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HPLC analysis and *in vitro* cytotoxic potential of different extracts of *Ixora parviflora* Lam. against human breast adenocarcinoma cell lines

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Abstract

Natural products utilization in prevention or treatment of cancer is at interest to man as they hold variety of potent phytoconstituents. *Ixora parviflora* Lam. is being used traditionally by most of the tribal people to treat various health problems. Hexane, ethyl acetate and methanol extracts of leaves of the plant were used for this study. HPLC analysis, *in vitro* antimitotic activity by seed germination assay and *Allium cepa* L. root tip assay were carry out. *In vitro* cytotoxic activity of extracts was performed by MTT assay against MCF-7 and MDA-MB-231 cell lines. HPLC analysis showed many peaks with different retention times. Antimitotic activity by seed germination assay showed significant decrease in seed weight after 24 h, 48 h and 72 h with different concentrations of extracts treatment. *A. cepa* root tip assay showed significant decreased in mitotic index 58.6 ± 0.6, 56.6 ± 0.8 and 54.3 ± 0.5 (p<0.01) at 12 h, 24 h and 48 h, respectively. The methanolic extract was more cytotoxic against breast cancer cell lines with 192.3 ± 1.0 IC_{50} µg/ml on MCF-7 and 189 ± 1.1 on MDA-MB-231 compared to other extracts.

1. Introduction

Although, there is a tremendous development in sector of synthetic drugs, there are some or other side effects, whereas plants hold their unique place by having no side effects (Hoareau and Dasilva,1999). In the treatment of complex cases such as cancer, components of the plants proved to be very effective. It has been proven that plants characterize by their ability to prevent some diseases (Yudharaj *et al.*, 2016).

Plants have set a base for different medicine systems providing remedies from past (Arpita Roy *et al.*, 2017). Inception of major anticancer agents from natural source earns more research for upcoming drugs to treat cancer (Sumner, 2000). The side effects and expensive treatment of allopathy has made the focal point of researches on herbals. The increasing side effects and expensive treatment has made the focus of researches on herbal medicines.

Ixora has about 500 species, consists of tropical evergreen shrubs, three of which were cultivated in Egypt are: *Ixora coccinea, Ixora finlaysoniana and Ixora undulata* (Hortus, 1976). The Ayurvedic system of medicine includes *Ixora* to treat variety of ailments (Usha *et al.*, 2016). Ethnobotanical uses of *Ixora* include leaves for antimicrobial, diarrhoea and anti-inflammatory, whooping cough and anaemia (Thakur and Harsha Kumar, 2014), flowers used in catarrhal bronchitis, cytotoxic, dysentery and antitumor, roots in scores, hiccough, fever, ulcers and skin diseases (Sunitha *et al.*, 2015), aerial parts were used as antioxidative, antibacterial, gastroprotective, hepatoprotective, antidiarrhoeal, antinociceptive, antimutagenic, antineoplastic and some metabolic disorders (Hortus, 1976; Kharat *et al.*, 2013). Tribes of Nellore district, Andhra Pradesh, used root bark infusion as ethnic practice to cures jaundice and burning micturition (Srinivas, 2011). Various parts of this plant is also used traditionally in malnutrition, locally to treat chronic wounds, urinary diseases, skin diseases, pulmonary troubles, liver disorder, hair tonic, sedative, diuretic, diarrhoea, dysentery, leucorrhoea and venereal diseases (Srinivas and Baboo, 2011). Antitumor activity of *I. coccinea* flowers was studied in comparison to intraperitonial transplanted daltons lymphoma and ehrlich ascites carcinoma tumors in mice and found that the flower extract showed considerable antitumor principle (and Panikar, 1998). The activity was due presence of camptothecin (Saravanan and Boopalan, 2011).

I. parviflora is rich in polyphenols and effectively a hepatoprotective (Naiwenkan *et al.*, 2013). Leaves possess potent antiinflammatory, analgesic and antipyretics effects (Sumanta, 2014). Major compounds like β-sitosterol, β-sitosterol-β-D-glucoside, kaempferoland kaempferol-7-O-methyl ether were isolated (Bachheti *et al.*, 2011). Flavonoids, glycosides, saponins and tannins, phenols and triterpinoids produced a significant dose dependent increase in the enzymatic antioxidants of liver like superoxide dismutase, catalase and glutathione levels. The hepatoprotective and antioxidant activity produced by whole plant of methanolic extract of *Ixora pavetta* (synonym of *I. parviflora*) may be due to the presence of flavonoids, glycosides, saponins and tannins, phenols and triterpinoids (Suneeta and Tirupathi, 2020). *I. parviflora* with high polyphenol content exhibited antioxidant activity and reducing UVB induced intracellular

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reactive oxygen species production (Kuochingwen *et al.*, 2011). *I. parviflora* extract showed antioxidant activity in a cell-free system and erythrocytes and inhibited reactive oxygen species generation in human fibroblasts after ultraviolet exposure. By this, it can said that it is a powerful absorber and neutralizer of free radicals, indicating that it is a potential antiageing and antiphotoaging agent (KuoChing wen *et al.*, 2011). From the literature, it was suggested that the plant *I. parviflora* consists of major secondary metabolites and possess potent biological activities. Hence, the plant was selected for further investigation. HPLC analysis and assessment of antimitotic activity and cytotoxic activity of *I. parviflora* was performed in this study.

2. Materials and Methods

2.1 Plant collection and processing

Fresh leaves of *I. parviflora* were collected from Manuguru forest, Telangana and authenticated by Dr. P. Laxman, Associate Professor, Govt. Degree College, Kukatpally, Telangana. The leaves were shade dried for 15 days and made into powder. Each 300 g powder was extracted with hexane, ethyl acetate and methanol (1000 ml) using Soxhlet apparatus. Excess solvent was removed under rota evaporator and dried extracts were put in desiccators until further use. Extracts were named as IPHE, IPEAE and IPME for hexane extract, ethyl acetate extract and methanol extract, respectively.

2.2 HPLC analysis for standardization of *I. parviflora* extracts

The HPLC analysis was performed on Shimadzhu LC-Prominence 20AT using SGE protecol PC18GP120 (250 mm × 4.6 mm, 5 µm) column. The mobile phase consists of acetonitrile to water (60:40 v/v) on isocratic mode. Elution was performed at a flow rate of 1.0 ml/min and detection was done at 275 nm by UV detector (Tripath *et al.*, 2012).

