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Anticancer evaluation of *Moringa oleifera* leaf extracts against pc3 cell lines

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Abstract
Moringa *oleifera* is a promising anticancer plants especially its leaf extracts having anticancer activities. In our study, we evaluate the anticancer activity of Moringa *oleifera* leaf extracts against PC3 cell lines. The percentage of cell inhibition of methanolic extract was found to be high against PC3 Cancer cell lines.

Keywords: moringa *oleifera*, pc3, cancer cell lines

Introduction

There has been extensive interest in exploring the anticancer activities of various parts of the M. *oleifera* tree, and many published research articles describe promising results of *in vitro* and *in vivo* testing of various extracts from the Moringa plant. All parts of the M. *oleifera* tree have been tested for anticancer activity, including the leaves, seeds, bark, and roots. However, the most extensive research on the anticancer activities of M. *oleifera* has focused on the leaf extracts. The Moringa tree is an evergreen that grows new leaves year-round, with a projected production of six tons per hectare per year [1]. The leaves are rich in polyphenols and poly flavonoids, which are antioxidants and potential anticancer compounds [2]. Many researchers start by exploring the antioxidant activity and anti-inflammatory activity of the leaf extracts as a preliminary screening for anticancer activity.

One of the factors that cause cancer is oxidative stress which is an imbalance in the production of free radicals and oxidants and their elimination by antioxidants [3]. Antioxidants can disrupt the formation of free radicals and reduce oxidative stress, which ultimately prevents cancer

The next step often involves testing the effects of the leaf extracts *in vitro* on cancer cell lines by examining the extract's impact on growth and proliferation of cancerous cells and on cell morphology. If the leaf extracts show promising anticancer activity for a specific cancer cell line, the researchers usually proceed by identifying the specific pathways disrupted by the extract through molecular analysis. With sufficient evidence, some researchers will continue exploring the anticancer activity of the extract *in vivo*, usually in mouse or rat models, to observe the actions of the leaf extract in a living mammal, which is a more accurate representation of the human body. The research showing the anticancer activities of moringa leaf extracts *in vivo* and *in vitro* and the pathways/mechanisms of action through which these effects may occur.

Materials and Methods

The *Moringa oleifera* leaf extracts from methanol, ethanol and acetone were used for testing the anticancer activity. The anticancer activity was performed against four different types of cell lines namely PC3 (Human Prostrate Cancer Cells).

Anticancer Activity of Moringa *oleifera* Leaf Extracts

Cell lines

PC3 (human Prostrate Cancer Cells) was obtained from National Centre for Cell Science (NCCS), Pune and grown in Eagles Minimum Essential Medium containing 10% fetal bovine serum (FBS). The cells were maintained at 370ºC, 5% CO_2, 95% air and 100% relative humidity. Maintained cell cultures were passaged weekly, and the culture medium was changed twice a week.

Cell treatment procedure

The monolayer cells were detached with trypsin ethylene diamine tetraacetic acid (EDTA) to make single-cell suspensions. Viable cells were counted by trypan blue exclusion using a haemocyto meter and diluted with medium containing 5% FBS to give the final density of 1x105 cells/ml. One hundred microliters per well of cell suspension were seeded into 96-well plates at a plating density of 10,000 cells/well and incubated to allow for cell attachment at 37°C, 5% CO_2, 95% air and 100% relative humidity. After 24 h the cells were treated with serial concentrations of the test samples. They were initially dissolved in neat dimethyl sulfoxide (DMSO) and an aliquot of the sample solution was diluted to twice the desired final maximum test concentration with serum-free medium. Additional four serial dilutions were made to provide a total of five sample concentrations. Aliquots of 100 µl of these different sample dilutions were added to the appropriate wells already containing 100 µl of the medium, resulting in the required final sample concentrations. Following sample addition, the plates were incubated for an additional 48 h at 37°C, 5% CO_2, 95% air and 100% relative humidity. The medium containing without samples were served as control and triplicate were maintained for all concentrations.

MTT assay

MTT assay was performed based on the procedure described by (Mosmann, 1983). 3- [4, 5-dimethylthiazol-2-yl] 2, 5-diphenyltetrazolium bromide (MTT) is a yellow water-soluble tetrazolium salt. A mitochondrial enzyme in living cells, succinate- dehydrogenase, cleaves the

tetrazolium ring, converting the MTT to an insoluble purple formazan. Therefore, the amount of formazan produced is directly proportional to the number of viable cells. After 48 h of incubation, 15µl of MTT (5mg/ml) in phosphate-buffered saline (PBS) was added to each well and incubated at 370ºC for 4h. The medium with MTT was then discarded and the formed formazan crystals were solubilized in 100µl of DMSO and then measured the absorbance at 570 nm using a microplate reader. The % cell inhibition was determined using the following formula.

