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In Vitro Cytotoxic Activity on Root Extracts and Fractions of *Jatropha Gossypiifolia* Linn

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ABSTRACT

The present study was to evaluate preliminary phytochemical analysis and *in vitro* cytotoxic activity on root extracts and fractions of *Jatropha gossipiifolia* (family Euphorbiaceae). The Chloroform and ethanol extract was obtained by hot continuous percolation by soxhlet apparatus. The phytochemical analysis of both the extracts shows the presence of flavonoids, alkaloids, tannins, phenolic compounds and steroids. Both the extracts subjected to *in vitro* cytotoxic activity against breast cancer cell line (MCF–7) cell line by MTT assay. The active extract (ethanol) was then subjected to fractionation by column chromatography by gradient elution from n hexane – ethanol. The fractions (chloroform 100%, chloroform: ethyl acetate, ethyl acetate: ethanol, ethanol 100%) The fractions were then subjected to *in vitro* cytotoxic activity against MCF– 7 cell line by MTT assay. The ethanol extract shows good anticancer activity. The percentage growth of MCF-7 cells in chloroform and ethanol extract treated against MCF-7 cell line was found to be 49.59 and 28.46 respectively. The EC_{50} value of chloroform and ethanol extract was found to be 0.00018 & 0.00055mg/ml respectively. The ethanol 100% shows good anticancer activity compared to other fractions against MCF-7 cell line with the percentage growth of 14.650 and EC_{50} value was found to be 0.00087mg/ml.

Keywords: *Jatropha Gossypiifolia*, MTT assay, MCF–7 cell line, Column chromatography

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INTRODUCTION

The potential medicinal properties of plant species have contributed significantly in the development of various herbal therapies for a number of diseases across the globe. The benefits of herbal medicine over allopathic medicine have helped medicinal plants to regain their importance in the field of health and medicine. Cancer is one of the major health problems that have widely affected the world's population. There is a great need to combat this disease with better and more effective medication as compared to existing therapies. A vast number of medicinal plants are known to have biochemical constituents with anticancer properties. The chemical metabolites of natural origin that possess anticancer properties can serve as potential lead compounds in drug designing. This association of medicinal plants and cancer needs further research and experimentation in order to develop and design anticancer drugs.[1]

Jatropha gossypiifolia L. synonym. *Adenoropium gossypiifolium* (L.) Pohl. belongs to the family Euphorbiaceae commonly called as Bellyache-bush. Roots contain antileukemic and tumour-inhibitor macrocyclic diterpene, jatrophone and jatropholones A and B and flavonoids. Bark contains β-sitosterol. Leaves contain flavonoids, saponin, resin, tannin and triterpenes. They also contain flavonoids, vitexin, isovitexin and apigenin [2,3]

Jatropha gossypiifolia (Euphorbiaceae) is used for the treatment of various types of disorders in the ayurvedic and folklore system of medicine in India and Bangladesh. The leaves of the plant are traditionally being applied to boils, carbuncles, eczema, itches, and venereal diseases in Latin America and the Caribbean and also used as febrifuge, bark is used as emmenagogue.[4] Seeds are emetic, purgative and used for cancer and body pain. The leaves and seeds of *Jatropha gossypiifolia* are considered as a purgative and are widely used to treat obstinate constipation. Roots are used to treat leprosy and cancer. Stem latex possess coagulant activity.[5] To establish its traditional uses, *Jatropha gossypiifolia* has been investigated for its anti-allergic, molluscicidal, antimicrobial, insect repellent, Larvicidal, anti-feeding, coagulating, and anti-coagulating activities. One of the most well-known pharmacological activities of *Jatropha gossypiifolia* is its antineoplastic action, which is frequently associated with the content of lignoids and terpenoids. The ethanolic extract from roots, as well as the isolated diterpene jatrophone, exhibited significant inhibitory activity *in vitro* against cells derived from human carcinoma of the nasopharynx and lymphocytic leukemia P-388 and in vivo against four standard animal tumor systems, such as sarcoma 180, Lewis lung carcinoma, P-388 lymphocytic leukemia, and Walker 256 intramuscular carcinosarcoma.[6,7]

MATERIALS AND METHOD

Collection of Plant material and extraction

The roots of *Jatropha gossypiifolia* were collected from Chennai, Tamil Nadu, India. The plant material was identified and authenticated by Botanist Dr.Sasikala Ethirajulu, Research officer, CCRAS, Govt.of India, Chennai. The roots of *Jatropha gossypiifolia* were dried under shade, segregated, pulverized by a mechanical grinder and passed through a 40-mesh sieve. The dried powder of the root was extracted sequentially by hot continuous percolation method by Soxhlet apparatus, using chloroform and ethanol as solvent. The extracts were concentrated by using a rotary evaporator and kept in a desiccator.