2.3 Antimitotic activity of extracts

2.3.1 Seed germination assay

Green gram (*Vigna radiata*) seeds were purchased and individual seed was weighed. Approximate equal weights of the seeds were selected for the study. Seeds were allowed for imbibitions by soaking in water. They were placed in sample vial containing different extracts of plant. After 24 h seeds were weighed and time of sprouting was extended to 48 h and 72 h. Vincristine was used as standard. Weight of the seeds in all extracts was noted and percentage inhibition was calculated;

% inhibition = (wtD – wtE)/ (wtD – wtS) x 100

where wtD, weight of seed in distilled water; wtE, weight of seed in extract and wtS, weight of the seed in standard (Satyanarayana *et al.*, 2011; Mayur *et al.*, 2019)

2.3.2 *Allium cepa* L. root tip assay

The outer scales of the bulbs were removed not destroying root primordial and were kept in beaker containing distilled water in dark at room temperature (Tajudeen *et al.*, 2020). Onions having root length of 2-4 cm were incubated in different concentrations of *I. parviflora* extracts. At growth period, root tips of onion were fixed in the Carnoy's fixative (1:3 acetic acid: alcohol) for 24 h. Fixed roots were placed in petridish, hydrolyzed with 1N HCl and later heated to dissolve cell wall (Rajneet *et al.*, 2014) and washed with water. The roots were transferred on a glass slide, small section of root (1–2 mm) was cut with a new blade and dipped in a drop of 2% acetocarmine for 2 min and squashed. The slide cover was carefully placed over slide avoiding air bubbles (Maria Sabeen *et al.*, 2020; Waghulde Sandeep *et al.*, 2021). It was observed under the fluorescence microscope and photographs of cell division were captured. Change of chromosome phases was observed (Mayur Parmar *et al.*, 2021) and the mitotic index was calculated by:

%MI= No. of diving cells/ Total no. of cells x 100

Statistical analysis

Statistical analysis was performed using GraphPad Prism 9 software and all data expressed as mean values ± SD (n = 3) represented by error bars.

2.4 MTT Assay

2.4.1 Cell lines and culture medium

Human breast adenocarcinoma cell lines were procured from ATCC, MCF-7 stock cells were cultured in DMEM supplemented with 10% fetal bovine serum (FBS), penicillin (100 IU/ml) and streptomycin (100 µg/ml) in a humidified atmosphere of 5% CO_2 at 37°C until confluent. MDA-MB-231 cells were grown at 37°C in Leibovitz's L-15 medium supplemented with 2 mM glutamine and 15% FBS. The cells were dissociated with cell dissociating solution (0.2% trypsin, 0.02% EDTA, 0.05% glucose in PBS). The viability of the cells was checked and centrifuged. Further, 50,000 cells /well were seeded in a 96 well plate and incubated for 24 h at 37°C, 5% CO_2 incubator.

2.4.2 Procedure

The monolayer cell culture was trypsinized and cell count was adjusted to 5.0 x 10^5 cells/ml using respective media containing10% FBS. To each well of 96 well microtiter plate, 100 µl of the diluted cell suspension (50,000 cells/well) was added. After 24 h, when a partial monolayer was formed, the supernatant was flicked off, washed the monolayer once with medium and 100 µl of different test concentrations of *I. parviflora* extracts were added on to the partial monolayer in microtiter plates. The plates were then incubated at 37°C for 24 h in 5% CO_2 atmosphere. After incubation, 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 (Talib and Mahasneh, 2010). The plates were incubated for 4 h at 37°C in 5% CO_2 atmosphere. The supernatant was removed and 100 µl of DMSO was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 590 nm (Ala, 2018). The percentage growth inhibition was calculated using the following formula and the concentration of test drug needed to inhibit cell growth by 50% (IC_{50}) values is generated from the dose response curves for each cell line.

Calculating inhibition:

% inhibition = ((ODc – ODs)/ODs) × 100 where ODc is optical density of control and ODs is optical density of sample.

Statistics

IC_{50} values for cytotoxicity tests were derived from nonlinear regression analysis (curve fit) and computed using Graph Pad Prism 6 (Graphpad, San Diego, CA, USA).

3. Results

3.1 HPLC analysis for standardization of *I. parviflora* extracts

HPLC analysis of the plant extract with three solvents like hexane, ethyl acetate and methanol were done qualitatively. For standardization of plant extract, HPLC is an accurate tool widely used for the quality assessment of plant extract (Mahendra *et al*., 2011). Hexane extract showed the presence of constituents evident by chromatogram at different retention times (1.277, 1.590, 1.803, 2.037, 2.233, 2.397, 2.610, 3.040, 4.567, 5.350, 8.523, 10.380) given in Figure1 and Table1, for ethyl acetate extract (1.923, 2.423, 3.230, 3.540, 4.070, 4.990, 7.560) given in Figure 2 and Table2, for methanol extract (2.053, 2.717, 2.983, 3.440, 4.127, 4.413, 4.617, 5.073, 5.423, 6.130) given in Figure 3 and Table 3.

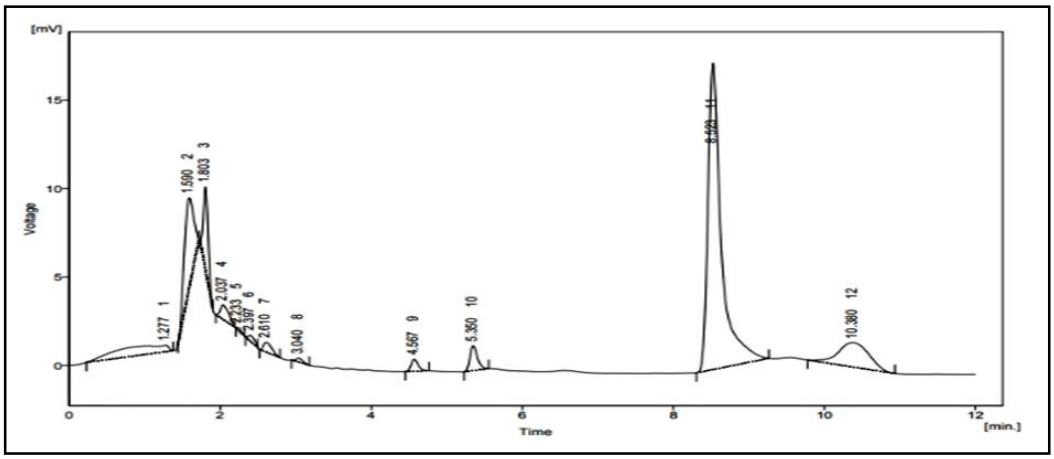


Figure 1: HPLC chromatogram of hexane extract of *I. parviflora*.

