Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 5, Issue 2, 2013

Research Article

CHARACTERISATION OF THE ACTIVE ANTIPROLIFERATIVE PRINCIPLES OF *JATROPHA CURCUS* AND *JATROPHA GOSSIPPIFOLIA* ON HELA CELL LINES

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Received: 20 Jan 2013, Revised and Accepted: 02 Mar 2013

ABSTRACT

Objective: The process of carcinogenicity presents a major challenge to scientists and provides limited tools for its control. An attempt has been made in the present research, to assess the cytotoxicity of *Jatropha curcus* and *Jatropha gossippifolia* on HeLa cell lines and also to isolate and characterise the active principle having highest anticancer activity in the methanolic and ethanolic extract of *Jatropha curcus and Jatropha gossipifolia* which will be certainly useful for the prevention and treatment of cancers.

Methodology: Crude methanolic and ethanolic fractions of *Jatropha curcus* and *Jatropha gossippifolia* stem were examined for their anticancer activity. The anticancer activity was determined for different concentrations of the crude extract on HeLa cancer cell line by 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay.The extracts were then subjected to high performance liquid chromatography LC Column: Reverse Phase C-18 was used with a detection range of 254 nm.

Results: *Jatropha curcus* and *Jatropha gossippifolia* extracts showed a significant antiproliferative activity with IC50 value of 98.18 µg/ml and 110.6 µg/ml respectively and a dose dependent effect was observed The sample contains a majority of polar compounds because RP Column was able to separate the compounds fast. Of the 55 samples collected from the ethanolic extract of *Jatropha curcus,* the third aliquot was found to have the lowest cell viability. The thirty fifth aliquot out of the 49 isolates from the methanolic extract of *Jatropha curcus* was found to have the lowest cell viability. Out of the 39 isolates from the ethanolic extract of *Jatropha gossipifolia*, the fourteenth aliquot was found to have the lowest cell viability and the second isolate among the 37 isolates from the methanolic extract of *Jatropha gossipifolia* was found to have the lowest cell viability. These four aliquots were then used for further analysis and identification of the compounds responsible for the anticancer property.

Conclusion: Hence the present study revealed that the isolated compounds from ethanolic and methanolic extracts of *Jatropha curcus* and *Jatropha gossypifolia* posses chemoprotective effect and can be used as a potent agent in treating cancer.

Keywords: Anticancer activity, *Jatropha curcus, Jatropha gossypifolia,* 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay, Mitochondrial leakage method (LDH), HPLC, HeLa cancer cell lines.

INTRODUCTION

The process of carcinogenicity presents a major challenge to scientists and provides limited tools for its control. As cancer progresses, it metastasizes – invading the surrounding tissues, entering the blood stream, spreading and establishing colonies in distant parts of the body [1]. Cervical cancer is the principal cause of cancer-related mortality in women of the developing countries that contribute more than 85% of global disease burden [2].

The greatest burden of cervical cancer is found in underserved, resource-poor populations, in which 80% of all incident cervical cancer and related mortality occurs [3]. There is good evidence that in some age groups there has been a large increase in the incidence of carcinoma *in situ* of the cervix. The extent of this effect cannot be quantified precisely because of uncertainties concerning the natural history of cervical cancer, differences in risk for different cohorts, and the possible effects of other factors[4]. Any practical solution in combating this dreadful disease is of paramount importance to public health [5]. The median survival rate of one year has remained essentially unchanged for a number of years despite aggressive treatment regimens that include surgery, radiation and chemotherapy [6]. This neoplastic is an excellent model for studying the mechanisms involved in cancer maintenance, because the Human Papilloma Virus (HPV) is the etiology factor in most cases [7].

HPV- vaccines have been shown to be quite safe in preventing cervical cancer and genital warts. Although long-term protection is a key-point in evaluating HPV-vaccine over time, there is currently inadequate information on the duration of HPV vaccine-induced immunity and on the mechanisms related to the activation of immune-memory [8]. Though the cervical cancer therapy is in advance, side effects due to the non-specific cytotoxicity of drugs and resistance to treatment represent a great problem in the cervical cancer management. Therefore, development and search of novel and effective anticancer agents, which in addition should overcome resistance, have become very important issues [9].