Preliminary phytochemical Screening

The extracts obtained were subjected for phytochemical screening using standard procedure. The dried extracts were dissolved in sufficient amounts of respective solvents and tested for various constituents such as alkaloids, glycosides, flavonoids, steroids, saponins, phenolic compounds.[8]

Fractionation by Column Chromatography.

Ethanol extract was subjected for column chromatographic isolation and wet packing method was followed. Initially 3/4th of the column is filled with hexane and then silica gel (100–200 mesh size) is added slowly to ensure uniform packing. 20gms of ethanol extract was chromatographed over a column of 400gms silica gel by gradient elution. The column was developed by elution with hexane (100%) followed by hexane: pet ether (160:40, 150:50, 100:100), pet ether (100%) and pet ether: chloroform (160:40, 150:50, 100:100), chloroform (100%) and chloroform: ethyl acetate (160:40, 150:50, 100:100), ethyl acetate (100%) and ethyl acetate : ethanol (160:40, 160:40, 150:50, 100:100) and finally ethanol 100%. The yellowish green fraction was started eluting from fractions (chloroform 100%), chloroform: ethylacetate (160:40), (150:50), (100:100) and ethyl acetate (100%). The brown colour fraction was started eluting from fractions (ethylacetate: ethanol) and ethanol (100%).

TLC was determined for the above fractions. The fractions were collected 200ml each. TLC was determined for the above fractions. The fractions with a same R_f value were mixed together to finally contain 4 fractions i.e fraction 1 (Chloroform 100%), fraction 2, (Chloroform: Ethyl acetate), fraction 3, (Ethyl acetate: Ethanol), fraction 4 (Ethanol 100%). All these fractions were subjected to *in vitro* anti-cancer activity against MCF-7 cell line to determine the active fraction against MCF-7 cell line.

***In vitro* cytotoxic activity by MTT assay**

In vitro cytotoxic effect on extract and fractions of *Jatropha gossypiifolia* was evaluated by using MTT assay In brief, the trypsinized cells from T-25 flask were seeded in each well of 96-well flat bottomed tissue culture plate at a density of 5x103 cells/well in growth medium and cultured at 37°C in 5% CO2 to adhere. After 48hr incubation, the supernatant was discarded and the cells were pretreated with growth medium and were subsequently mixed with different concentrations of test compounds (0, 0.025, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5mg/ml) in triplicates to achieve a final volume of 100µl and then cultured for 48hr. The extract and fractions was prepared as 1.0 mg/ml concentration stock solutions in PBS. Culture medium and solvent are used as controls. Each well then received 5µl of fresh MTT (0.5mg/ml in PBS) followed by incubation for 2hr at 37°C. The supernatant growth medium was re-moved from the wells and replaced with 100µl of DMSO to solubilize the colored formazan product. After 30min incubation, the absorbance (OD) of the culture plate was read at a wavelength of 572 nm on an ELISA reader, Anthos 2020 spectrophotometer. The percentage cell growth is determined and EC50 is determined.[9,10]

RESULTS AND DISCUSSION

Table -2 depicts the percentage growth of MCF-7 cells in chloroform and ethanol extract treated against MCF-7 cell line was found to be 49.59 and 28.46 respectively. The EC50 value of chloroform and ethanol extract was found to be 0.00018 & 0.00055mg/ml respectively. This clearly indicates that ethanol extract have better anticancer activity on MCF-7 cell line than the chloroform extract. As it was found in preliminary phytochemical screening (Table -1), the ethanol extract shows the presence of flavonoids and phenolic compounds, the better anticancer activity of ethanol extract may be due to the presence of these active compounds. The dose response curve was show in fig 1 & 2 for the extracts.

Ethanol extract was subjected to column chromatography isolation by gradient elution technique. The fractions with the same Rf value were mixed to contain totally of 4 fractions. Fraction 1 (chloroform fraction), fraction 2 (chloroform: ethyl acetate), fraction 3 (ethyl acetate: ethanol) and fraction 4 (ethanol 100%).

(Table -3) & Figure 3 – 6 of dose response curves depicts the *in vitro* anticancer activity from fractions from column chromatography on MCF-7 cell line. The fraction 4 (Ethanol 100%) shows the good anticancer activity compared to other fractions against MCF-7 cell line. The percentage growth of MCF-7 cells in fractions (1 -4) was found to be 24.527, 49.132, 36.909, 14.650 respectively. The EC50 value was found to be for fractions (1 to 4) were found to be 0.00082, 0.1728, 0.00028, 0.00087mg/ml respectively. The cytotoxic effect on maximum concentration

0.5mg/ml of fractions treated against MCF–7 cells was seen in fig 6. This clearly indicates that fraction (IV) shows good anticancer activity against MCF-7 cell line when compared to other 3 fractions. Hence fraction (1V) is an active fraction.

