The Pharma Innovation Journal 2019; 8(3): 38-41

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2019; 8(3): 38-41

www.thepharmajournal.com
Received: 19-01-2019
Accepted: 23-02-2019

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In-vitro anticancer activity of few plant extracts against MCF-7, MDA-MB468 and MDA-MB231 cell lines

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Abstract
In-vitro anticancer activity of four angiosperm plants *Crotalaria longipes*, *Petiveria alliacea*, *Caralluma umbellata*, *Niebuhria apetala* and a pteridophyte plant *Marsilea quadrifolia* against MCF-7, MDA-MB468 and MDA-MB231 cell lines was studied. During the present investigation methanol extracts of selected plants were prepared using soxhlet apparatus. Preliminary phytochemical studies revealed the presence of alkaloid, flavonoid, saponin, steroid, phenol, tannin, glycoside and terpenoid. In-vitro anticancer activity of selected plants extracts at various concentrations (50-250 mg/ml) was studied against the chosen cell line using MTT assay. The methanol extract of *Crotalaria longipes* (aerial part) exhibited highest percentage of inhibition against MCF-7 cell line whereas methanol extract of *Niebuhria apetala* (whole plant) showed highest percentage of inhibition against MDA-MB468 cell line. Similarly the methanol extract of *Caralluma umbellata* (whole plant) exhibited highest percentage of inhibition against MDA-MB231 cell line with efficient IC50 values.

Keywords: Cytotoxicity, MTT assay, methanol, cell lines

1. Introduction
Cancer is the leading cause of mortality worldwide. As per the cancer reports published by the World Health Organization (WHO) and the World Cancer Research Fund the occurrence of cancer is still increasing especially due to diet, environment and carcinogenic virus diseases [1, 2]. More than 30% of cancer is caused by modifiable behavioural and environmental threat factors, including tobacco and alcohol use, dietary factors, inefficient regular consumption of fruit and vegetables, overweight, obesity, physical inactivity, chronic infections, environmental and occupational risks including exposure to ionizing and non-ionizing radiation [3]. In hospitals conservative drugs are commonly given to cancer patients. Nevertheless, due to less toxic and adverse effects of phytochemicals the research on medicinal plants and cancer has been deepened [4].
Cell lines are extensively used in many aspects of laboratory research and mostly as *in-vitro* models in cancer research. They have a number of advantages; for example they are easy to handle and represent an unlimited self replicating source that can be grown in almost infinite quantities. In addition, they exhibit a relatively high degree of homogeneity and are easily replaced from frozen stocks if lost through contamination [5]. Studies with cell lines can reuse as an initial screen for agents that might regulate drug resistance. To establish more appropriate models of drug resistance and explore the differences that exist in the different drug resistant sublines selected by different treatments [6].
The aim of the present study was to examine the *in-vitro* cytotoxicity studies using three cell lines MCF-7, MDA-MB468 and MDA-MB231 in different parts of four angiosperm plants and one pteriodophyte plant namely *Crotalaria longipes* (aerial part), *Petiveria alliacea* (whole plant), *Caralluma umbellata* (whole plant), *Niebuhria apetala* (leaf) and *Marsilea quadrifolia* (leaf) respectively. Further there have no detailed *in-vitro* studies on anticancer properties of above said plants. Hence the current study is aimed on the evaluation of anticancer activity of five selected plants.

2. Material and Methods
The whole plant of *Petiveria alliacea* (PA),) *Niebuhria apetala* (NA) were collected from Petchiparai, the whole plant of *Caralluma umbellata* (CA) was collected from Parvathipuram, Nagercoil and the leaf of *Marsilea quadrifolia* (MQ) was collected from Puthalam, Kanyakumari District. The aerial part of *Crotalaria longipes* (CL) was collected from Kothagiri, Nilgiri Biosphere Reserve, Western Ghats, Tamil Nadu.

The collected plants were washed thoroughly in tap water in the laboratory to remove dust and shade dried in a well ventilated place at room temperature. The dried plants were ground to a coarse powder and subjected to solvent extraction.

2.1 Solvent extraction

Methanol was used as solvent to prepare the plant extracts. The different plant materials were direct soaked for 12 hrs in 500 ml methanol and then subjected to extraction by reflexing for 6 to 8 hrs below the boiling point of the solvent. The extracts were subjected to qualitative test for the identification of various phytochemical constituents as per the standard procedures [7, 8, 9]. The methanol extracts were concentrated by evaporating at a reduced pressure using rotary evaporator. The concentrated extracts were further dried at 37^0C for 3 to 4 days in order to facilitate complete evaporation of the solvents.

