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RESEARCH ARTICLE

In vitro Anti-Cancer Study of *Vitis viniferae, Ixora coccinea* and *Piper longum* Extract on Human Breast Carcinoma Cells

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ABSTRACT:

Herbal constituents have got some attention because of their chemopreventive action and cost effectiveness to reduce cancer. One of the leading causes of death in women worldwide is breast cancer. The aim of present work is to find out the invitro cytotoxic effect of the constituents of polyherbal extract on MCF-7 human breast cancer cells. Here, we investigated the anti-cancer effects of 3 drug extracts-*vitis viniferae*-Grape seed (GSE), *Ixora coccinea* flower and *Piper longum* root extract against breast cancer treatment. cells was cultured in DMEM supplemented with 10% inactivated Fetal Bovine Serum (FBS), 1% penicillin in an humidified atmosphere of 5% CO2 at 37 ºC. The IC_{50} of a drug can be determined by constructing a dose-response curve.The percentage inhibition of *vitis viniferae* (10–320 µg/ml),*Ixora coccinea* (10–320 µg/ml) and *Piper longum* (10–320 µg/ml) treatment of cells resulted in 10–90%, 6-53% and 14-90% growth inhibition respectively.Cell proliferation decreased in a concentration and time-dependent manner. In conclusion, results showed that 3 drug extracts exhibits cytotoxic activity to MCF-7 cells and it could be a potential anticancer drug. Further studies are needed to confirm the mechanism of action of drug extracts.

KEYWORDS: MCF-7 cells, phenolic compounds, flavonoids, alkaloids, *vitis viniferae*, *Ixora coccinea* and *Piper longum*.

INTRODUCTION:

Breast cancer is one of the leading death cause in women in comparison with other cancer.Cancer is characterized by local tissue invasion and uncontrolled growth of body cells[(1)]. in terms of morbidity and mortality,breast cancer is globally ranked 2 amongst all cancer[(2)]. Mammary cancers exhibit heterogeneity not only with respect to hormones- oestrogen, progesterone and receptors -human epidermal growth factor-2 (HER-2) expression but also with tumour size, grade and nodal status,hence different treatment is needed[(3)].

Thus, breast cancer is a diverse phenotype mixture, for which different treatment needed and there is no cure for breast cancer at the present moment[(4)], thus new drug search having lesser side effects such as herbal remedies is needed to treat and to control the disease. In earlier report all 3 plant (*vitis viniferae*-Grape seed (GSE), *Ixora coccinea* flower and *Piper longum* root)extract showed anticancer activity[(5,6,7)] as because of phenolic components, flavonoids and alkaloid respective active constituents. These.Some reports also proved effective in suppressing 62.5% tumour formation in not only in MCF-7 cells but also in LNCaP and PC-3 prostate cancer cell lines by increasing the death of cultured[(8)]. LNCaP androgen-dependent human prostate carcinoma cells[[(9)]. Zhao et al.[(10)] is used further to confirm Cytotoxic activity. The aim of the present work was to find out the plant extracts invitro cytotoxic activity on MCF-7 cells.

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MATERIAL AND METHODS:

Dried seeds of *vitis viniferae*, flowers of *Ixora coccinea* and roots of *piper longum* were collected in the month of may from Kerala. Morphological and microscopical characters of the plant was first identified with description given in the different literature review[11,12,13]. Later plants were authenticated by Prof. M.D. Rajanna at Botonical garden, University of agricultural sciences, GKVK, Bangalore, Karnataka. All the plant parts were shade dried and reduced to powder separately and stored at room temperature in airtight containers. All 3 plant parts were extracted by ethanol separately.

Method[14,15]: MTT Powder [3-{4,5-dimethylthiazol-2-yl}-2,5-diphenyltetrazolium bromide], CO2 incubator, 70% Ethanol, DMEM(Dulbecco's Modified Eagle's Medium), Microplate reader (Tecan). Cell lines and culture medium. Stock cells was cultured in DMEM supplemented with 10% inactivated Fetal Bovine Serum (FBS), 1% penicillin (100 IU/ml), and streptomycin (100 µg/ml) in an humidified atmosphere of 5% CO2 at 37 ºC. The cell was dissociated with TPVG (Trypsin Phosphate Versene Glucose)solution (0.2 % trypsin, 0.02 % EDTA, 0.05 % glucose in PBS). The viability of the cells are checked using trypan blue(dye) and centrifuged. Further, 50,000 cells / well of L929 (mouse fibroblast cell line: Adherent cells) was seeded in a 96 well plate and incubated for 24 hrs at 37ºC, 5% CO2 incubator.

Cell line: Human breast adenocarcinoma MCF 7 cell line was procured from ATCC (American Type Culture Collection).