Human cancer cell lines represent a mainstay of tumour biology and drug discovery through facile experimental manipulation, global and detailed mechanistic studies, and various high-throughput applications. Numerous studies have used cell-line panels annotated with both genetic and pharmacological data, either within a tumour lineage [10,11].

For the last few decades, phytochemical examination has been making rapid progress and herbal products are becoming popular as sources of possible anticancer compounds [12]. Phytochemicals like alkaloids and flavanoids help in preventing the formation of potential carcinogens (substances that cause cancer), block the action of carcinogens on their target organs or tissue, or act on cells to suppress cancer development [13]. Some of the links between individual phytochemicals and cancer risk found in laboratory studies are compelling and make a strong case for further research [14]. The interaction between certain phytochemicals and the other compounds is proved to be dangerous and it is very much necessary to devise a standardized extraction procedure for crude drug from the medicinal plant parts to attain the therapeutically desired portion [15]. Pharmaceutical research across the world shows that natural products are potential sources of novel molecules for drug development[16]

Numerous cancer research studies have been conducted using traditional medicinal plants in an effort to discover new therapeutic agents that lack the toxic side effects associated with current chemotherapeutic agents [17]. Therefore the identification of active plant chemicals is an essential component of modern pharmacognosy and medical effects are not necessarily restricted to a single plant chemical. The biological activity and clinical value of the whole plant, as in medicinal herbalism, is also being pursued [18].

Among the different species of *Jatropha* reported, *Jatropha curcus* and *Jatropha gossipifolia* is considered the most important for its medicinal properties. *Jatropha curcus* possess anti-bacterial, anticancer and immunosuppressive activity [19] while Ethno botanical uses of *J. gossypifolia* has been reported for cancer, diarrhoea, dysentery, skin diseases leprosy), arthritis, ulcer, gum infections and wound healing [20,21,22]. The phytochemical investigation of the genus *Jatropha* has identified more than 150 compounds with varying structural patterns. Among these constituents, saponins, steroids, alkaloids, phenolic groups and flavanoids form the major class [19].

Despite their widespread use, however, no scientific assessment for anticancer effect has been conducted in most cases. It has been found that the studies carried out in *Jatropha curcus* and *Jatropha gossippifolia* had not explored the anticancer activities of these plants and have not made any attempt to characterise and explore the active principles responsible for combating this dreadful disease. Though ample literatures on therapeutic application of these plants are available but data on the proximate compounds responsible for the anticancer activity of these plants are very scarce. Considering their increasing recognition, the present study was undertaken to evaluate the anticancer potential of these plant extracts in the inhibition of cell proliferation.

An attempt has been made in the present research, to assess the cytotoxicity of *Jatropha curcus* and *Jatropha gossippifolia* on HeLa cell lines and also to isolate the aliquot having highest anticancer activity using high performance liquid chromatography in the methanolic and ethanolic extract of *Jatropha curcus and Jatropha gossipifolia* which will be certainly useful for the prevention and treatment of cancers.

MATERIALS AND METHODS

Phytochemical analysis of *Jatropha Curcus* and *Jatropha Gossipifolia*

Test for Alkaloids

Dragendroff's test

8g of Bi(NO3)3.5H2O was dissolved in 20 ml of HNO3 and 2.72g of KI in 400ml of water. These were mixed and allow to stand when KNO3 crystals out. The supernatant was decanted off and made up to 100ml with distilled water. The alkaloids were regenerated from the precipitate by treating with sodium carbonate followed by extraction of the liberated base with ether. To 50ml of alcoholic solution of extract was added to 2ml of HCl. To this acidic medium 1ml of reagent was added. An orange red precipitate produced immediately indicates the presence of alkaloids.

Wagner's test (Iodine- Potassium iodide solution)

1.2 gm of Iodine and 2gm of H2SO4 and the solution was diluted to 100ml. 10ml of alcoholic extract was acidified by adding 1.5% v/v of HCl and a few drops of Wagner's reagent. Formation of yellow or brown precipitate confirmed the presence of alkaloids.

