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Cytotoxicity determination of leaves extract of *Anisomeles malabarica* (L.) R.Br. ex sims against breast cancer cell line (MCF 7) using MTT assay

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Abstract

Anisomeles malabarica is a perennial herbaceous shrub. To test cell viability, breast cancer cells (MCF 7) were treated with different doses of plant leaves extract and known standard compounds including cis -vaccenic acid and linoleic acid. The cells were analyzed after 24 hours treatment and notable morphological changes were observed. Treated cells were showing drastic morphological changes such as rounding up of cells, connection and contact were lost with adjacent cells and observed to be easily detachable from the dish. It is a remarkable evident that it can be used as alternate source of medicine in the breast cancer treatment.

Keywords: MTT assay, MCF 7, cis-vaccenic acid, linoleic acid, breast cancer, IC_{50} value

Introduction

Anisomeles malabarica, more commonly known as the Malabar catmint (USDA, 2015), is a species of herbaceous shrub in the family Lamiaceae. *Anisomeles malabarica* is a perennial herbaceous shrub that ranges from 0.9 to 2.0m in height (Aluri *et al*., (1998). The thick, petiolate leaves are a narrow oval shape, tapering to a point at each end; with a width of 1.5–3 cm and a length of 3-8 cm (Ling, S.K. (2001) [11], Annapoorani, S. (2019) [3]. The base of the leaves are narrowly cuneate or attenuate (Bean, Anthony R. (2015).They are pale above, white below, crenated and woolly, with pinnate venation (Singh *et al*., 2003) [12]. The leaves are also lobed, with a prominent gland less than 1 mm deep at the peak of each lobe, and 14–29 lobes on each side of the leaf. The transition from leaves to bracts is abrupt, and the petioles are 9-13 mm long.

A. malabarica is used for medicine, fragrances, and cosmetics (Aluri *et al*., (1998), Ling, S.K. (2001) [3]. It has been used for centuries as a medicinal herb in Indian and Sri Lankan folk medicine, with all components, the leaves and roots in particular, being used to treat a range of conditions including congenital mental disabilities, fevers arising from teething, and swelling (Khare, C. (2007) [10]. The decoction of the leaf and essential oil are also used externally in rheumatic arthritis. There is evidence to support most of these applications, in addition to being effective for epilepsy, intestinal worms, halitosis, and gout (Annapoorani, (2019) [3]. It has also been shown to have anti-cancer, anti-inflammatory, antiallergic, anti-anaphylactic, and anti-bacterial properties (Lavanya *et al*., (2010) [8].

Plants are valuable sources of a vast array of chemical compounds and accumulates in various parts of the body (Girija S and Ravindran., 2011) [7]. In the fields of biology, medicine and pharmacy natural products have provided a rich source of compounds which have various importance. The antimutagenic and anticarcinogenic activities are found in a wide group of plants. Plants have played a crucial role as sources of efficient anticancer agents, and it is clearly noticeable that over 60% of anticancer agents that are used at the present time are obtained by some means or the other from natural sources, which includes marine organisms, plants and microorganisms. (Kinghorn AD, *et al.*, 2009). The n-hexane and chloroform extracts of *Anisomeles malabarica* were shown to be cytotoxic to cervical cancer cell lines. They inhibited proliferation and induced apoptosis of cervical cancer cells. MTT assay, Acrylamide Orange, Ethidium Bromide, Hoechst 33258 staining, Annexin V-Cy3, JC 1 and Comet assay were used (Preethy *et al.*, 2013) [9]. Anisomelic acid is a major component of *A. malabarica*, a cembrane-type diterpenoid which can be synthesised chemically. Anisomelic acid isolated from *A. malabarica* and *A. indica* has been shown to be cytotoxic to KB cells (Arisawa *et al.*, 1986) [4]. In a 2013 study, Anisomelic acid extracted from *A. malabarica* extracted using LC was shown to be cytotoxic and apoptosis inducing in ER-positive and negative breast cancer and HPV-positive cervical cancer cell lines. MTT, AO, EB staining, comet assays were used to test the cytotoxicity (Preethy *et al.*, 2013) [9].

Methodology

Healthy samples of *Anisomeles malabarica* (*L*.) was collected from the surroundings of Kavalkinaru, Tirunelveli District. Leaves were separated from the plant and shade dried. Dried leaves were pulverized into fine powder filtered through a mesh. The extraction was made using distilled water.

Cell culture

MCF 7 cell lines were procured from the cell repository of National Centre for Cell Sciences (NCCS), Pune, India.

