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Cytotoxic activity of methanolic extract of *Artocarpus heterophyllus* against A549, HeLa and MCF-7 cell lines

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ABSTRACT

Research is focusing on the search for new types of natural chemotherapeutic agents derived from plants which are proving to be excellent sources of new compounds. The present research article was aimed to study the cytotoxic activity of methanolic extracts of *Artocarpus heterophyllus* plant by various *in vitro* cytotoxic assays like MTT and SRB against different cell lines like HEK293, A549, HeLa and MCF-7. The IC₅₀ values of methanolic extract of *Artocarpus heterophyllus* were found 35.26 µgm/ml and 35.27 µgm/ml against A549 cell line by MTT and SRB assay methods respectively whereas this extract was found to be non toxic to normal cells (HEK293), proved that the methanolic extract exhibited significant anti cancer potential with no toxicity on normal cell line. The methanolic extract had no activity against HeLa and MCF-7 cell line.

Key words: Cytotoxicity, SRB, MTT, Extraction.

INTRODUCTION

Many medicinal and food plants contain large amounts of chemical components having broad spectrum of pharmacological activities like anti cancer, anti tumor and anti oxidant activities. The anti cancer activities are mainly due to Phenolic acids, flavonoids, and phenolic diterpenes. Natural products are reportedly beneficial to physiological health. Various flavonoids and non-flavonoids have been reported as showing anti cancer, anti tumor and anti oxidant activities. Moraceae is large family comprising sixty genera and nearly 1400 species, including important group such as *Artocarpus*, *Morus*, and *Ficus*. *Artocarpus heterophyllus* or Jackfruit (family of Moraceae) is a monoecious evergreen tree that is grown in several tropical countries. It produces a large pear or barrel-shaped fruit that can grow up to 90 cm long, 50 cm thick and having a weight of 20 kg. Several individual fruits, covered with fleshy and juicy perianths, are found under the spiny surface. The seed is large, oblong and has a slimy membranous testa and a brown tegmen. *A.heterophyllus* is widely distributed in tropical region and has been used as traditional folk medicine against inflammation, malarial fever and so on. In addition, the function of *Artocarpus heterophyllus* in human health such as pulp and seed for tonic; root for diarrhea, fever; wood for muscular contraction; leaves for activating milk in women and animal, anti-syphilis, vermifuge; leaf ash for ulcers and wound. Moraceae plants including *A. heterophyllus* are rich sources of the isoprenylated phenolic compounds, including flavonoids (Acedo, 1992; Jarrett, 1959; Piga, 2004). The objective of this study is to investigate cytotoxic activity of crude extracts from seeds of *Artocarpus heterophyllus* on A549, HeLa and MCF-7 cell lines using the MTT assay and SRB

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assay methods. The overall result indicates the promising baseline information for the potential uses of crude extract from the tegmen of *A. heterophyllus* as an antitumor agent.

MATERIALS AND METHODS

Plant material

Artocarpus heterophyllus seeds were obtained from pujan nursery, Ahmedabad, Gujarat. The seeds were washed with sterile water, dried in shade, finely powdered & stored in air tight bottles.

Preparation of Plant extract

10 gm of dried, finely grounded powder of *Artocarpus heterophyllus* seeds was immersed in 150 ml of methanol and extracted by reflux extraction at 40 °C for 3 hours. After extraction, the extract was filtered through Watmann filter paper and evaporated till dryness. 100 Microgram of plant extract was dissolved in the 1 ml DMSO and then 1:3 dilution of test compound was prepared for MTT and SRB assay.

Phytochemical investigation of *Artocarpus heterophyllus* seeds

Test for steroids: Liebermann –Burchard reaction : Mix 2ml extract with chloroform .Add 1-2 ml acetic anhydride and 2 drops conc. H₂SO₄ from the side of test tube. First red, then blue and finally green color appears.

Test for Saponins: Foam test: shake the drug extract or dry powder vigorously with water. Persistent foam observed.

Test for Flavonoids: *Shinoda test:* To dry powder or extract, add 5ml 95%ethanol, few drops conc. HCl and .5gms magnesium turnings, Pink color observed.