% Cell Inhibition = 100- Abs (sample)/Abs (control) x100.

Nonlinear regression graph was plotted between % Cell inhibition and Log concentration and IC50 was determined using Graph Pad Prism software 8.

Results

Average percentage cell inhibition of Methanolic leaf extract of Moringa *oleifera* leaf against PC3 cells showed linear relationship, where the increased concentration of the extract increases the percentage of cell inhibition with an R2 value of about 0.985 (Figure.1). Ethanolic leaf extract of Moringa *oleifera* leaf against PC3 cells showed a linear relationship, where the increased concentration of the extract increases the percentage of cell inhibition with an R2 value of about 0.975 (Figure. 2). Acetone leaf extract of Moringa *oleifera* leaf against PC3 cells showed a linear relationship, where the increased concentration of the extract increases the percentage of cell inhibition with an R2 value of about 0.976 (Figure. 2). The 50% cell inhibition for the Moringa *oleifera* leaf extracts at different concentrations for the methanol, ethanol and acetone against PC3 cells was determined. The cell inhibition was higher in the acetone leaf extract followed with the ethanol and methanol leaf extracts (Figure. 3). The percentage of cell inhibition at different concentrations like 50, 100, 150, 200, 250 and 300 µg/ml against PC3 cell line was calculated. At all concentrations, the percentage of cell inhibition was higher in the Methanolic extract with the range of about 13.30 ± 0.56, 23.54 ± 1.19, 32.52 ± 0.68, 52.11 ± 0.66, 68.21 ± 0.23 and 77.97 ± 0.61 with the p-value of about <0.0007 at 50 µg/ml and same p-value of about <0.0001 for the other concentrations and different f values like 30.551, 54.199, 114.13, 167.66, 481.78 and 66.879. The cell inhibition was very low in the acetone extracts in all the concentrations with the range of about 10.56 ± 0.40, 16.18 ± 0.71, 25.17 ± 0.44, 41.44 ± 1.02, 52.50 ± 0.57 and 69.37 ± 1.05 respectively (Table. 1). The inhibitory concentration (IC_{50}) for the leaf extract of Moringa *oleifera* against PC3 cells in different solvents showed higher inhibitory concentration rate at acetone extract with the IC_{50} of about 233.46 ± 2.89 whereas it was very low in the Methanolic leaf extract with IC_{50} of about 195.42 ± 1.86 and it was found to be 207.41 ± 3.05 in the ethanolic leaf extract with the p-value of about < 0.0001 and f value of about 161.22 (Table 2). The significance rate of percentage of cell inhibition at different concentrations for the different solvent extracts of *Moringa oleifera* leaf against PC3 cells was determined. At the concentration of 50 µg/ml, it was highly significant with methanol and acetone whereas non-significant in ethanol with acetone. 100, 150 and 200 µg/ml showed highly significant with all the solvents like methanol with ethanol and acetone, ethanol with acetone.

Table 1: Percentage cell inhibition of different solvent leaf extract of M. *oleifera* (µg/ml) against PC3 cells

Concentrations	Methanol	Ethanol	Acetone	*p* value	*f* value
50	13.30 ± 0.56	11.60 ± 0.30	10.56 ± 0.40	<0.0007	30.551
100	23.54 ± 1.19	18.65 ± 0.64	16.18 ± 0.71	<0.0001	54.199
150	32.52 ± 0.68	28.13 ± 0.65	25.17 ± 0.44	<0.0001	114.13
200	52.11 ± 0.66	48.86 ± 0.36	41.44 ± 1.02	<0.0001	167.66
250	68.21 ± 0.23	65.21 ± 0.96	52.50 ± 0.57	<0.0001	481.78
300	77.97 ± 0.61	73.29 ± 1.01	69.37 ± 1.05	<0.0001	66.879
p value	<0.0001	<0.0001	<0.0001		
f value	379.71	671.53	569.88		

Table 2: Fifty percent inhibition (IC_{50}) concentration of M. *oleifera* crude extract with different solvents for PC3 cells

Solvents	IC_{50} (µg/ml)
Methanol	195.42 ± 1.86
Ethanol	207.41 ± 3.05
Acetone	233.46 ± 2.89
p value	< 0.0001
f value	161.22