Table: 1 Phytochemical Screening on root extracts of *Jatropha gossipiifolia* Linn

S.No.	Name of the test	Chloroform	Ethanol
1.	Liberman Burchard (for terpenes & steroid)	+	-
2.	Salkowski (for steroid & terpenes)	+	-
3.	Fixed oils and fats.	-	-
4.	Mayer's (for alkaloids)	-	-
5.	Molisch (for carbohydrates)	-	-
6.	Fehlings (for carbohydrates)	-	-
7.	Baljet's test for glycosides	+	+
8.	Legal,s test for glycosides	+	+
9.	Test for Phenolics ($FeCl_3$)	-	+
10.	Shinoda (for flavonoids)	-	+

+ = Presence, - = Absence

Table: 2 Effect of Cytotoxity on MCF -7 Cell line of root fractions of *Jatropha gossypiifolia*

Concentration mg/ml	Vehicle	Fraction 1	Fraction 2	Fraction 3	Fraction 4
	Percentage growth	**Percentage growth**	**Percentage growth**	**Percentage growth**	**Percentage growth**
0	100.0000	100.00 ± 0.33	100.00 ± 0.26	100.00 ± 0.35	100.00 ± 0.23
0.025	98.82798	45.93 ± 0.24	94.49 ±0.33	89.79 ± 0.25	31.73 ± 0.16
0.05	96.75803	40.38 ± 0.45	89.27 ± 0.50	74.10 ± 0.52	26.67 ± 0.32
0.1	94.35255	39.01 ± 0.36	81.99 ± 0.52	67.55 ± 0.45	25.00 ± 0.10
0.2	92.88752	27.29 ± 0.33	73.41 ± 0.45	61.62 ± 0.35	17.76 ± 0.25
0.3	92.06079	27.05 ± 0.42	67.96 ± 0.42	45.84 ± 0.55	17.34 ± 0.35
0.4	91.80057	24.64 ± 0.36	54.86 ± 0.35	37.21 ± 0.16	17.15 ± 0.22
0.5	90.40643	**24.52 ± 0.16** **EC_{50}: 0.00082 mg/ml**	**49.13** ± 0.23 **EC_{50}:0.1728 mg/ml**	**36.90** ± 0.20 **EC_{50}: 0.00028 mg/ml**	**14.65** ± 0.10 **EC_{50}:0.00087 mg/ml**

(a): average of three determinations[Mean ± S.E.M. a]

Table: 3 Effect of cytotoxicity on MCF cell line of extracts of *Jatropha gossypiifolia* Linn

Concentration mg/ml	Vehicle		Chloroform extract		Ethanol extract	
	Absorbance	Percentage growth	Absorbance	Percentage growth	Absorbance	Percentage growth
0	1.05800	100.0000	1.05800	100.00 ±0.42	1.05800	100.00 ± 0.45
0.025	1.04560	98.82798	1.05300	99.52 ± 0.33	0.78225	73.93 ± 0.35
0.05	1.02370	96.75803	0.83300	78.73 ± 0.45	0.75222	71.09 ± 0.26
0.1	0.99825	94.35255	0.68400	64.65 ±0.25	0.70518	66.65 ± 0.35
0.2	0.98275	92.88752	0.52203	49.34 ±0.35	0.56840	53.72 ± 0.23
0.3	0.97400	92.06079	0.52012	49.16 ± 0.16	0.52187	49.32 ± 0.25
0.4	0.97125	91.80057	0.51475	48.65 ± 0.26	0.37820	35.74 ± 0.45
0.5	0.95650	90.40643	0.52475	**49.59** ± 0.16	0.30120	**28.46 ± 0.12**