Table 1: RT and area of peaks of hexane extract of *I. parviflora*

S. No.	Retention time (min)	Area (mV.s)	Height (mV)	Area (%)
1	1.277	23.791	0.366	6.4
2	1.590	43.136	5.064	11.6
3	1.803	20.999	4.639	5.7
4	2.037	6.613	0.826	1.8
5	2.233	0.647	0.113	0.2
6	2.397	2.028	0.328	0.5
7	2.610	4.328	0.545	1.2
8	3.040	1.427	0.225	0.4
9	4.567	4.388	0.678	1.2
10	5.350	9.615	1.395	2.6
11	8.523	214.200	17.325	57.8
12	10.380	39.266	1.378	10.6

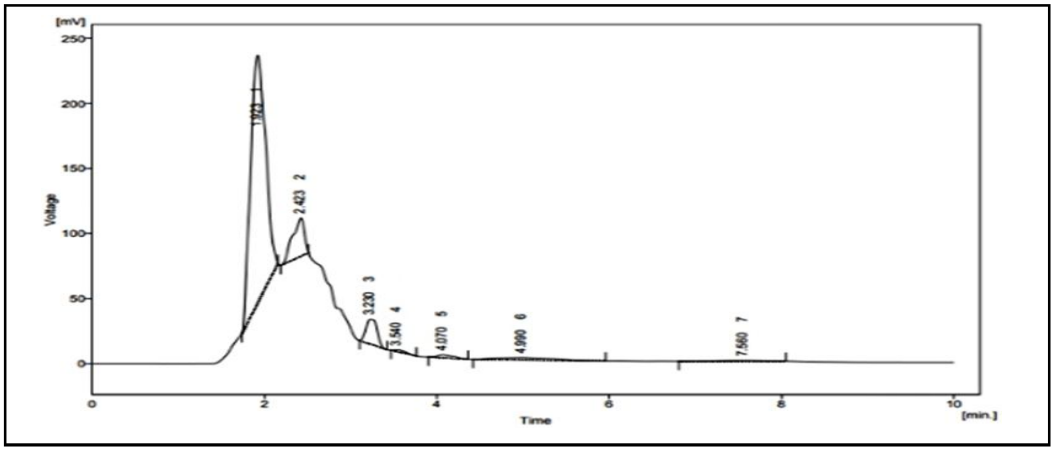


Figure 2: HPLC chromatogram of ethyl acetate extract of *I. parviflora*.

Table 2: RT and area of peaks of ethyl acetate extract of *I. parviflora*

S.No	Retention time (min)	Area (mV.s)	Height (mV)	Area (%)
1	1.923	2296.294	189.994	79.2
2	2.423	279.296	29.298	9.6
3	3.230	174.336	19.180	6.0
4	3.540	15.224	1.280	0.5
5	4.070	30.777	2.311	1.1
6	4.990	80.666	1.803	2.8
7	7.560	23.839	0.606	0.8

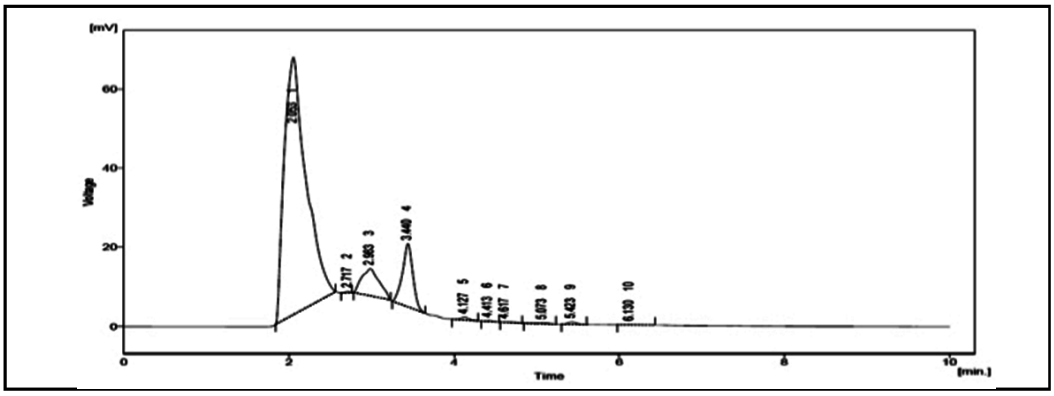


Figure 3: HPLC chromatogram of methanol extract of *I. parviflora*.

Table 3: RT and area of peaks of methanol extract of *I. parviflora*

S.No	Retention time (min)	Area (mV.s)	Height (mV)	Area (%)
1	2.053	1194.320	64.961	62.4
2	2.717	1.350	0.338	0.1
3	2.983	100.914	6.850	7.0
4	3.440	139.783	15.905	9.6
5	4.127	4.711	0.716	0.3
6	4.413	1.359	0.1844	0.1
7	4.617	1.009	0.065	0.1
8	5.073	1.916	0.170	0.1
9	5.423	3.798	0.509	0.3
10	6.130	1.111	0.104	0.1

Table 4: Seed germination assay of hexane extract of *I. parviflora*

Extract	Dose (mg/ml)	Weights (mg) (mean ± SD) after treatment					
		24 h		48 h		72 h	
		Seed wt (mg)	% inhibition	Seed wt (mg)	% inhibition	Seed wt (mg)	% inhibition
Control (distilled water)	0	188.6 ± 0.4	-	195.3 ± 2.4	-	208.3 ± 3.4	-
Std(V)	0.1	97.6 ± 2.4	-	94.3 ± 2.4	-	92.6 ± 2.4	-
IPHE	2	187.6 ± 0.2**	1.7 ± 0.1	184.6 ± 0.4**	10.9 ± 0.2	182.3 ± 2.0**	22.7 ± 0.1
IPHE	4	186.6 ± 0.4**	2.8 ± 0.3	180.3 ± 0.5**	14.8 ± 0.4	176.6 ± 1.6**	27.9 ± 0.3
IPHE	6	182.3 ± 1.1**	7.2 ± 0.6	172.3 ± 0.6**	22.8 ± 0.6	160.2 ± 2.4**	41.7 ± 0.2
IPHE	8	174.5 ± 0.4**	16.5 ± 0.7	168.3 ± 1.2**	26.8 ± 0.2	156.3 ± 0.4**	45.2 ± 0.8
IPHE	10	162.6 ± 0.4**	29.23 ± 0.2	150.2 ± 0.4**	44.6 ± 0.1	145.7 ± 2.4**	54.7 ± 0.7

3.2 Seed germination assay

Germination of green gram increased its weight in distilled water as the time progressed (at 24 h, 48 h, 72 h), whereas the germination was reduced in hexane, ethyl acetate and methanol extracts of *I. parviflora* which was calculated as % inhibition. The results of seed weight and % inhibition was represented in Tables 4, 5 and 6 for hexane extract, ethyl acetate extract and methanol extract, respectively. The assay was done in triplicate.