Dulbecco`s Modified Eagle Media (DMEM) was used for maintaining the cell line, which was supplemented with 10% Fetal Bovine Serum (FBS). Penicillin (100U/ml), and Streptomycin (100µg/ml) were added to the medium to prevent bacterial contamination. The medium with cell lines was maintained in a humidified environment with 5% CO_2 at 37°C.

Cytotoxicity determination

The cytotoxicity of selected plant materials and standard compounds on MCF-7 cells were determined by the method of Mosmann, (1983). The yellow 3-4, 5 Dimethylthiozol-2-Yl)-2, 5 Diphenyltetrazolium bromide (MTT) is reduced by mitochondrial dehydrogenase of viable cells yielding a measurable purple formation product. Viable cells contain NAD (P) H-dependent reductase, which reduce the MTT reagent to Formazon, with a deep purple colour. Formazon crystals are then dissolved using solubilizing solution and absorbance is measured at 500-600nm by plate reader.

Stock solution

MTT (50mg) dye was dissolved in 10mL of PBS. After Vortexing for 1 minute, it was filtered through 0.45 micro filters. The bottle was wrapped with aluminium foil to prevent light, as MTT was light sensitive. The preparation was stored at 4°C.

Procedure involved

Cell viability assay, MCF-7 viable cells were harvested and counted using hemocyto meter diluted in DMEM medium to a density of 1×10^4 cells/ml was seeded in 96 well plates for each well and incubated for 24 hours to allow attachment. After MCF-7 cells treated with control and the containing different concentrations of both plant extract (25-300µg/ml) and standard compounds (5 - 50µg/ml) were applied to each well. MCF-7 cells were incubated at 37°C in a humidified 95% air and 5% CO_2 incubator for 24 hours.

After incubation, the drug-containing cells wash with fresh culture medium and the MTT (5mg/ml in PBS) dye was added to each well, followed by an incubation for another 4 hours at 37°C. The purple precipitated formazon formed was dissolved in 100 µl of concentrated DMSO and the cell viability was absorbance and measured 540 nm using a multi-well plate reader. The results were expressed at the percentage of stable cells with respect to the control. The Half Maximal Inhibitory Concentration (IC_{50}) values were calculated and the optimum doses were analysed at different time period.

$$\text{Inhibitory of cell proliferation (\%)} = \frac{\text{Mean absorbence of the control} - \text{Mean absorbence of the sample}}{\text{Mean absorbence of the control}} \text{ X } 10$$

The IC_{50} values were determined from both the plant extract and saponin dose Responsive curve where inhibition of 50% cytotoxicity compared to vehicle control cells.

Results

MTT comparision-control-*Anisomeles malabarica*-Cis-vaccenic acid-linoleic acid compounds inhibit the survival of breast cancer

