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ANTICANCER POTENTIAL OF *Elephantopus scaber L.* LEAVES AGAINST MCF -7 CELL LINES

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

This study aimed to evaluate the cytotoxicity of *Elephantopus scaber* leaves against breast cancer cell lines. Leaves of the plant were collected, washed, shade dried and powdered. The ethanol extract of the leaf powder was used for the study. The anticancer activity of the ethanol extract of the leaves was evaluated using MTT assay against MCF-7 cells and was confirmed by ETBR-AO staining. The study confirms that the leaf extract of *E.scaber* has pronounced anticancer potential against MCF-7 cell lines. The plant investigated possesses remarkable anticancer activity, where the effect was dose dependent and the IC50 value was found to be 79.56 µg /ml. Since the preliminary data is promising, isolation of the compound may contribute to the development at a novel and natural phytomedicine for the disease. Additional studies are needed to determine its mode of action.

Keywords: *Elephantopus scaber*; MTT assay; anti-cancer; MCF-7; ETBR-AO stain; ethanol extract.

1. INTRODUCTION

According to World Health Organization [1], more than 14 million people were diagnosed with cancer and 8 million died in 2012. Current treatments for cancer include chemotherapy, radiotherapy and chemically derived drugs. Treatments such as chemotherapy can put patients under a lot of strain and further damage their health [2]. Therefore, there is a focus on using alternative treatments and therapies against cancer. Medicinal plants have been used for thousands of years in folk medicines in Asian and African populations and many plants are consumed for their health benefits in developed nations. Recently, there has been an increased interest in the study of materials from plant source as an anticancer compound. Several studies have found the role of medicinal plants in treatment of cancer [3] . Therefore, development and research into naturally derived compounds to be used for anticancer treatment is becoming high in demand with a focus on those derived from plant species and their natural products. Medicinal herbs and their derivative phytocompounds are being increasingly recognized as useful complementary treatments for cancer. Plant-derived drugs are desired for anticancer treatment as they are natural and readily available. They can be readily administered orally as part of patient's dietary intake. Also, being naturally derived compounds from plants they are generally more tolerated and non-toxic to normal human cells [4].

Elephantopus scaber L**.** is a species of flowering plant in the asteraceae family. It is found in Tropical Africa,

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Eastern Asia, Indian Subcontinent, Southeast Asia, and Australia. Research on the medicinal properties of *Elephantopus* species and isolation of various compounds from the plant has been carried out [5]. Such studies have been carried out based on the knowledge of the solubility of specific compounds in specific solvents also known as solvent fractionation. Research on the medicinal properties of *Elephantopus* species and isolation of various compounds from the plant has been carried out [6,7]. Also, the whole plant has been reported to be useful for curing insomnia, diabetes, bronchitis, viral or bacterial infection, leukaemia, rheumatism, snake bite, diuresis, antipyresis, to eliminate bladder stones and for filariasis. The leaves crushed and mixed with salt is used to treat dysentery [8], while the water extract of the leaves is applied externally to treat eczema and ulcers and against bacteria [9,10]. The Murut people of Sabah Malaysia used the roots of the *Elephantopus* species to treat bloody stool [11]. Also, the plant has been reported to possess hypoglycemic activity [12]. In conclusion, *Elephantopus sp* has wide traditional and pharmacological uses in various disease conditions.

2. MATERIALS AND METHODS

2.1 Plant Material

Elephantopus scaber L. plants were collected from the Kerala, South India and were authenticated. and voucher specimen was deposited. The leaves were collected separately and washed. The leaves were dried under shade and mechanically reduced to moderate coarse powder and sieved. The leaves chosen for the study had been washed, macerated and lyophilised. About 500g of the leaves yielded 33g powder. The procedure was repeated to collect the needed quantity. 100g of the powdered leaves were extracted in Soxhlet apparatus separately using 1 L of ethanol for 18h and then filtered. The filtrates were evaporated to dryness under reduced pressure and at a lower temperature in a rotary evaporator. The dried residues were stored in airtight containers for further use.

2.2 *In vitro* – MTT Assay

E. scaber leaf acetone extract was tested for *in vitro* cytotoxicity, using MCF-7 cells by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Briefly, the cultured MCF-7 cells were harvested by trypsinization, pooled in a 15 ml tube. Then, the cells were plated at a density of 1×10^5 cells/ml cells/well (200 µL) into 96-well tissue culture plate in DMEM medium containing 10 % FBS and 1% antibiotic solution for 24-48 hour at 37°C. The wells were washed with sterile PBS and treated with various concentrations of the leaf extract in a serum free DMEM medium. Each sample was replicated three times and the cells were incubated at 37°C in a humidified 5% CO2 incubator for 24 h. After the incubation period, MTT (20 µL of 5 mg/ml) was added into each well and the cells incubated for another 2-4 h until purple precipitates were clearly visible under an inverted microscope. Finally, the medium together with MTT (220 µL) were aspirated off the wells and washed with 1X PBS (200 µl). Furthermore, to dissolve formazan crystals, DMSO (100 µL) was added and the plate was shaken for 5 min. The absorbance for each well was measured at 570 nm using a micro plate reader (Thermo Fisher Scientific, USA) and the percentage cell viability and IC50 value was calculated using GraphPad Prism 6.0 software (USA).

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3. RESULTS AND DISCUSSION

3.1 Anticancer activity-MTT Assay

The MTT (3-4, 5 dimethylthiazol-2yl-2, 5-diphenyl tetrazolium bromide) cell viability assay is widely used in determining drug sensitivity in primary screening of potential chemotherapeutic drugs. Mitochondrial dehydrogenase enzyme of viable cells to cleave the tertrazolium rings of the pale yellow MTT and form a dark blue colored formazan crystals which is largely impermeable to cell membranes, thus resulting in its accumulation within healthy cells.