2.2 Cell lines and culture medium

The human breast cancer cell line (MCF-7, MDA-MB 468 and MDA-MB-231) was obtained from National Center for Cell Sciences (NCCS), Pune and grown in Eagles minimum essential medium (EMEM) containing 10% Fetal Bovine Serum (FBS). All cells were maintained at 37^0 C, 6.5% CO_2, 95% air and 100% relative humidity. Maintenance cultures were passage weekly, and the culture medium was changed twice a week.

2.3 MTT assay

The cells were preincubated at a concentration of 1×10^6 cells/ml in culture medium for 3 hrs at 37 °C and 6.5 % CO_2. Then, the cells were seeded at a concentration of 5×10^4 cells/well in 100 µl culture medium and at various concentrations (50-250 µg/ml) of plant extract and standard drug Doxorubicin (dissolved in 2 % DMSO (dimethylsulphoxide) solution into microplates (tissue culture grade, 96 wells, flat bottom) and incubated for 24 hrs at 37 °C and 6.5 % CO_2. The cell proliferation is based on the ability of the mitochondrial succinate-terazolium reductase system to convert 3-(4, 5- dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to a blue colored formazan. The test denotes the survival cells after toxic exposure. Then, 10 µl MTT mixture was added and incubated for 4 hrs at 37 °C and 6.5 % CO_2. Each experiment was done in triplicates. Then 100 µl of solubilization solution was added into each well and incubated for overnight. The spectrophotometric absorbance of the samples was measured using a microplate (ELISA) reader. The wavelength to measure absorbance of the formazan product in between 550 and 600 nm according to the filters available for the ELISA reader was used. The reference wavelength should be more than 650 nm.

2.4 IC_{50}

The concentration of compound required to inhibit 50 % cell growth, was determined by plotting a graph of Log (concentration of compound) vs % cell inhibition. A line drawn from 50 % value on the Y axis meets the curve and interpolate to the X axis. The X axis value gives the Log (concentration of compound). The antilog of that value gives the IC_{50}, value. Percentage inhibition of unknown extract against the two cell lines was calculated using the following formula:

$$\% \text{ cell survival} = \frac{(At - Ab)}{(Ac - Ab)} \text{ x } 100$$

Where,
At = Absorbance of Test,
Ab= Absorbance of Blank (Media),
Ac= Absorbance of control (cells)
% cell inhibition = 100 − % cell survival

3. Results and Discussion

The preliminary phytochemical screening of the methanol extracts of all the plants revealed the presence of alkaloid, flavonoid, saponin, steroid, phenol, tannin, glycoside and terpenoid (Table 1). The effect of methanol extract of *Petiveria alliacea* (PA), *Niebuhria apetala* (NA), *Caralluma umbellata* (CA), *Crotalaria longipes* (CL) and *Marsilea quadrifolia* (MQ) and doxorubicin (standard) on the growth of MCF-7, MDA-MB468 and MDA-MB231 cell lines were examined by the MTT assay. Dose dependent response were obtained between the range 50-250 µg/ml for plant extracts and doxorubicin (standard-control), expenses decreasing number of viable cells with increasing concentration of plant extracts as well as doxorubicin. Calculation of IC_{50} value was done using Graph pad Prison Software (ver.5.01). The susceptibility of cells to the different plant extracts and doxorubicin was characterized by IC_{50} and R2 values (Tables 2 - 4). An IC_{50} value of MCF cell line was 34.93, 32.41, 38.12, 40.51, 39.93, 38.06 µg/ml and R2 value was 0.7936, 0.7684, 0.8134, 0.8292, 0.8188 and 0.8112 for NA, PA, CU, CL, MQ and doxorubicin respectively. The IC_{50} value of MDA-MB 468 cell line was 39.65, 32.16, 39.13, 35.19, 34.26, 38.54 µg/ml and R2 values was 0.8122, 0.7084, 0.8096, 0.7912, 0.7854 and 0.8067 for NA, PA, CU, CL, MQ and doxorubicin respectively while the IC_{50} value of MDA-MB 231 cell line was 33.28, 31.98, 39.63, 39.12, 32.63, 39.21 µg/ml and R2 value was 0.7893, 0.7767, 0.8186, 0.8096, 0.7816 and 0.9186 for NA, PA,CU, CL, MQ and doxorubicin respectively.

