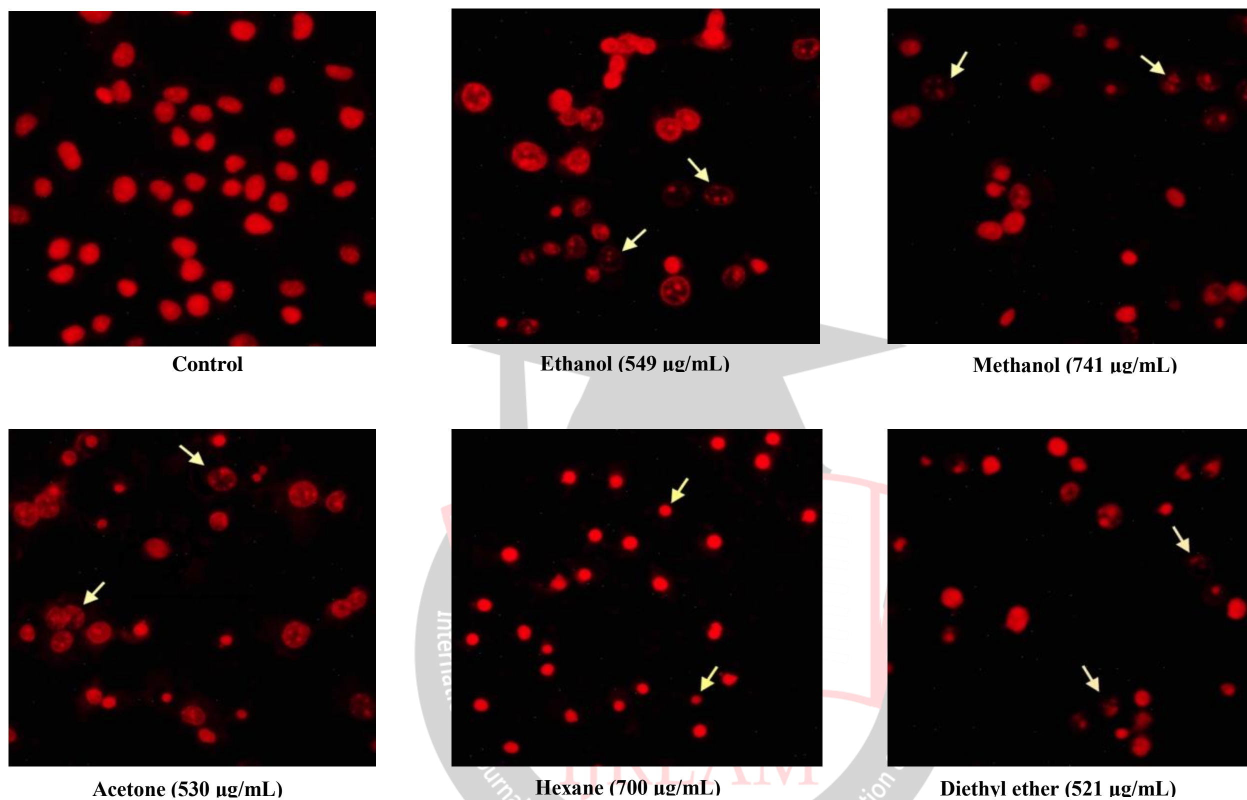

Figure 3: Morphological changes in propidium iodide stained MCF -7 cells exposed to IC$_{50}$ concentrations of *S. portulacastrum* extracts.

IV. CONCLUSION

The present study revealed that the extracts of *S. portulacastrum* showed dose dependent anticancer activity. MCF – 7 cells showed apoptotic related morphological changes on treatment with the extracts. Among all the extracts, diethyl ether extract of S. portulacastrum showed the highest anticancer activity against human breast adenocarcinoma cells, MCF – 7. Further separation of the extract has to be done to isolate, purify and characterize the bioactive compound having anticancer potential.

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