Cell culture

Human embryonic kidney cell line (HEK 293T), lung adeno carcinoma cell line (A549 cell line), cervical cancer cell line (Hela cell line) and breast cancer cell line MCF-7 was grown in DMEM (Dulbecco's modifications of eugal's medium with L-glutamine & 4.5G/L glucose) supplemented with fetal bovin serum 100 units/ml of penicillin G and 0.1 mg/ml of streptomycin sulfate in a humidified atmosphere of a 5% CO₂ at 37°C.

Cytotoxicity assay by MTT (3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyltetrazolium Bromide) assay method

The monolayer cell culture was trypsinized and the cell count was adjusted to 3 lac cells/ ml using medium containing 10% newborn calf serum. Pre incubate cells at a concentration of 1× 10⁶ cells/ml in culture medium for 3 h at 37°C and 6.5% CO₂. The cells were seeded at a concentration of 5× 10⁴ cells/well in 100 µl culture medium and incubated at 37°C in 5 % CO₂ incubator for 24 hrs. After 24 hours, when the monolayer formed, the supernatant was flicked off and added previously diluted with media of 100µl of different concentrations of test extract in microtitre plates and kept for incubation at 37°C in 5 % CO₂ incubator for 72 hour and cells were periodically checked for granularity, shrinkage,

swelling. After 72 hour, the sample solution in wells was flicked off and 10µl of MTT dye was added to each well. The plates were gently shaken and incubated for 4 hours at 37°C in 5% CO₂ incubator. The supernatant was removed and 100 µl of Isopropanol was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at 590 nm with a reference filter of 620 nm. (Mossmann, T., (1983; Knick, V.C. 1995; Skehan, P.2003; Skehan P, 1990).

The percentage cell growth inhibition or percentage cytotoxicity was calculated by following formula:

$$\% \text{ Growth inhibition} = 100 - \frac{\text{Mean OD of individual Test Group}}{\text{Mean OD of control Group}} \times 100$$

Cytotoxicity assay by SRB method

The monolayer cell culture was trypsinized and the cell count was adjusted to 0.5-1.0 x 10⁵ cells/ml using medium containing 10% new born sheep serum. To each well of the 96 well microtitre plate, 0.1ml of the diluted cell suspension (approximately 10,000 cells) was added. After 24 hours, when a partial monolayer was formed, the supernatant was flicked off, washed once and 100µl of different test compound concentrations were added to the cells in microtitre plates. The plates were then incubated at 37°C for 72 hours in 5% CO₂ incubator and microscopic examination was carried out and observations recorded every 24 hours. After 72 hours, 25µl of 50% trichloroacetic acid was added to the wells gently such that it forms a thin layer over the test compounds to form a over all concentrations 10%. The plates were incubated at 40°C for one hour. The plates were flicked and washed five times with tap water to remove traces of medium, sample and serum, and were then air-dried. The air dried plates were stained with 100µl SRB and kept for 30 minutes at room temperature. The unbound dye was removed by rapidly washing four times with 1% acetic acid. The plates were then air-dried. 100µl of 10mM Tris base was then added to the wells to solubilize the dye. The plates were shaken vigorously for 5 minutes. The absorbance was measured using microplate reader at a wavelength of 540nm (Vanicha V. 2006; Skehan, P.2003; Skehan P, 1990)

The percentage growth inhibition was calculated using following formula,

$$\% \text{ Growth inhibition} = 100 - \frac{\text{Mean OD of individual Test Group}}{\text{Mean OD of control Group}} \times 100$$

RESULTS AND DISCUSSION

In vitro cytotoxic Activity of methanolic extract of *Artocarpus heterophyllus* by MTT assay

The effect of methotrexate and *Artocarpus heterophyllus*, was performed on four different cell lines by MTT assay. Dose response curves constructed for MTT method between the range of 5.0-100000 n gm/ml. Calculation of IC₅₀ and R² values was done using graphs generated from Microsoft excel 2007 edition .The

susceptibility of cells to the drug exposure was characterized by IC_{50} values. Results indicate that the antiproliferative effect strengthens with increase in the concentration of drug.