MTT cytotoxicity assay used to measure the cytotoxic effect of NA, PA, CU, CL and MQ plant on three breast carcinoma (MCF-7, MDA-MB 468 and MDA-MB231) cells. In the preliminary screening result, the methanol extracts exhibited broad spectrum cytotoxic activity and it had most active cytotoxic activity on all the tested cell lines. The MTT cytotoxic activity was analysed by using various cell lines. In the present study methanol extract of NA, PA, CU, CL and MQ revealed a significant growth inhibition in all the cell lines at lower concentration of IC50 values. The IC_{50} values of extract on cell line less than 100µg/ml is categorized as a potential cytotoxic substance [10]. NA, PA, CU, CL and MQ extract treatment on cancer cell lines showed significant decrease in growth rate compared with control. On the other hand the percentage of non-viable cell lines increased with the increasing concentration of extracts. These results were in concordance with the studies investigated on the cytotoxic effect of *Goniothalamin* towards human breast cancer cells by Al-Qubaisi *et al*. [11].

In conclusion the ethanol extract of NA, PA, CU, CL and MQ were found to be cytotoxic towards all the tested cell lines in MTT assay and the strong cytotoxic activity on all the breast cancer cell lines may be through DNA fragmentation which is related to the induction of apoptosis. The movement may be due to the company of one or more phytochemical

constituents present in the extracts. Supplementary studies warranted, for isolation of the constituents accountable for the activity and also to discover the exact mechanism of achievement of the activity. Hence there is a trust in the pharmaceutical industry, that even more commanding commercial drugs can be developed sooner, using plant derivatives, to effectively cure cancer and save mankind.

Table 1: Phytochemical screening of methanol extracts of NA, PA, CU, CL and MQ

Phytochemicals	NA	PA	CU	CL	MQ
Alkaloid	+	+	+	+	+
Anthraquinone	-	+	-	+	-
Catecin	-	-	+	-	-
Coumarins	-	+	-	-	-
Flavonoid	+	+	+	+	+
Quinine	-	+	-	+	-
Phenol	+	+	+	+	+
Saponin	+	+	+	+	+
Steroid	+	+	+	+	+
Tannins	+	+	+	+	+
Terpenoid	+	+	+	+	+
Sugar	+	+	+	+	+
Glycoside	+	+	+	+	+
Xanthoprotein	+	-	+	-	-
Fixed oil	+	+	-	+	+

+ indicates the Presence - indicates the absence
NA: *Niebulria apetala*
PA: *Petiveria alliacea*
CU: *Caralluma umbellata*
CL: *Crotalaria longipes*
MQ: *Marsilea quadrifolia*

Table 2: *In vitro* Cytotoxic activity of different plant extract (NA, PA, CU, CL and MQ) on MCF-7 cell lines

S. No	Concentration (µg / ml)	% inhibition on MCF -7 cell lines					
		NA	PA	CU	CL	MQ	STD (DOX)
1	50	21.53	18.94	20.96	22.92	21.98	23.86
2	100	39.51	37.56	38.54	40.56	39.66	38.54
3	150	53.93	50.33	54.88	59.33	58.54	51.63
4	200	64.33	62.98	67.56	69.54	68.50	63.92
5	250	75.41	70.36	82.16	86.34	84.63	81.66
IC_{50}	-	34.93	32.41	38.12	40.51	39.93	38.06
R_2	-	0.7936	0.7684	0.8134	0.8292	0.8188	0.8112

Table 3: *In vitro* Cytotoxic activity of different plant extract (NA,PA,CU,CL and MQ) on MDA-MB 468 cell lines

S. No	Concentration (µg / ml)	% inhibition on MDA-MB 468 cell lines					
		NA	PA	CU	CL	MQ	STD (DOX)
1	50	22.96	16.84	21.94	20.63	19.56	23.84
2	100	41.54	29.56	40.88	37.65	34.81	40.16
3	150	57.66	41.28	58.94	55.23	53.28	54.84
4	200	68.36	56.93	67.31	67.89	65.76	69.63
5	250	83.92	69.34	82.22	79.56	78.22	80.26
IC_{50}	-	39.65	32.16	39.13	35.19	34.26	38.54
R_2	-	0.8122	0.7084	0.8096	0.7912	0.7854	0.8067

Table 4: *In vitro* cytotoxic activity of different plant extract (NA, PA, CU, CL and MQ) on MDA-MB-231 cell line

S. No	Concentration (µg / ml)	% inhibition on MDA-MB-231 cell lines					
		NA	PA	CU	CL	MQ	STD (DOX)
1	50	19.86	18.22	22.84	21.63	18.93	23.63
2	100	33.27	30.67	41.56	39.54	31.56	41.92
3	150	51.92	40.92	60.22	58.11	40.54	62.66
4	200	63.27	59.67	68.92	67.54	58.67	69.54
5	250	79.92	71.26	84.23	81.31	73.94	83.96
IC_{50}	-	33.28	31.98	39.63	39.12	32.63	39.21
R_2	-	0.7893	0.7767	0.8186	0.8096	0.7816	0.9186

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