Table 5: Seed germination assay of ethyl acetate extract of *I. parviflora*

Extract	Dose (mg/ml)	Weights (mg) (mean ± SD) after treatment					
		24 h		48 h		72 h	
		Seed wt (mg)	%inhibition	Seed wt (mg)	%inhibition	Seed wt (mg)	%inhibition
Control (distilled water)	0	188.6 ± 0.4	-	195.3 ± 2.4	-	208.3 ± 3.4	-
Std(V)	0.1	97.6 ± 2.4	-	94.3 ± 2.4	-	92.6 ± 2.4	-
IPEAE	2	182.6 ± 0.2**	7.2 ± 0.4	180.6 ± 0.4**	14.8 ± 0.3	170.5 ± 2.0**	33.1 ± 0.5
IPEAE	4	174.6 ± 0.4**	16.0 ± 0.6	168.3 ± 0.5**	26.8 ± 0.6	162.6 ± 1.6**	40.0 ± 0.3
IPEAE	6	168.3 ± 1.10**	22.6 ± 0.3	1156.3 ± 0.6**	38.7 ± 0.4	150.2 ± 2.4**	50.3 ± 0.6
IPEAE	8	156.5 ± 0.4**	35.8 ± 0.6	142.3 ± 1.2**	52.6 ± 0.7	134.3 ± 0.4**	64.2 ± 0.4
IPEAE	10	143.6 ± 0.4**	50.1 ± 0.3	134.2 ± 0.4**	60.5 ± 0.2	122.7 ± 2.4**	74.5 ± 0.2

Table 6: Seed germination assay of methanolic extract of *I. parviflora*

Extract	Dose (mg/ml)	Weights (mg) (mean ± SD) after treatment					
		24 h		48 h		72 h	
		Seed wt (mg)	% inhibition	Seed wt (mg)	% inhibition	Seed wt (mg)	% inhibition
Control (distilled water)	0	188.6 ± 0.4	-	195.3 ± 2.4	-	208.3 ± 3.4	-
Std(V)	0.1	97.6 ± 2.4	-	94.3 ± 2.4	-	92.6 ± 2.4	-
IPME	2	180.6 ± 0.1**	8.7 ± 0.5	178.6 ± 0.4**	16.8 ± 0.6	165.3 ± 2.0**	37.1 ± 0.7
IPME	4	172.6 ± 0.7**	17.5 ± 0.7	164.3 ± 0.5**	30.6 ± 0.4	158.6 ± 1.6**	43.2 ± 0.5
IPME	6	164.3 ± 1.10**	26.3 ± 0.4	150.3 ± 0.6**	44.5 ± 0.7	148.1 ± 2.4**	59.1 ± 0.6
IPME	8	142.3 ± 0.4**	50.5 ± 0.6	134.3 ± 1.2**	60.3 ± 0.3	128.3 ± 0.4**	68.9 ± 0.4
IPME	10	132.6 ± 0.4**	61.5 ± 0.2	121.3 ± 0.4**	73.2 ± 0.1	118.6 ± 2.4**	77.5 ± 0.2

**Value of p less than 1% (*i.e.*, $p<0.01$) was considered statistically significant.

3.3 *Allium cepa* L. root tip assay

The results showed significant abnormalities in cell division of onion root tip. The cell division decreased gradually as the time progressed at 12 h, 24 h and 48 h in all the extracts. Results of mitotic index were represented in Tables 7, 8 and 9 and graphical representation was given in Figures 4, 5 and 6.

Table 7: %Mitotic index of hexane extract of *I. parviflora*

Extract	Dose (mg/ml)	% Mitotic index (mean ± SD) after treatment		
		12 h	24 h	48 h
Control	0	73.6 ± 0.88**	87.1 ± 0.5**	94.3 ± 0.8**
Std (V)	0.1	55.3 ± 0.8**	46.6 ± 0.8**	38.3 ± 0.6**
IPHE	2	72.2 ± 1.2**	69.9 ± 0.5**	64.1 ± 0.5**
IPHE	4	71.6 ± 0.1**	67.4 ± 0.2**	63.2 ± 0.3**
IPHE	6	69.2 ± 1.5**	65.6 ± 0.8**	61.6 ± 0.5**
IPHE	8	66.3 ± 0.6**	63.3 ± 0.8**	60.6 ± 0.3**
IPHE	10	64.6 ± 1.5**	61.6 ± 0.6**	58.3 ± 0.3**

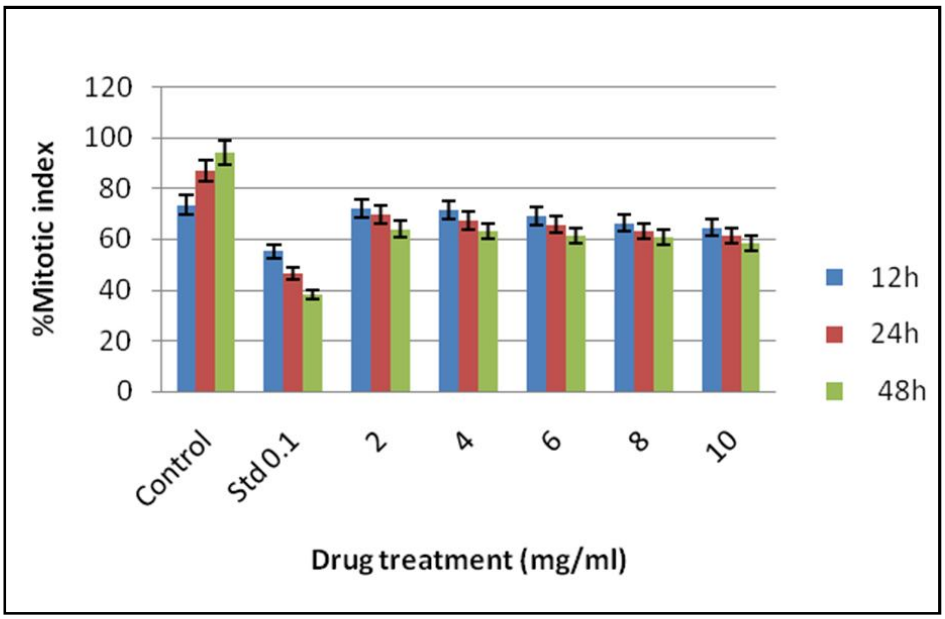


Figure 4: %Mitotic index of hexane extract of *I. parviflora.*

Table 8: %Mitotic index of ethyl acetate extract of *I. parviflora*

Extract	Dose (mg/ml)	% Mitotic index (mean ± SD) after treatment		
		12 h	24 h	48 h
Control	0	73.6 ± 0.88**	87.1 ± 0.5**	94.3 ± 0.8**
Std(V)	0.1	55.3 ± 0.88**	46.6 ± 0.8**	38.3 ± 0.6**
IPEAE	2	70.2 ± 0.3**	68.3 ± 0.6**	63.4 ± 0.4**
IPEAE	4	69.3 ± 0.5**	66.6 ± 0.4**	61.6 ± 0.6**
IPEAE	6	67.6 ± 0.7**	64.6 ± 0.2**	60.9 ± 0.6**
IPEAE	8	65.3 ± 0.1**	61.3 ± 0.4**	59.1 ± 0.7**
IPEAE	10	62.6 ± 0.4**	58.6 ± 0.7**	56.3 ± 0.9**