Test for Glycosides

A small amount of alcoholic extract was dissolved in 1.0ml of water and the aqueous NaOH solution was dissolved in 1.0ml of water and the aqueous NaOH solution was added. Formation of yellow color indicates the presence of glycosides.

Test for Tannins

Ferric chloride test

To 1-2 ml of aqueous extract few drops of 5% aqueous $FeCl_2$ solution was added. A bluish black color disappears on addition of a few ml of H_2SO_4 and when there is no formation of the yellow brown precipitate indicates the presence of tannins.

Test for Flavanoids

In a test tube containing 0.5ml of alcoholic extract, 5-10 drops of dilute HCl and small piece of ZnCl or magnesium were added and the solution was boiled for a few minutes. In the presence of flavanoids, reddish pink or dirty brown color was produced.

Test for Saponins

In a test tube containing 0.5ml of aqueous extract a drop of sodium bicarbonate was added. The mixture was shaken vigorously and kept for 30 minutes. A honey comb like froth was formed which showed the presence of saponins.

Test for Steroid

Salkowski test

To 2ml of chloroform extract, 1ml of concentrated sulphuric acid was added carefully along the sides of the test tubes. A red color was produced in the chloroform layer in the presence of steroids.

Test for Phenols

Ferric chloride

To 10ml of alcoholic solution of extract, 2ml of distilled water followed by drops of 10% aqueous FeCl3 solution were added. Formation of blue or green indicates the presence of phenols.

Invitro antioxidative effect of *Jatropha Curcus* and *Jatropha Gossipifolia* extract

Nitric oxide radical scavenging assay [23]

Sodium nitro prusside in aqueous solution at physiological pH spontaneously generates nitric oxide which interacts with O_2 to produce nitrite ions, which can be measured at 540nm spectrophotometrically in the presence of Griess reagent.

Procedure: 5mg of extract was dissolved and made up to 10ml with methanol. The sample was made completely soluble. 50µl of 10mM sodium nitro prusside and 50µl test solution of various concentrations are illuminated using fluorescence light at room temperature for 150 minutes. Following incubation, 125µl of Griess reagent was added and incubated for 30 minutes at room temperature. The absorbance was measured at 546nm.

(Griess reagent: 1% sulphanilic acid, 2% phosphoric acid and 0.1% N-1- napthyl ethylene diamine dihydrochloride)

Reducing power [24,25]

The sample solution was mixed with 2.5ml of 0.2M of phosphate buffer (pH 6.6) and 2.5ml of 1% potassium ferricynaide. The mixture was incubated at 50^0 C for 20 minutes and aliquot (2.5ml) of 10% TCA was added to mixture followed by centrifugation at 3000rpm for 10 minutes. The upper layer of solution was mixed with 2.5ml of distilled water and 2.5ml of 0.1% $FeCl_3$ and absorbance at 700nm. Increased absorbance shows increase in reducing power.

Lymphocyte Culture Medium

Human peripheral lymphocytes were cultured in R P M I 1640 MEDIUM (Himedia) Supplemented with inactivated fetal bovine Serum (Himedia), antibiotics (Streptomycin and Penicillin) Phyto haemagglutinin (Himedia), was used as the stimulant for proliferations of lymphocytes. The culture was filter sterilized using 0.2µm pore size cellulose acetate filter (Sartorius) Fresh plasma was aseptically added to the culture flasks at a concentration of IX 10 G cells / ml the culture flasks were incubated for 72 hours.

Effect of *Jatropha Curcus* and *Jatropha Gossipifolia* Extract on Mitogen Activated Human Peripheral Lymphocyte

Effect of Concentration of *Jatropha Curcus* and *Jatropha Gossipifolia* on total cell Viability of Human Peripheral Lymphocyte

Invitro cytotoxicity testing was based on the effect of Jatropha curcas on lymphocyte cell viability. A standard cytotoxicity assay was employed to determine the effective 50mg/dl, 100mg/dl, 200mg/dl and 400mg/dl concentrations that reduces the lymphocyte cell vaibility.The cell suspension was prepared for trypan blue assay.