EC50: 0.00018 mg/ml **EC_{50}: 0.00055 mg/ml**

(a): average of three determinations

Dose response curve of extract and fractions against MCF -7 cell line

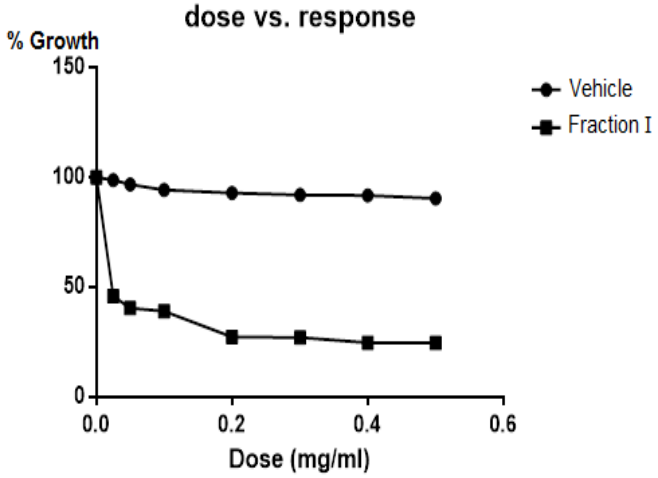

Figure: 1 Chloroform extract against MCF-7 cell

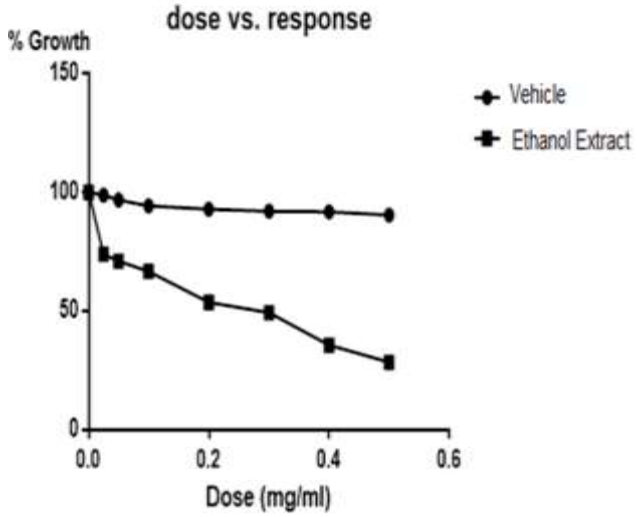

Figure: 2 Chloroform extract against MCF-7 cell

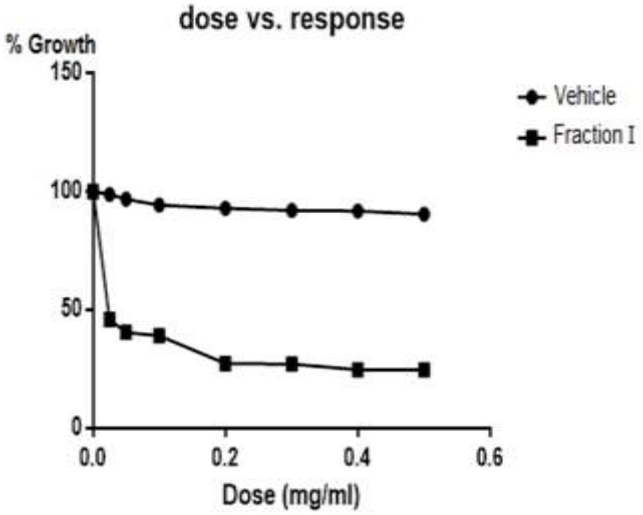

Figure: 3 Fraction 1 against MCF-7 cell

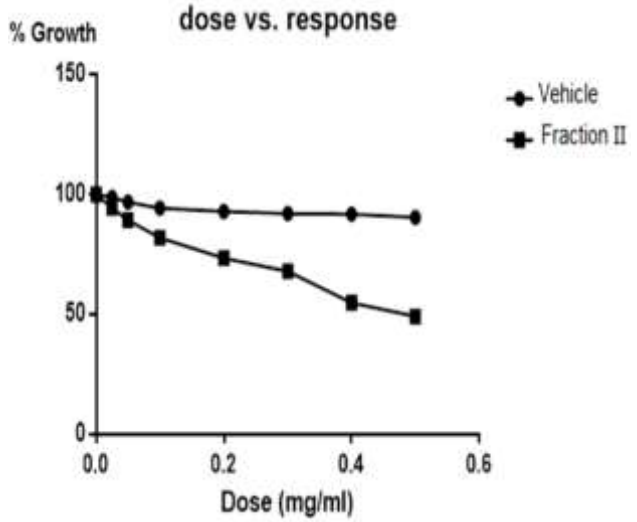

Figure: 4 Fraction 2 against MCF-7 cell

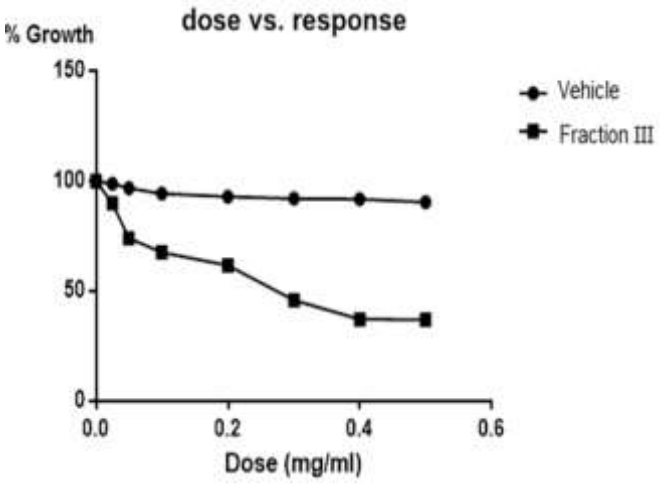

Figure: 5 Fraction 3 against MCF-7 cell

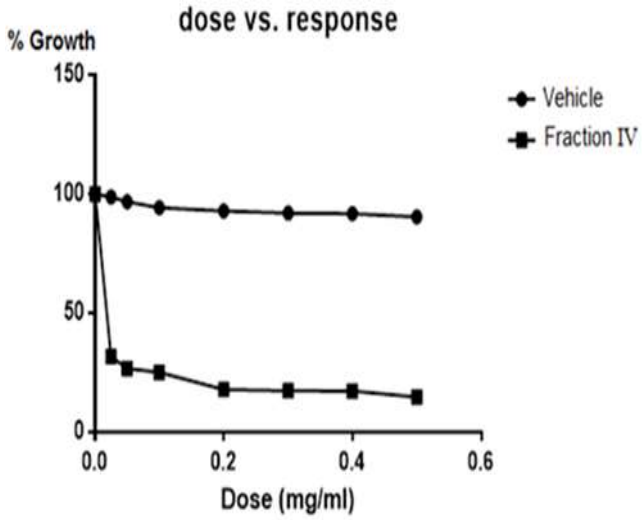

Figure: 6 Fraction 4 against MCF-7 cell

CONCLUSION

The conclusion of the study present study is the ethanol extract shows good anticancer activity against MCF-7 cell line than chloroform extract. The flavonoids and phenolic compounds present in the ethanol extract may be the reason for the activity. The ethanol fraction of *Jatropha gossypifolia* obtained from the active extract by column chromatography is proved to have good anticancer activity against MCF-7 cell line than the other fractions. More research has to be carried out to isolate the active compound from this active fraction from column chromatography.

REFERENCES

1. Ahmedin Jemal DVM, Rebecca Siegel MPH, Jiaquan Xu, MD. Elizabeth Ward. Cancer Statistics. Ca cancer J Clin.2010; 60:277–300
2. Anonymous *Jatropha gossypiifolia* L., Euphorbiaceae. Pacific Island Ecosystems at Risk (PIER): Plant threats to Pacific ecosystems. Institute of Pacific Islands Forestry, Hawaii, USA.2006.