From table 1, observed that the highest cytotoxic activity was found with methotrexate against A549 and HeLa having IC_{50} : 9.70 and 27.90 μ gm/ml respectively. Lower activities were in HEK-293 and MCF-7 having IC_{50} :45.42 and 49.42 μ gm/ml.

From figure 1a, it showed Methotrexate has the dose-effect co-relation with maximum linearity in case of A549, MCF-7, HEK 293 and HeLa, respectively with increasing with increase in the concentration & attains linearity till IC_{50} value is reached.

Interestingly, observed that methotrexate showed cytotoxic activity against A549, HeLa, MCF-7 cancer cell lines and also the normal kidney cell line i.e., HEK-293T cell line show better results in terms of IC_{50} and regression due to its antimetabolite activity which inhibit dihydro folate reductase enzyme.

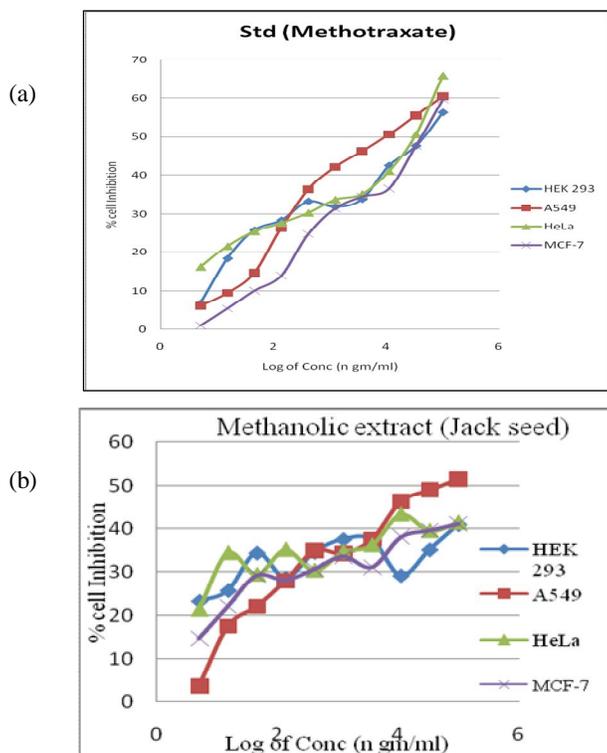


Fig 1(a) & (b): % Growth inhibition of methotrexate and methanolic extract of *Artocarpus heterophyllus* against HEK 293, A549, HeLa, and MCF-7 by the MTT assay.

From table 1, observed that cytotoxic activity of Methanolic extract of *Artocarpus heterophyllus* have found against only A549 cell line with IC_{50} : 35.26 μ gm/ml. Whereas this extract not showed the activity against other cell lines.

From figure 1b, it showed that methanolic extract of *Artocarpus heterophyllus* showed the dose-effect co-relation with maximum linearity in case of A549 of the four cell lines at value being 0.9117. The % inhibition is increasing with increase in the conc. linearly.

On the other hand, Methanolic extract of *Artocarpus heterophyllus* against HeLa and MCF-7 show insignificant regression with non linearity in the values of change of %

inhibition with the increase in concentration, due to that they are not reach upto 50% cell growth inhibition. Normal cell line HEK 293 also show non linearity with insignificant regression, which is less toxic to human health.

In vitro metabolic assay of *Artocarpus heterophyllus* extracts and methotrexate by SRB assay

The effect of Methotrexate and *Artocarpus heterophyllus*, extracts was performed on four different cell lines by SRB assay. Dose response curves constructed for SRB method between the range of 5.0-100000 n gm/ml. Calculation of IC_{50} and R^2 values was done using graphs generated from Microsoft excel 2007 edition. The susceptibility of cells to the drug exposure was characterized by IC_{50} values. Results indicate that the antiproliferative effect strengthens with increase in the concentration of drug. The IC_{50} values of test extract as well as standard drug (methotrexate) were summarised in table 2 and figure 2(a) & (b).