Trypan Blue Cell Exclusion Assay [26]

A 1:1 dilution of the culture cell suspension with a 0.4% tryphan blue solution (1:1 dilution PBS) was charged on to the counting chamber of

a haemocytometer and counted at 40x. Stained viable cells and dead cells are counted and the percentage of viability is calculated.

Viability was calculated as per the formula given below

Viability = A/B ×100

A-Number of viable cells

B-Total number of viable and dead cells.

Effect of Incubation Time of *Jatropha Curcus* and *Jatropha Gossipifolia* Extract on Human Peripheral Lymphocytes

The cultured human peripheral lymphocytes were exposed to concentrations of Jatropha curcas and Jatropha gossipifolia at different time intervals of growth incubation (24th, 48th and 72nd hour) as per standard protocols. (Preston et al., 1987) This is the optimum time for addition of the compounds. If the chemical is given in the Go phase, the response to PHA is affected leading to delay in mitosis of growing lymphocytes. In addition, if the agent is short lived, the cells are only exposed in G_0/G_1 phase, which for many compounds is a relatively insensitive stage of the cell cycle. Control culture flasks without treatment Jatropha curcas were also maintained.

Effect of *Jatropha Curcus* and *Jatropha Gossipifolia* Extract on Antioxidant Enzymes of Mitogen Activated Human Peripheral Lymphocytes

Estimation of superoxide dismutase activity [27]

SOD is a well-known antioxidative enzyme with the activity to convert superoxide into hydrogen peroxide. The super oxide dismutase assay is used as a parameter indicating free radical scavenging efficiency

SOD uses the photochemical reduction of riboflavin as oxygen generating system and catalyses the inhibition of NBT reduction, the extent of which can be analysed spectrophotometrically at 600 nm. 0.5ml of plant extract was pipetted into the test tube containing 300 µl potassium phosphate buffer, 1.35 µl methionine, 0.159 µl riboflavin and 2.52 µl NBT and the change in absorbance was read spectrophotometrically

Determination of reduced glutathione [28]

The tripeptide GSH is present in cells at millimolar concentrations. Under normal conditions, most of the GSH is maintained in its reduced form by glutathione reductase. GSH reacts readily with reactive oxygen species thereby providing protection against oxidative injury. Continual production of reactive oxygen species, such as H_2O_2, OH and lipid peroxides lead to accumulation of oxidized glutathione, and a concomitant reduction in the level of GSH provides a relevant and accurate measure of the oxidative state of the cell.

1 ml plant extract was pipetted into the test tube containing 0.5 ml phosphate buffer, 1.3 ml distilled water and 0.2 ml DTNB and read the absorbance at 420 nm.

Estimation of lipid peroxidation [29]

An invitro model of Human peripheral lymphocyte cell extract was used for induction of lipid peroxidation.The presence of oxidative compound will result in peroxidation of intracellular lipids. 50µl of supernatant was taken for the induction of lipid peroxidation. The volumes in the test tubes were made up to 500 µl with cold TBS. Incubated the tubes at 37^0C for 1 hour. Following the incubation period 500 µl of 70% alcohol was added to all the tubes to stop the reaction. 1.0ml of TAB was added to all the tubes followed by boiling in a hot bath for 20 minutes. After cooling to room temperature added 50µl of acetone and measured the TBARS at 535 nm in a spectrophotometer.

Invitro anticancer effect of *Jatropha Curcus* and *Jatropha Gossipifolia* extract on hela cell lines

MTT Assay [30]

The cell culture suspension was washed with 1X PBS and then added with 200µl MTT solution to culture (MTT – 5mg/vol dissolved in PBS).Then incubated at 37^0C for 3hrs. Removed all MTT, wash with 1X PBS and add 300µl DMSO to each culture. Incubated at room temperature for 30min until the cell gets lysed and colour is obtained. The solution was transferred to centrifuge tubes and centrifuged at top speed for 2 min to precipitate cell debris. OD is measured at 540nm using DMSO blank.