Table 3: Comparative significance percentage cell inhibition of Different solvent leaf extract of M. *oleifera* (µg/ml) against PC3 cells

Solvents	Ethanol	Acetone	Ethanol	Acetone	Ethanol	Acetone
	50		100		150	
Methanol	**	***	**	***	***	***
Ethanol		ns		*		**
Acetone						
	200		250		300	
Methanol	**	***	**	***	**	***
Ethanol		***		***		**
Acetone						

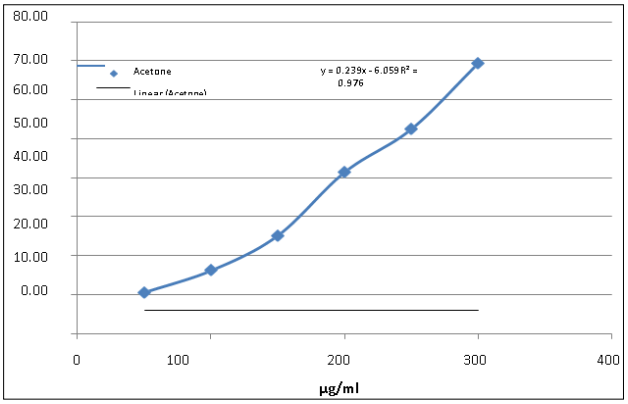

Fig 1: Average percentage cell inhibition of acetone leaf extract of M. *oleifera* against PC3 cells

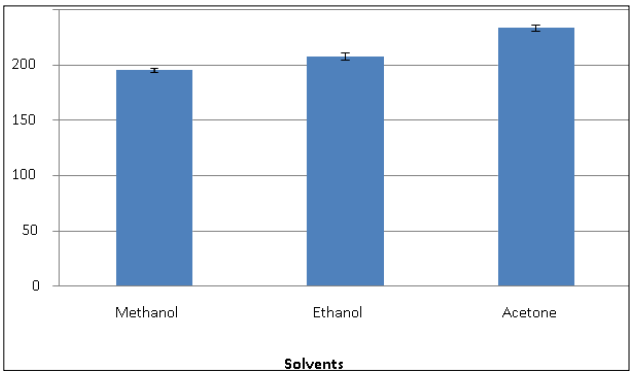

Fig 2: Fifty percent inhibition (IC_{50}) concentration of M. *oliefera* crude extract with different solvents for PC3 Cells

Discussion

Moringa *oleifera* was established for its prospective medicinal values, more researches have to be carried out to study its antioxidant and anticancer activities. Hence, the methanol, ethanol and acetone extracts of Moringa *oleifera* leaf was evaluated for its antioxidant and cytotoxicity activities.

From the earlier research, it was clear that the free radicals were found to be the key factor for the cause of DNA mutation, as it triggers the gene responsible for cancer (Johnson, 2007). These free radicals can be controlled by the exogenous and endogenous substances obtained from the natural sources (Johnson, 2004). Many researchers reported that the Moringa *oleifera* leaf possesses the antioxidant property due to the occurrence of surplus secondary metabolites such as Flavonoids and polyphenols (Luqman, *et al*., 2012; Santos, *et al*., 2012). As reported earlier, the Methanolic leaf extract of Moringa *oleifera* possesses the antioxidant property.

The presence of many organosulphur compounds in the Moringa *oleifera* leaves was highly responsible for the prevention of the cancer cells (Aja, *et al*., 2014) [4]. Hence as per the earlier report, the present investigations revealed that the cell inhibition increases by increased concentrations of the Moringa *oleifera* leaf extract with different solvents

The *in vivo* study of the methanolic and ethanolic extracts of Moringa *oleifera* leaf delays the cancer cell proliferation in various cancer cells and increases the survival rate of the cancer patients (Purwal, *et al*., 2010). Hence the present investigation also states that the percentage of cell inhibition of methanolic extract was found to be high against the PC3 cell lines. Recent researches suggested that the compounds like niazimicin and the isothiocyanate present in the ethanolic extract of the Moringa *oleifera* leaf possessed to contain the antioxidant and anticancer activities against many cancer cell lines like breast, lung and colorectal cancers (Hermawan, *et al*., 2012) [3, 6]. Another report suggested that the antioxidant activity was found to be higher in the methanolic extract when compared with the other extract used in the previous study (Abdulaziz, *et al*., 2015) [1].

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Conflict of Interest

Authors shows no conflict of interest

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