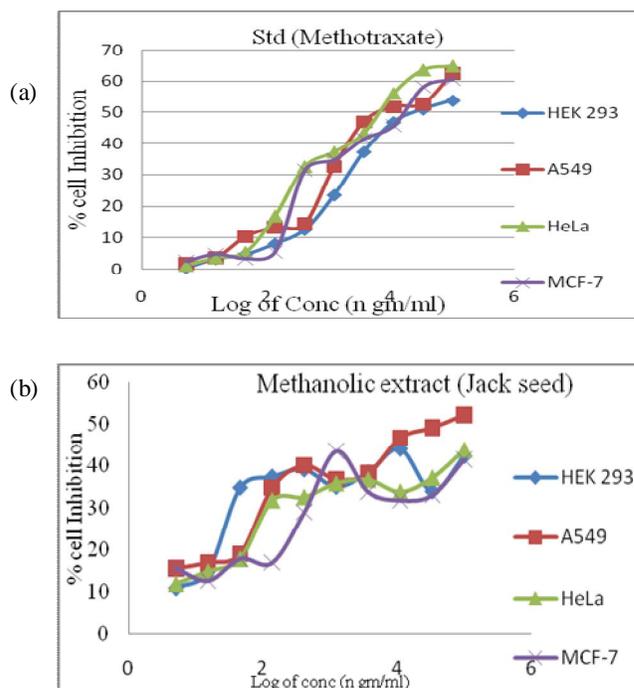


Fig 2(a) and (b): % Growth inhibition of methotrexate and methanolic extract of *Artocarpus heterophyllus* against HEK 293, A549, HeLa, MCF-7 by SRB assay

From table 2, observed that highest activity of Methotrexate have found against HeLa and A549 having IC_{50} : 7.7643 and 16.112 respectively. Lower activities were in MCF-7 and HEK 293 (IC_{50} : 16.531 and 45.671). From figure 2a, it showed that methotrexate has the dose-effect co-relation with maximum linearity in case of HeLa of the four cell lines at value being 0.9747. The % inhibition is increasing with linearity till IC_{50} value is reached. Linearity in case of A549, HEK 293 and MCF-7 cell lines with increasing with increase in the concentration & attains linearity till IC_{50} value is reached respectively.

So, methotrexate shows cytotoxic activity against A549, HeLa, MCF-7 cancer cell lines and also the normal kidney cell line

Table 1: % growth inhibition and IC₅₀ values of methotrexate and methanolic extract of *Artocarpus heterophyllus* by MTT assay.

Compound name	Conc. ng/ml	% Growth Inhibition				IC ₅₀ (µg/ml)			
		HEK-293	A549	HeLa	MCF-7	HEK-293	A549	HeLa	MCF-7
Methotrexate	5.08053	6.859	6.117	16.283	0.997	45.42	9.70	27.907	49.42
	15.24158	18.361	9.530	21.552	5.449				
	45.72474	25.782	14.725	25.411	10.124				
	137.1742	28.379	26.375	27.637	14.057				
	411.5226	33.277	36.319	30.308	24.743				
	1234.568	31.867	42.107	33.722	31.495				
	3703.704	33.870	46.263	35.131	34.538				
	11111.11	42.478	50.641	40.994	36.542				
	33333.33	47.673	55.465	50.641	47.747				
	100000	56.207	60.437	65.854	59.620				
<i>Artocarpus heterophyllus</i>	5.08053	23.259	3.519	21.329	14.651	-	35.26	-	-
	15.24158	25.708	17.471	34.538	21.997				
	45.72474	34.538	22.071	29.492	29.269				
	137.1742	28.750	28.008	35.280	28.231				
	411.5226	34.686	35.058	30.457	30.754				
	1234.568	37.655	34.464	34.761	33.722				
	3703.704	37.506	37.729	36.319	31.198				
	11111.11	29.269	46.411	43.369	38.100				
	33333.33	35.280	49.157	41.811	39.659				
	100000	40.327	51.680	39.584	41.365				

Table No. 2: % growth inhibition and IC₅₀ values of methotrexate and methanolic extract of *Artocarpus heterophyllus* by SRB assay.