Isolation of the active principles from *Jatropha Curcus a*nd *Jatropha Gossipifolia* using high performance liquid chromatography (HPLC)

Prep HPLC Parameters

LC Column: Reverse Phase C-18

Pump: SPD 10 AVP

Mobile phase: Water: Methanol (50:50)

Ionisation Mode: not applied

Injection Volume: 5 ml

Concentration: Total extract in 5 ml

Flow rate: 2 ml/min

Column Dimension: 50 cm X5 mm

LC Detection : 254 nm

Soft ware: Class V P Integrated.

Collector: Automatic GD fraction Collector.

Collected volumes: 1 ml (Ependroffs tube)

Run Time: 25 Minutes

Total fractions: Varies on samples

Code Specification: Sample 1= A-1…………A-50 *J.curcus* (ethanol)

Sample 2= B-1………..B-50 *J.curcus (*methanol)

Sample 3 = C-1……..C-30 *J.gossipifolia (*ethanol)

Sample 4 = D-1………..D-30 *J.gossipifolia* (methanol)

Anticancer activity of the Isolated active principle from *Jatropha curcus* and *Jatropha gossipifolia* using MTT and Neutral Red

MTT Assay

Neutral red Assay

The response of the cultures to cadmium chloride solutions will be examined.The cultures containing different concentrations of the extract is exposed to Neutral red, to a final concentration of 50 µg/ml. After an additional 1 hour exposure, the medium is removed, rinsed the cells and determined the Neutral red uptake. Followed the protocol below (rinse and extraction was done carefully as weakened and dying cells may easily get dislodged from the plate and loss of cells will distort the results). The amount of NR will be determined spectrophotometrically.

RESULTS AND DISCUSSION

Preliminary photochemical screening of *Jatropha curcus a*nd *Jatropha gossipifolia*

Table: 1 and 2 shows that the ethanolic and methanolic extracts of Jatropha *curcus and Jatropha gossipifolia* have the maximum phytochemicals such as alkaloids and flavanoids and hence these can be taken for further assays.

Traditional indigenous medicine is limited to small tribal and geographical areas called "little traditions" are an excellent repository of knowledge about medicinal properties of botanical sources. (Kamboj, 2000) stated that the bioactive extract should be standardized on the basis of phytochemical compounds

Flavonoids are important antioxidants and help in removal of oxidative stress. The main drawback with the utilization of synthetic antioxidants is the side effects associated with them when taken *in vivo* (Chen et al., 1992). During the present study,

it was found that ethanolic and methanolic extracts of *Jatropha curcus and Jatropha gossipifolia* possessed phenolic, flavonoid and alkaloid content and therefore, could be potential sources of antioxidants.

Figure 1 showing the Nitric oxide scavenging assay gave the IC50 value of 37.20 µg/ml for *Jatropha curcas* and >250 µg/ml for *Jatropha gossypifolia*. Reducing power assay gave IC50 of 41.20 µg/ml for *Jatropha curcas* and 25.33 µg/ml for *Jatropha gossypifolia* [Figure 2]. The reducing power increases with increasing concentration which are in par with the results of Nagarajan[31].

Suppression of released NO may be partially attributed to direct NO scavenging, ethanolic and methanolic extracts of *Jatropha curcus and Jatropha gossipifolia* decreased the amount of nitrite generated from the decomposition of SNP *in vitro.* The scavenging of NO by the extracts was increased in dose dependent manner. Figure-1 illustrates a significant decrease in the NO radical due to the scavenging ability of ethanolic and methanolic extracts of *Jatropha curcus and Jatropha gossipifolia*. ethanolic and methanolic extracts of *Jatropha curcus and Jatropha gossipifolia* showed IC50 value of 37.20 µg/ml for *Jatropha curcas* and >250 µg/ml for *Jatropha gossypifolia*.