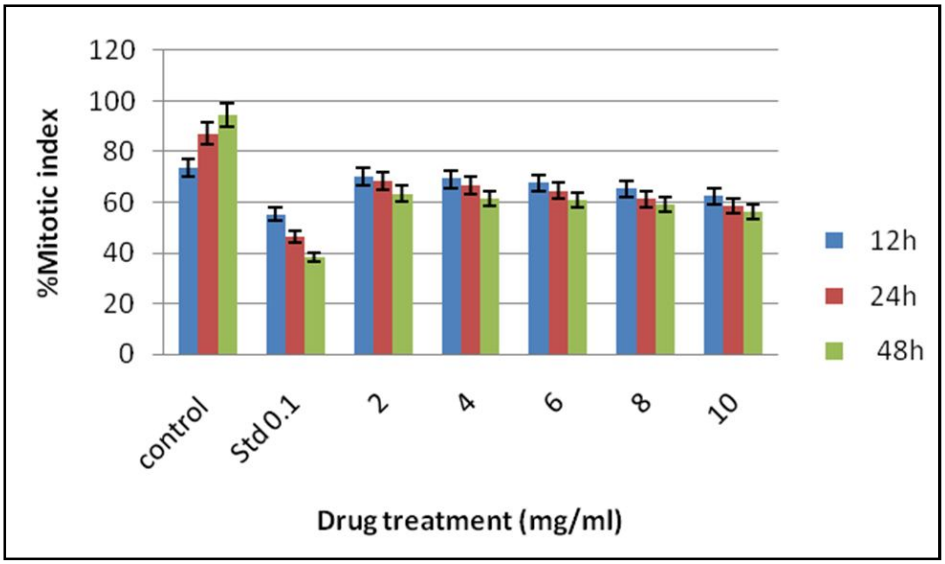


Figure 5: %Mitotic index of ethyl acetate extract of *I. parviflora.*

Table 9: %Mitotic index of methanolic extract of *I. parviflora*

Extract	Dose (mg/ml)	% Mitotic index (mean ± SD) after treatment		
		12 h	24 h	48 h
Control	0	73.6 ± 0.88**	87.1 ± 0.5**	94.3 ± 0.8**
Std(V)	0.1	55.3 ± 0.88**	46.6 ± 0.8**	38.3 ± 0.6**
IPME	2	69.8 ± 0.4**	67.3 ± 0.7**	60.9 ± 0.5**
IPME	4	67.3 ± 0.1**	64.6 ± 0.3**	59.6 ± 0.6**
IPME	6	66.9 ± 1.5**	61.6 ± 0.9**	57.6 ± 0.5**
IPME	8	62.3 ± 0.3**	59.3 ± 0.8**	56.6 ± 0.4**
IPME	10	58.6 ± 0.6**	56.6 ± 0.8**	54.3 ± 0.5**

**Value of p less than 1% (*i.e.*, $p<0.01$) was considered statistically significant.

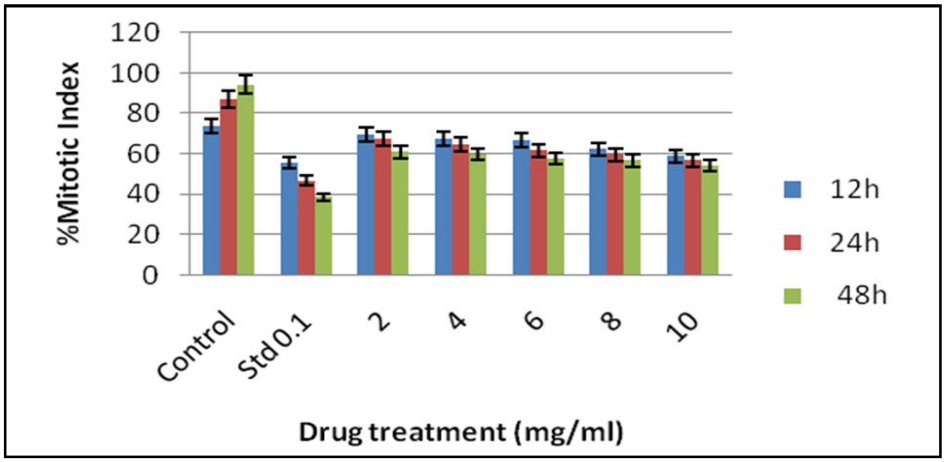


Figure 6: %Mitotic index of methanol extract of *I. parviflora.*

Table 10: MTT assay of *I. parviflora* extracts against MCF-7

Sample	Conc. (µg/ml)	OD at 590 nm	% inhibition	IC_{50} µg/ml
IPHE	10	0.936	2.58	284.1 ± 2.6
	20	0.897	8.79	
	40	0.822	14.81	
	80	0.752	26.81	
	160	0.692	40.82	
	320	0.541	45.58	
IPEAE	10	0.869	2.96	252.5 ± 1.8
	20	0.768	9.93	
	40	0.666	18.85	
	80	0.623	29.62	
	160	0.555	47.74	
	320	0.523	50.51	
IPME	10	0.896	3.54	192.3 ± 1.0
	20	0.836	10.47	
	40	0.763	29.62	
	80	0.689	34.28	
	160	0.615	58.64	
	320	0.446	68.78	

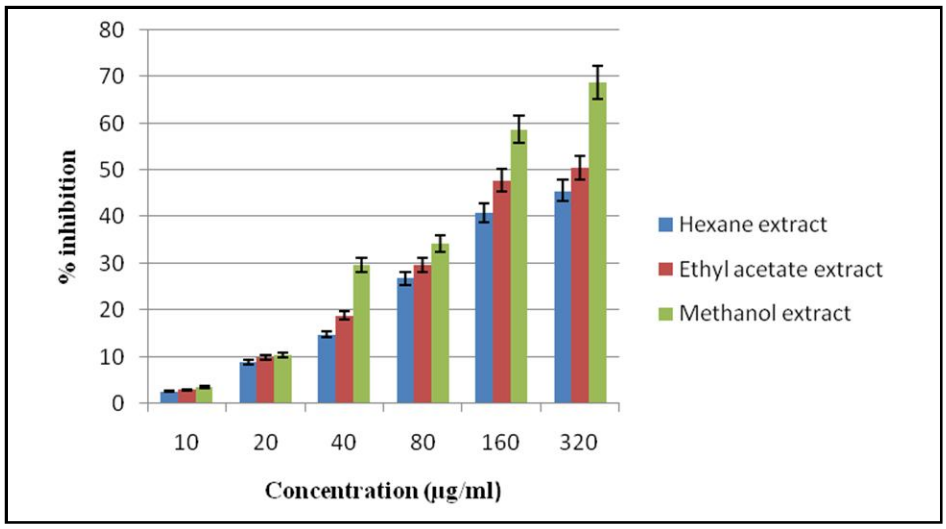


Figure 7: Graphical representation of MTT assay of *I. parviflora* extracts against MCF-7.