The anticancer activity of leaf extract was carried out against MCF-7 cell line at different concentrations to determine the IC50 (50% growth inhibition) by MTT assay. MTT assay of leaf extract showed significant effect on MCF-7 cell line at a concentration range of 70 µg/ml to 87 µg/ml compared with control. Leaf extract exerts high cytotoxicity in 200 µg/ml concentration against MCF-7 cell line with 76.69 percent of cell growth inhibition. The IC_{50} value of a leaf extract on MCF-7 was 79.56 µg /ml. The apoptotic activity was found to be dose dependent. Lupeol, an active compound isolated from *Elephantopus scaber* leaves has been observed to inhibit the proliferation of gastric tumor cells in a dosedependent manner [13].

Ethanol extract of the plant and subsequent fractions reportedly also inhibited the growth of HCT116 human colorectal carcinoma cells and HT-29 cells and induced apoptosis [14]. Lupeol is another compound that has been isolated from the plant. The effect of lupeol has been studied on MCF-7 breast cancer cells. Lupeol induced an effective change in the cell viability of MCF-7 cells with IC50 concentration as

80 µM/ml [15], which is quite less effective than our extract. Normal tumor cells, early and late apoptotic cells, and necrotic cells were examined using fluorescent microscopy. Early-stage apoptotic cells were marked by a crescent-shaped or granular yellow-green acridine orange nuclear staining. Late-stage apoptotic cells were marked with concentrated and asymmetrically localized orange nuclear ethidium bromide staining. Necrotic cells increased in volume and showed uneven, orange-red fluorescence at their periphery. Other additional studies are under progress to determine the mode of action.

Table 1. OD values of plant extract treated MCF 7 cells

S. No	Tested sample concentration (µg/ml)	OD Value at 570 nm (in triplicates)		
1.	Control	0.428	0.460	0.433
2.	200 µg/ml	0.119	0.108	0.100
3.	180 µg/ml	0.259	0.287	0.273
4.	160 µg/ml	0.255	0.232	0.282
5.	140 µg/ml	0.302	0.319	0.297
6.	120 µg/ml	0.321	0.336	0.286
7.	100 µg/ml	0.353	0.347	0.366
8.	80 µg/ml	0.390	0.334	0.399
9.	60 µg/ml	0.386	0.392	0.404
10.	40 µg/ml	0.411	0.417	0.400
11.	20 µg/ml	0.412	0.471	0.437

Table 2. Cell viability of plant extract treated MCF 7 cells

S. No	Tested sample concentration (µg/ml)	Cell viability (%) (in triplicates)			Mean Value (%)
1.	Control	100	100	100	100
2.	200 µg/ml	27.02	24.52	22.71	24.75
3.	180 µg/ml	58.82	65.18	62.00	62.00
4.	160 µg/ml	57.91	52.69	64.04	58.21
5.	140 µg/ml	68.58	72.45	67.45	69.49
6.	120 µg/ml	72.90	76.31	64.95	71.38
7.	100 µg/ml	80.17	78.80	85.05	81.34
8.	80 µg/ml	88.57	75.85	90.62	85.01
9.	60 µg/ml	87.66	89.03	91.75	89.48
10.	40 µg/ml	93.34	94.70	90.84	92.96
11.	20 µg/ml	93.57	106.97	99.25	99.93

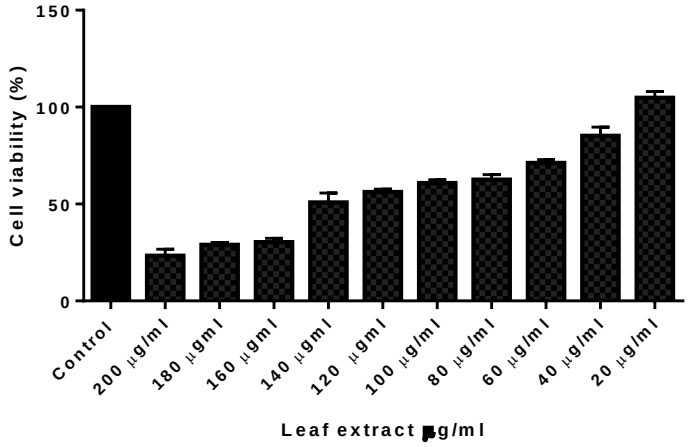

Fig. 1. Effect of varying concentrations of plant extract at 24hrs on MCF-7 cells

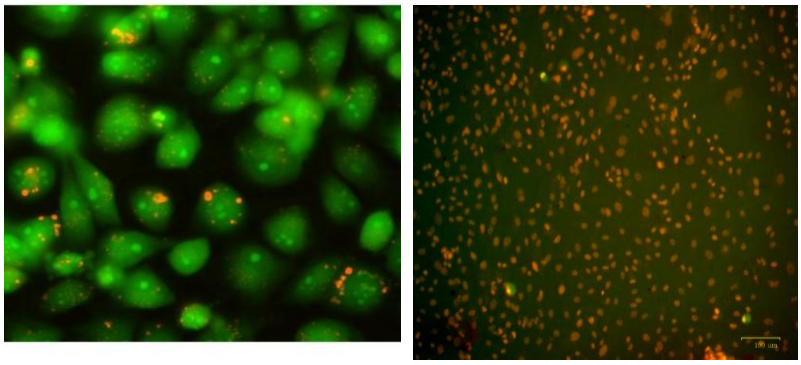

Fig. 2. Effect of *E.scaber* leaf extract (79.56 µg/ml) on MCF-7 cells

4. CONCLUSION

The results are promising and suggests the anticancer property of *Elephantopus scaber* leaves and further studies are needed to establish its action.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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