Table 1: Phytochemical Analysis of *Jatropha curcus*

Phytochemicals	Methods employed	Petroleum extract	Chloroform extract	Methanol extract	Ethanol extract	Aqueous extract
Alkaloids	Dragendorff's Test	-	-	+	+	-
	Wagner's Test	-	-	+	+	+
Glycosides	Bromine water test	-	-	+	-	-
Tannins	Ferric Chloride Test	-	-	+	-	-
Flavonoids	Zinc Hydrochloride Reduction Test	-	-	+	+	-
Saponins	Froth test	-	-	-	-	-
Steroids	Salkowski test	-	-	+	+	-
Carbohydrates	Molisch Test	-	-	-	-	-
Phenols	Ferric Chloride Test	-	-	+	+	-

Table 2: Phytochemical Analysis of *Jatropha gossipifolia*

Phytochemicals	Methods employed	Petroleum extract	Chloroform extract	Methanol extract	Ethanol extract	Aqueous extract
Alkaloids	Dragendorff's Test	-	-	+	+	-
	Wagner's Test	-	-	+	+	-
Glycosides	Bromine water test	-	-	+	-	-
Tannins	Ferric Chloride Test	-	-	-	-	-
Flavonoids	Zinc Hydrochloride Reduction Test	-	-	+	+	+
Saponins	Froth test	-	-	-	-	-
Steroids	Salkowski test	-	-	+	+	-
Carbohydrates	Molisch Test	-	-	-	-	-
Phenols	Ferric Chloride Test	-	-	+	+	-

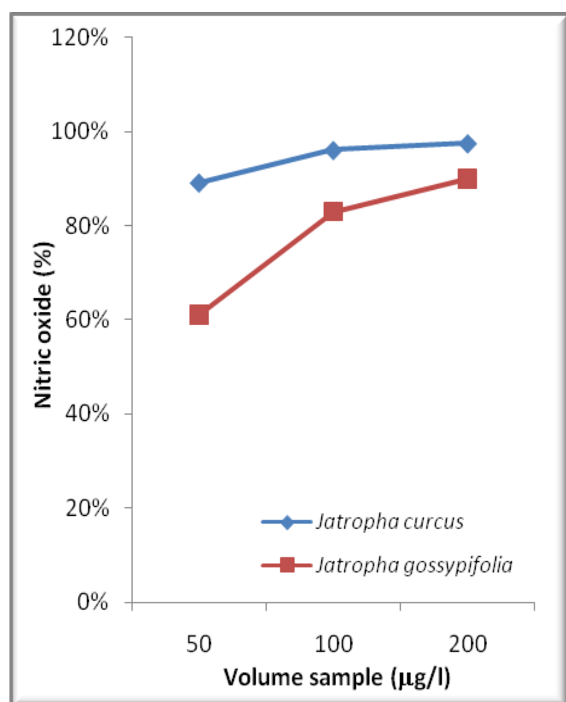


Fig. 1: Nitric oxide scavenging assay

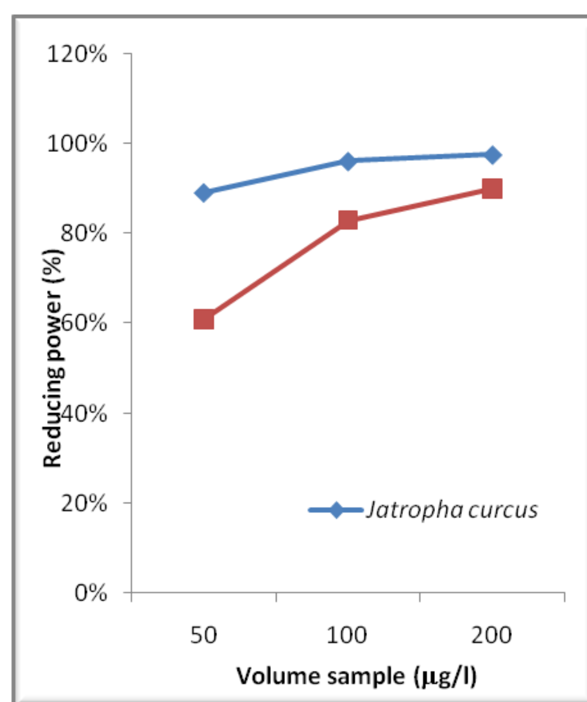


Fig. 2: Reducing Power

Fig 1 & 2: Invitro Antioxidative effect of *Jatropha curcus* and *Jatropha gossipifolia* extract