3.4 MTT assay

After 24 h of extracts treatment, 50% growth inhibition concentrations (IC_{50}) results were observed in dose dependent manner. The methanol extracts showed highest cytotoxic activity of 192.3 ± 1.0 against MCF-7 and 189.1 ± 1.1 against MDA-MB-231 cell lines. For hexane extract it was 284.1 ± 2.6 and for ethyl acetate extract, it was 252.5 ± 1.8 against MCF-7. The values against MDA-MB-231 cell lines for hexane and ethyl acetate extracts were reported as 280 ± 2.1 and 250 ± 1.2, respectively. Results of MTT assay of extracts were presented in the Table 10, Table 11 and standard readings in Table 12 and Table 13. Images of morphology of cell lines were presented in the Figures 9 and 10. Standard vincristine images were given in Figure 11.

Table 11: MTT assay of *I. parviflora* extracts against MDA-MB-231

Sample	Conc. (µg/ml)	OD at 590 nm	% inhibition	IC_{50} µg/ml
IPHE	10	0.905	3.36	
	20	0.885	10.32	
	40	0.835	16.56	280 ± 2.1a
	80	0.768	27.91	
	160	0.698	42.37	
	320	0.586	47.97	
IPEAE	10	0.805	4.96	250 ± 1.2
	20	0.748	12.93	
	40	0.712	20.85	
	80	0.657	32.62	
	160	0.611	49.74	
	320	0.486	52.51	
IPME	10	0.889	5.54	189.1 ± 1.1
	20	0.863	14.47	
	40	0.815	31.62	
	80	0.725	36.28	
	160	0.508	62.64	
	320	0.371	70.78	

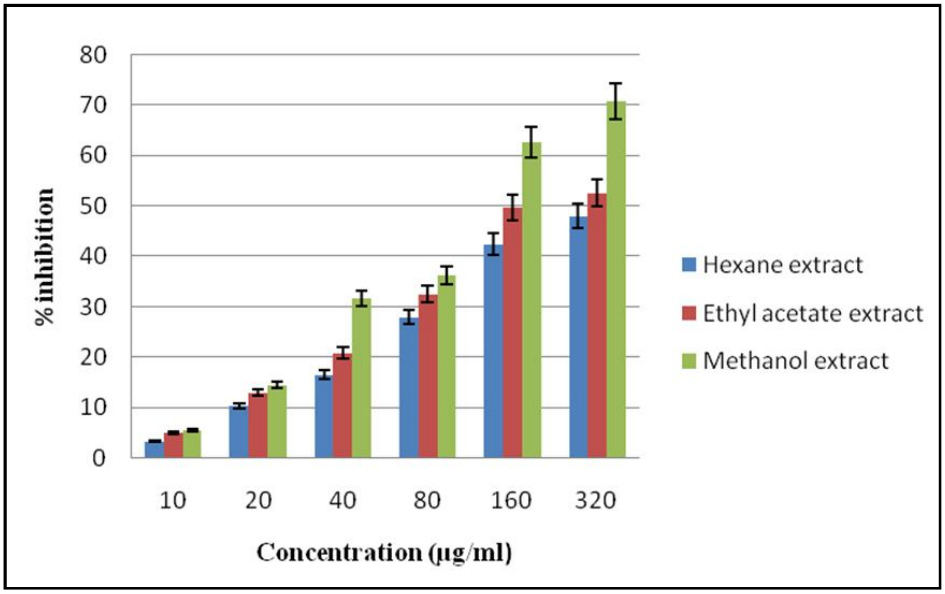


Figure 8: Graphical representation of MTT assay of *I. parviflora* extracts against MDA-MB-231.

Table 12: IC_{50} of vincristine on MCF-7 cell lines

MCF-7				
Sample	**Conc. µM**	**OD at 590 nm**	**% inhibition**	**IC_{50} µM**
Control	0	0.936	0	
Vincristine	3.125	0.873	6.73	24.56
	6.25	0.835	10.79	
	12.5	0.648	30.79	
	25	0.419	55.26	
	50	0.248	73.50	
	100	0.102	89.11	

Table 13: IC_{50} of vincristine on MDA-MB-231 cell lines

MDA-MB-231				
Sample	**Conc. µM**	**OD at 590 nm**	**% inhibition**	**IC_{50} µM**
Control	0	0.985	0	
Vincristine	3.125	0.886	7.92	22.85
	6.25	0.859	11.58	
	12.5	0.680	32.64	
	25	0.520	57.15	
	50	0.456	76.45	
	100	0.348	90.21	