Compound name	Conc. ng/ml	% Growth Inhibition				IC ₅₀ (µg/ml)			
		HEK-293	A549	HeLa	MCF-7	HEK-293	A549	HeLa	MCF-7
Methotrexate	5.08053	0.372	1.627	1.0479	2.399	45.67	16.11	7.76	16.53
	15.24158	3.268	3.558	3.558	4.716				
	45.72474	4.716	10.314	5.295	3.268				
	137.1742	8.094	13.403	16.878	5.392				
	411.5226	12.631	14.465	32.515	31.357				
	1234.568	23.538	32.708	37.244	34.832				
	3703.704	37.341	46.993	43.326	41.492				
	11111.11	46.897	51.820	56.067	45.739				
	33333.33	51.048	52.689	63.596	58.191				
	100000	53.847	62.631	64.851	60.701				
<i>Artocarpus heterophyllus</i>	5.08053	10.990	15.527	12.052	15.527	-	-	-	-
	15.24158	14.947	16.878	14.948	12.631		35.27		
	45.72474	34.735	19.001	17.939	17.843				
	137.1742	37.341	34.735	31.839	16.878				
	411.5226	39.078	40.044	32.612	28.847				
	1234.568	34.928	36.762	35.894	43.519				
	3703.704	38.403	38.114	36.762	33.769				
	11111.11	44.001	46.704	33.867	31.743				
	33333.33	33.867	49.117	37.244	32.997				
	100000	42.264	52.206	43.905	41.492				

i.e., HEK-293T cell line show better results in terms of IC₅₀ and regression due to its antimetabolite activity which inhibit dihydro folate reductase enzyme.

From table 2, observed that cytotoxic activity of methanolic extract of *Artocarpus heterophyllus* have found against only A549 cell line IC₅₀: 35.27 µ gm/ml. where as other cell lines have no activity.

From figure 2b, showed that methanolic extract of *Artocarpus heterophyllus* has the dose-effect co-relation with maximum linearity in case of A549 of the four cell lines at value being 0.9117. The % inhibition is increasing with increase in the conc. linearly. The other cell lines showed insignificant regression with non linearity in the values of change of % cells inhibition not reach up to 50% with the increase in concentration.

DISCUSSION

The results of cytotoxicity study of methanolic extract of *Artocarpus heterophyllus* showed significant cytotoxicity against

A549 cell line, whereas this extract had no activity against HeLa, MCF-7 cell lines. This methanolic extract of *Artocarpus heterophyllus* is non toxic to normal cells, but showed excellent toxicity on cancer cells. The cytotoxicity of methanolic extract of *Artocarpus heterophyllus* may be due to the presence of flavonoids having mono to poly phenolic groups in the structure. The flavonoids have reported for their cytotoxic activity due to presence of phenolic groups. (Matsuo M., 2005).

The extractive value, total polyphenolic content and anti cancer activity was at its peak in methanolic extract indicating that most of the active components are extracted with methanol. Cytotoxic changes observed was cell aggregation, cell rounding and cell death. The overall results indicates the promising baseline information for the potential uses of the methanol extracts of tegmen of *Artocarpus heterophyllus* seed as an anti cancer agent.

CONCLUSION

From the results obtained from MTT and SRB assay methods, by the comparison of the IC₅₀ values and linearity of the

activity, the methanolic extract of *Artocarpus heterophyllus* showed excellent cytotoxicity against the A549 cell line, but had not activity against HeLa and MCF-7 cell lines. and HeLa cancerous cell line respectively. The IC₅₀ value found for methanolic extract of *Artocarpus heterophyllus* on A549 cell line was 35.26 µg/ml and 36.119 µg/ml by MTT and SRB assay respectively. From results it found that the methanolic extract had no cytotoxicity against HEK293, whereas methotrexate showed toxicity with IC₅₀ of 45.42 µg/ml and 45.67 µg/ml against HEK 293 by MTT and SRB assay methods respectively. It proved that the methanolic extract of *Artocarpus heterophyllus* had potential cytotoxicity against lung cancer, but non toxic to the normal cells (HEK293 cell line) as compared to methotrexate.

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