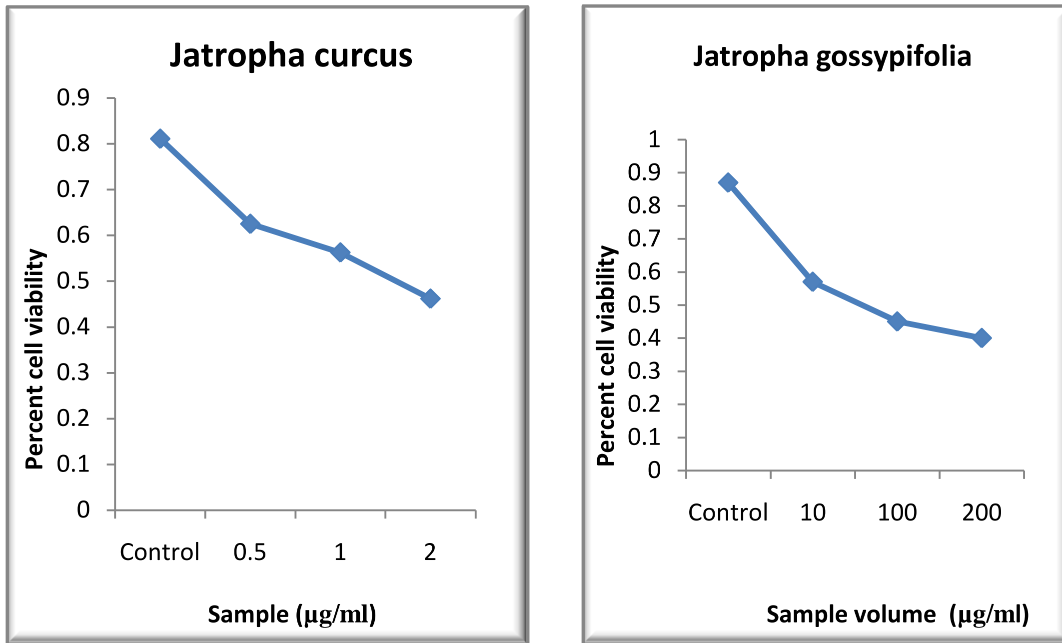


Fig. 3 & 4: Effect of *Jatropha curcus* and *Jatropha gossipifolia* extract on mitogen activated Human Peripheral Lymphocyte using Trypan Blue assay

From the figure 3 & 4, it can be observed that increase in concentration of *Jatropha curcus* and *Jatropha gossypifolia* extract decreases the total cell viability.

Using the trypan blue test, the ethanolic and methanolic extract of *Jatropha curcus* and *Jatropha gossypifolia* extract exhibited a remarkable reduction against Human peripheral lymphocytes in a concentration- and time-dependent manner (Fig. 3 & 4). The mean 50% inhibitory concentration (IC 50) of both ethanolic and methanolic extract of *Jatropha curcus* and *Jatropha gossypifolia* on cell viability was at a concentration of 200 µg/ml at 48 hours.

Figure 5 & 6 shows a maximum reduction of superoxide dismutase and reduced glutathione at a concentration of 100 µg/ml and 200 µg/ml respectively for *Jatropha curcus* and *Jatropha gossypifolia* extract.

As per our results the increase in the *Jatropha curcus* and *Jatropha gossypifolia* extract concentration decreased the SOD and glutathione levels.The results showed that both the both *Jatropha curcus* and *Jatropha gossypifolia* plants have good antioxidant activity.