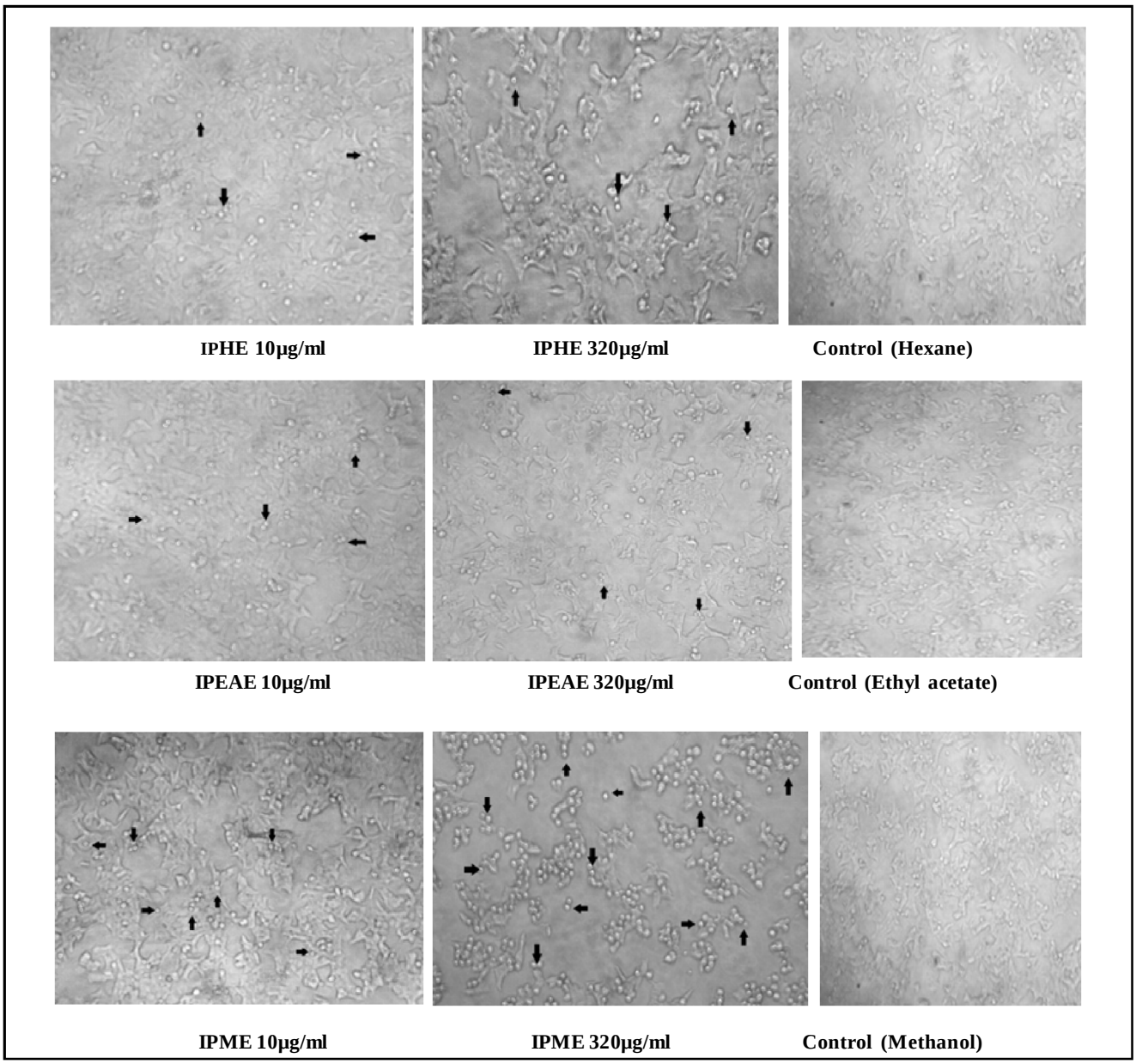
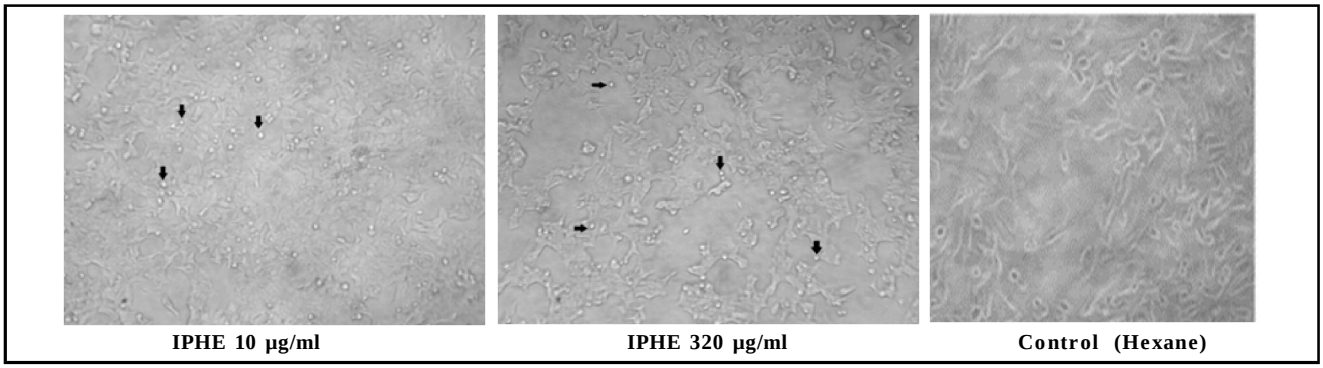


Figure 9: Phase contrast images of MCF-7 cells showing morphological changes in a dose dependent manner following treatment with extracts. They lose cell-to-cell contact and attain more rounded shape, prominent such as included cell shrinkage, membrane blebbing, cell fragmentations and detachment mimicking apoptosis were observed. Arrows indicate dead cells which appears round and translucent (Magnification 40x).



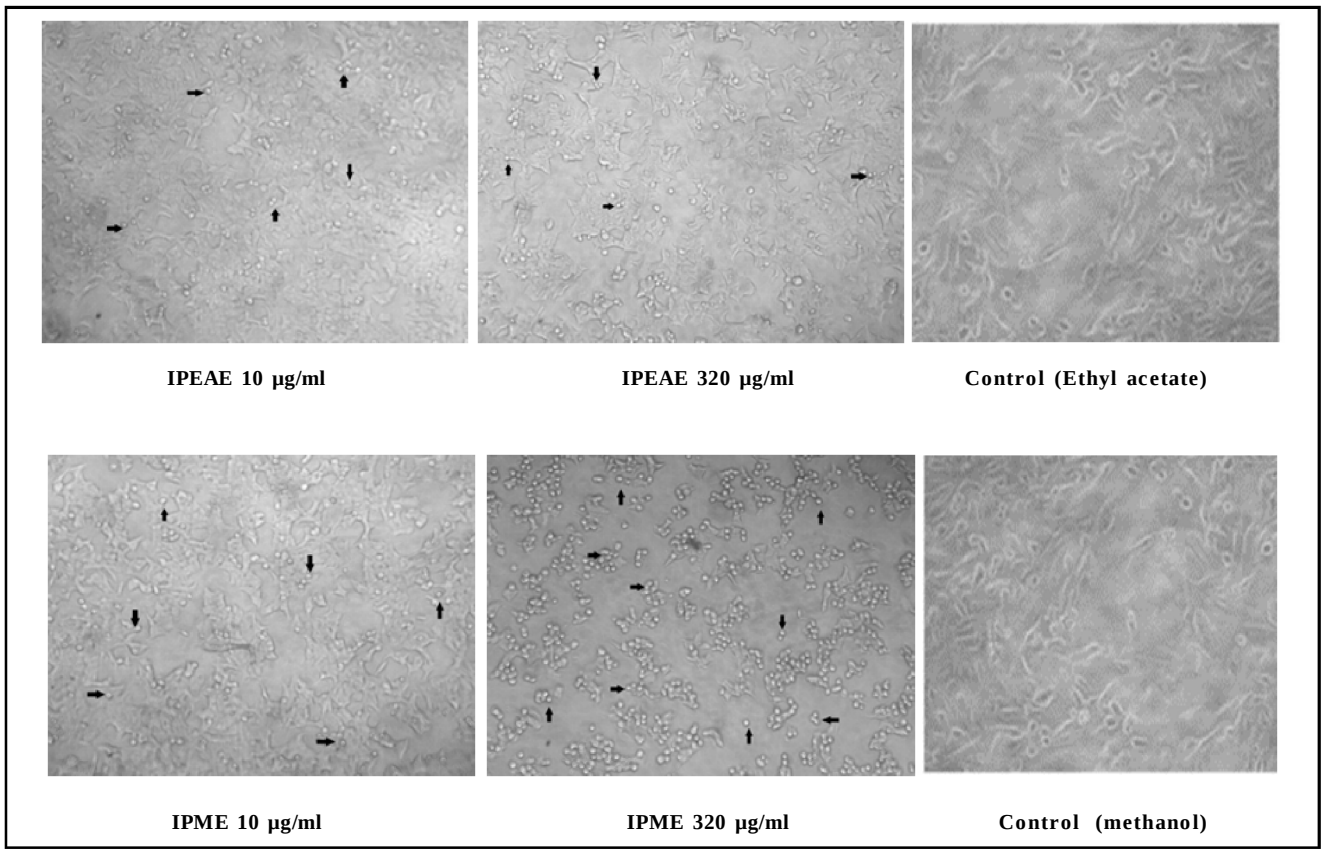


Figure 10: Phase contrast images of MDA-MB-231 cells showing morphological changes in a dose dependent manner following treatment with extracts. They lose cell-cell contact and attain more rounded shape, membrane blebbing, cell fragmentations and detachment mimicking apoptosis were observed. Arrows indicate dead cells which appears round and translucent (Magnification 40x).