Cell proliferation by MTT assay[16,17]: Test samples were placed in each well of the 96 well microtiter culture plate. The L929 monolayer cells were trypsinized and the cell count was adjusted to 5.0 x 105 cells/ml using DMEM containing 10% FBS.

To each well of the 96 well microtiter plate, 100 µl of the diluted cell suspension (50,000 cells/well) was seeded on each scaffold and cells seeded on cell culture plate. The plates were then incubated at 37ºC for 1 day in 5% CO2 atmosphere. After 24 h, the test solutions in the wells were discarded and 100 µl of MTT (5 mg/10 ml of MTT in PBS) was added to each well. The plates were gently shaken and incubated for 4 h at 37o C in 5% CO2 atmosphere. The mitochondrial dehydrogenase enzymes of viable cells cleave the tetrazolium ring to an insoluble purple formazan. The supernatant was removed and 100 µl of DMSO was added and the plates were gently shaken to solubilize the intracellular formed formazan and the absorbance was measured using a microplate reader at a wavelength of 590 nm. percentage inhibition was calculated using formula,Percent inhibition =(OD of control-OD of sample)×100.

RESULTS:

Cell proliferation by MTT assay: Cytotoxic activity of all 3 extracts on human (MCF-7) breast carcinoma cells using MTT was evaluated. IC50 values can be calculated for a given antagonist by determining the concentration needed to inhibit half of the maximum biological response of the agonist. Figure 1 shows that *Piper longum* having highest percentage inhibition (90.73%) than *Vitis viniferae* (89.76%) and *Ixora coccinea* (52.81%)at the dose of 120.5µg/ml,139.6µg/ml and 110µg/ml, respectively.

Table No:1:In-vitro cell line studies on MCF-7 cells showing percentage inhibition and IC50 values of ethanolic extracts of *Piper longum*,*Ixora coccinea* and *Vitis viniferae*.

	Conc. µg/mi	OD at 590nm	% inhibition	
Ethanolic extract of piper longum	20	0.71	14.03	**120.5µg/ml**
	40	060	26.59	
	80	0.44	46.86	
	160	0.18	78.42	
	320	0.08	9.73	
Ethanolic extract of Ixora coccinea	10	0.77	5.99	**110µg/ml**
	20	0.73	10.86	
	40	0.68	17.57	
	80	0.62	23.91	
	160	0.47	42.45	
	320	0.39	52.81	
Ethanolic extract of vitis vinifera	10	0.77	6.23	**139.6µg/ml**
	20	0.74	10.38	
	40	0.63	23.42	
	80	0.45	45.01	
	160	0.23	71.59	
	320	0.08	89.76	

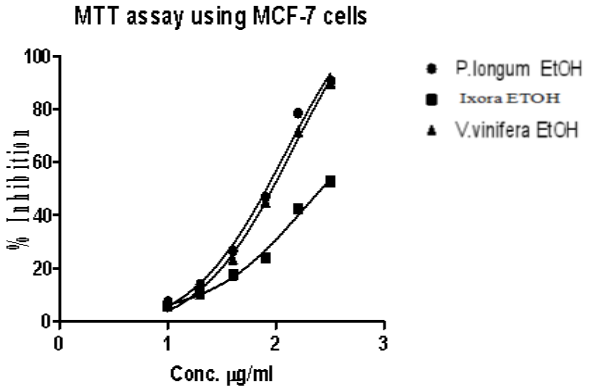

Figure 1: Dose response curve to calculate IC50 value of ethanolic extracts of *Piper longum*,*Ixora coccinea* and *vitis viniferae* on MCF-7 Cell lines.

DISCUSSION:

Different types of herbal drugs used as therapeutic agent in different parts of world.Derived drugs from herb may have therapeutic improtance in illness[18,19].The herbal extracts of *vitis viniferae*,flowers of *Ixora coccinea* and

roots of *piper longum* were reported to have potential anticancer activity. Cytotoxic activity on MCF-7 cells were screened with the above 3 extracts and results found to reduce viability of MCF-7 cells. In literature review above all 3 extracts reported to possess cytotoxic activity and normal cells are protected from cytotoxic activity.Anitcarcinogenic effcects of *vitis viniferae*,flowers of *Ixora coccinea* and roots of *piper longum* extracts are because of presence of phenolic compond,flavnoids and alkaloids respectively.

CONCLUSION:

Significant effect on cancer cells (in vitro) observed by herbal extracts by selecting increasing cytotoxicity without causing toxicities,all 3 extracts could render appropriate candidate for cancer therapy. Further more studies with specific methods needed to find out the constituents responsible for the activity.

CONFLICT OF INTEREST:

The authors confirm that this article has no conflict of interest

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