3. Rastogi RP, Mehroti BN. Compendium of Indian Medicinal Plants. 1990; p. 859
4. Dhale DA, Birari AR. Preliminary screening of antimicrobial and phytochemical studies of *Jatropha gossypifolia* Linn. Recent Res Sci Technol. 2010; 2:24–8.
5. Parvathi VS, Jyothi BS, Lakshmi T, Babu PS, Karthikeyan R. Morpho-anatomical and physicochemical studies of *Jatropha gossypifolia* (L.) Der Pharmacia Lettre. 2012; 4:256–62.
6. Oduola T, Avwioro OG, Ayanniyi TB. Suitability of the leaf extract of *Jatropha gossypiifolia* as an anticoagulant for biochemical and haematological analyses. Afr.J Biotechnol. 2005; 4:679–81.

7. Kupchan SM, Sigel CW, Matz MJ, Renauld JAS, Haltiwanger RC, Bryan RF. "Jatrophone, a novel macrocyclic diterpenoid tumor inhibitor from *Jatropha gossypiifolia*, Journal of the American Chemical Society. 1970; 92(14): pp. 4476– 4477.
8. Harborne JB. Phytochemical methods 11th ed.New York: In Chapman &, Hall;1984. 4-5.
9. Mosmann T, Rapid colorimetric assay for cellular growth and survival: application to proliferation and Cytotoxicity assays. Journal of Immunological Methods.1983; 65: 55-63.
10. Yousefi M, Safari M, Torbati MB, and Amanzadeh A. *In vitro* anti-proliferative activity of novel hexacoordinated triphenyltin (IV) Trifluoroacetate containing a bidentate n-donor ligand. Journal of Structural Chemistry.2014; 55(1): 101-106.

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