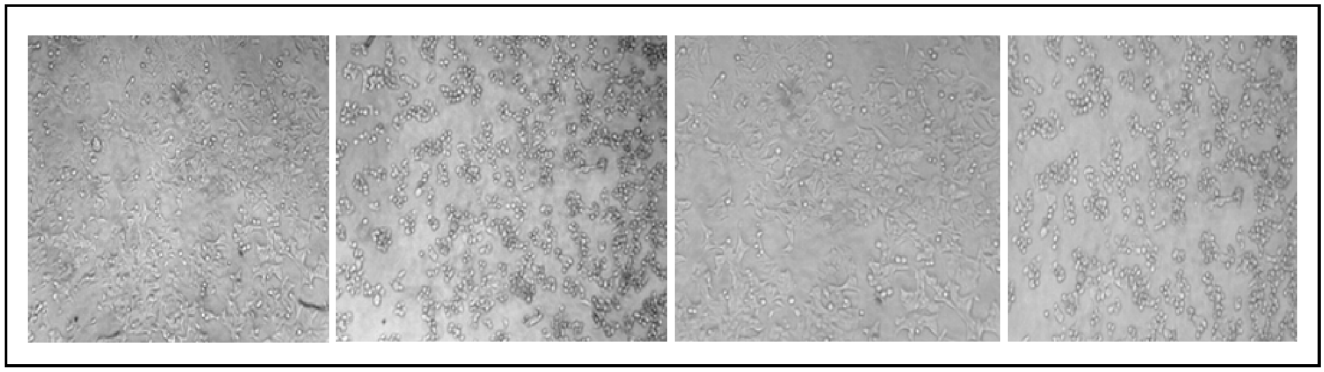


Figure 11: Morphological analysis of standard by phase-contrast microscopic images of MCF7 cells (a and b) and MDA-MB-231cells (c and d).

4. Discussion

The plant *I. parviflora* has many traditional uses as a folklore medicine since ancient times. As per literature, the plant possesses various biological activities like antioxidant activity, hepatoprotective activity, anti-inflammatory, analgesic and antipyretics. HPLC is a chromatographic technique that can separate a mixture of compounds and is used in phytochemical and analytical chemistry to identify, quantify and purify the individual components of the mixture (Yasodha and Suresh, 2017). This technique is gaining popularity among various analytical techniques as the main choice for fingerprinting study for the quality control of herbal plants (Fan *et al.*, 2006). Several authors describe the use of HPLC for characterization and quantification of secondary metabolites in plant extracts (Martin and Guiochon, 2005). The major compounds in the plant extracts can be determined by the representation of various peaks in the chromatogram of HPLC (Janovik *et al.*, 2012). Results of HPLC analysis of *I. parviflora* at 275 nm shows presence of various constituents as evidenced by chromatogram obtained at various retention times (1.277, 1.590,

1.803, 2.037, 2.233, 2.397, 2.610, 3.040, 4.567, 5.350, 8.523, 10.380 for hexane extract, 1.923, 2.423, 3.230, 3.540, 4.070, 4.990, 7.560 for ethyl acetate extract and 2.053, 2.717, 2.983, 3.440, 4.127, 4.413, 4.617, 5.073, 5.423, 6.130 for methanol extract). This shows the presence of various constituents in the plant. Here, HPLC analysis was performed only as quality control parameter. However, it must be coupled with advanced techniques like LC-MS/GC-MS and/or NMR to identify the compounds and get some insight into the structures. The antimitotic and antiproliferative effects are the important *in vitro* assays for the screening of anticancer compounds (Shwetha Saboo *et al.*, 2014). From the results, it can be suggested that *I. parviflora* extracts has antimitotic activity by green gram seed germination assay where the radicle length and weight of the seed got supressed with treatment of extracts by 72 h. The IPHE, IPEAE, IPME showed %inhibition of 54.7 ± 0.1, 74.5 ± 0.1, 77.5 ± 0.1, respectively, which was confirmed by onion root tip assay as the mitotic index was decreased as the contact time of extracts with the onion root increased. After 48 h treatment with extracts %MI resulted for IPHE, IPEAE, IPME as 58.3 ± 0.3, 56.3 ± 0.9 and 54.3 ± 0.5, respectively. The present study revealed that treatment of *A. cepa* root meristems with extracts containing both polar and non-polar fractions of *I. parviflora* leaves had a detrimental effect on root tips of *A. cepa*. Treatment not only brought down the frequency of dividing cells, but also produced a good number of anomalies in the mitotic cells. There was a marked decrease in the mitotic index from hexane (non-polar) extract to methanol (polar) extract (Table 9). By MTT assay, it was more confirmed that *I. parviflora* extracts showed good inhibition of cell growth against MCF-7 and MDA-MB-231. The IC_{50} values against MCF-7 were resulted as 284.1 ± 2.6, 252.5 ± 1.8, 192 ± 1.0 and 280 ± 2.1, 250 ± 1.2, 189.1 ± 1.1 against MDA-MB-231 for IPHE, IPEAE and IPME, respectively. In this study, mitotic index of extracts indicates the inhibition of growth of cancer cells either by affecting microtubules, thus stopping them from breaking. This makes the cells become so clogged with microtubules that they cannot continue to grow and divide. As a result of this, cells arrest in mitosis and die by apoptosis (Roberge *et al.*, 2000). The percentage of cytotoxicity and mitotic inhibition was found to be different in different extracts based on the polarity. The methanol extract which is a polar fraction showed the best activity.

5. Conclusion

Based on the obtained results, it can be confirmed that *I. parviflora* extracts showed good cytotoxic activity against human breast adenocarcinoma cell lines. The findings support the reported therapeutic use of this plant as an anticancer agent in the traditional system of medicine. The identification of important secondary metabolites responsible for cytotoxic effects must be done by various analytical techniques like FTIR, NMR, LCMS/GCMS, *etc*. Further, *in vitro* and *in vivo* methods are more needed to find out the exact mechanism of action of the plant in order to get the maximum benefits of the plant usage against the human breast cancer.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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