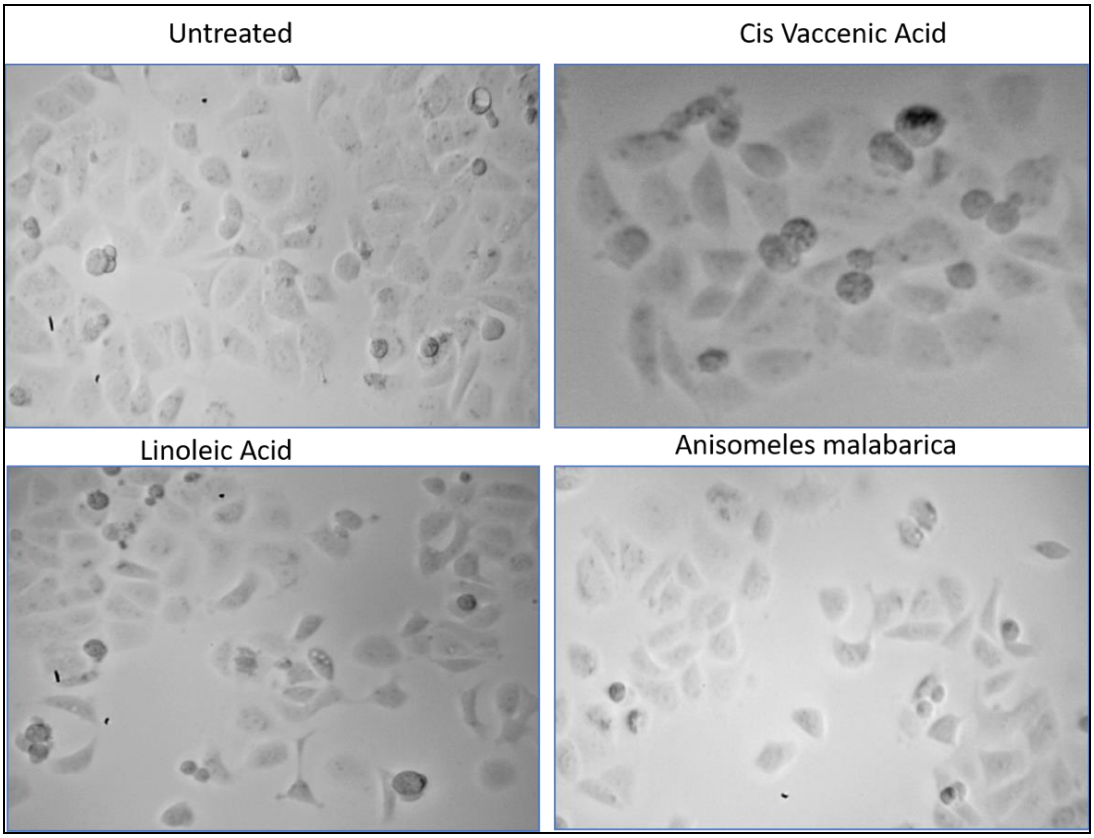

Fig 1

MTT Inhibition % of cis-vaccenic acid, linoleic acid and *Anisomeles malabarica* leaves

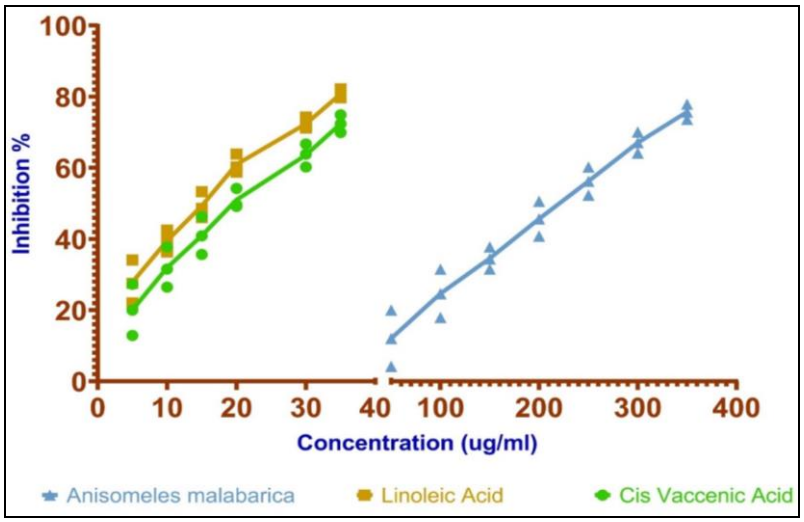

Fig 2

Table 1

Cis vaccenic acid				Linoleic acid				*Anisomeles malabarica*		
Control	0.9	0	0	0	0.09	-0.08	Control	0.02	0	-0.01
5 µg	12.9	27.28	20.01	22.08	34.12	27.53	50 µg	4.19	20.01	12.01
10 µg	26.49	37.79	31.57	39.69	42.53	36.35	100 µg	17.94	31.5	24.64
15 µg	35.67	46.29	40.92	46.06	53.3	48.63	150 µg	34.44	37.76	31.53
20 µg	49.17	54.23	49.65	58.76	63.9	60.29	200 µg	40.81	50.59	45.65
30 µg	66.73	63.87	60.26	71.2	74.28	71.71	250 µg	52.35	60.21	56.24
35 µg	69.94	74.9	72.39	79.7	82.22	80.44	300 µg	64.14	70.06	67.07
							350 µg	73.63	77.89	75.68

Discussion

Most chemotherapeutic agents have some side effects. Our aim was to determine the cytotoxicity of the aqueous extract of *Anisomeles malabarica* using MTT assay. The leaves of *Macaranga barteri* displayed strong inhibition of lipid peroxidation in linoleic acid system and moderate reducing properties (Adesegun *et al*., (2007) [2]. Anticancer activity of C.intybus commonly known as chicory has been recognized against some cancer cell lines such as breast cancer MCF-7 (Abu-Dahab and Afifi, 2007) [1], MTT assay showed *in vitro* cytotoxicity of E. camaldulensis extract against human breast and colon cancer cell lines (Hrubik and Jovin, 2012, Singab *et al*., 2011) [13]. er, MTT assay showed *In vitro* cytotoxicity of E. camaldulensis extract against human breast and colon cancer cell lines (Hrubik and Jovin, 2012, Singab *et al*., 2011) [13].

Conclusion

A.malabarica leaves extract treated cells were showed cytoplasmic condensation, shrinkage, tendency to float in the medium, and reduction in size compared to the mock cells. $IC_{50\ value}$ revealed that plant leaves extract and standard test compounds inhibits the viability of breast cancer cell lines in a dose dependent manner. IC_{50} was calculated and results showing doses of 219 ug/ml for MCF 7 worked efficiently.

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