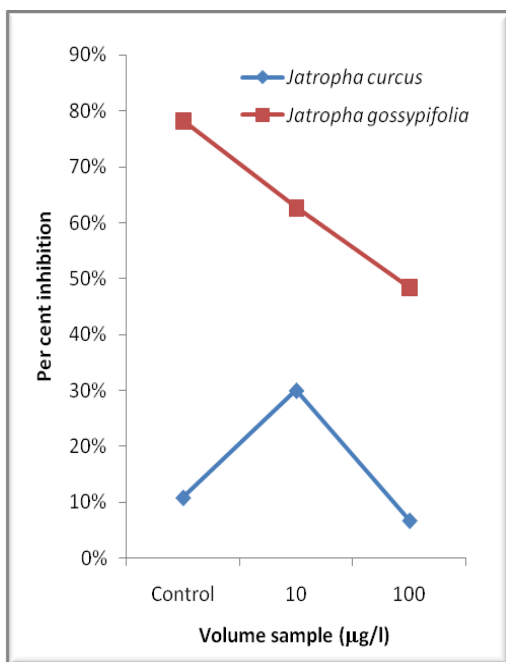


Fig. 5: Superoxide dismutase

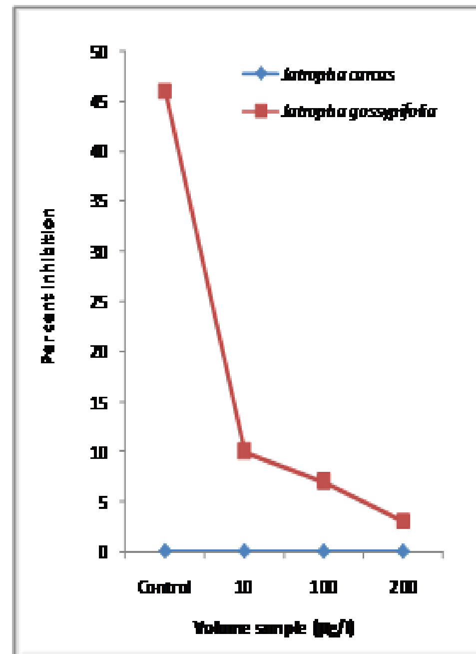


Fig. 6: Reduced glutathione

Fig. 5 & 6: Effect of *Jatropha Curcus* and *Jatropha Gossipifolia* extract on antioxidant enzymes of mitogen activated human peripheral lymphocytes

Table 3: Lipid peroxidation

Sample Volume (μg/ml)	*Jatropha curcus*	*Jatropha gossipifolia*
	Enzyme units/ml	Enzyme units/ml
Control	78 units/ml	83 units/ml
10	56 units/ml	67 units/ml
100	44 units/ml	58 units/ml
200	38 units/ml	41 units/ml

Table 3 depicts a maximum production of TBARS at a concentration of 200μg/ml from the stock of *Jatropha curcus* and *Jatropha gossypifolia*.

The increase in concentration of the extract showed the proportional increase in TBARS production suggesting induction of toxicity by the study *Jatropha curcus* and *Jatropha gossypifolia* extract.

Invitro anticancer effect of *Jatropha curcus* and *Jatropha gossipifolia* extract on HeLa cell lines

Cell viability is measured by the metabolism of 3-(4, 5-dimethyl-2-thiazolyl)-2, 5diphenyl-2*H* tetrazolium bromide (MTT) (Mosmann, 1983) and neutral red uptake (NRU) ((Nabavi *et al,* 2008). The ethanolic and methanolic extracts of *Jatropha curcus* and *Jatropha gossypifolia* induced a dose dependent inhibitory effect against HeLa cell lines. Although there was a suppression effect on cell replication for the concentration 100 μg/ml, it could not be expressed as a reasonable anticancer activity. The significant inhibition of cancer cell growth was found at a concentration of 300 μg/ml, whose estimated IC_{50} (98.18 μg/ml and 110.6 μg/ml) exhibited the highest cytotoxicity (> 50%) in all the extracts.

Isolation of the active principles from *Jatropha Curcus* and *Jatropha Gossipifolia* using high performance liquid chromatography (HPLC)

Figure 8 shows the *Jatropha curcus* separation on the mobile phase which a mixture of methanol – acetonitril –water (60:20:20:v/v/v) under other chromatographic conditions showed high performance in the separation of the flavonoid compounds.

Table 4: MTT assay - methanolic extract of *Jatropha curcus*

Concentration	18.75 μg	37.5 μg	75 μg	150 μg	300 μg	Cont μg
ABS	0.428	0.387	0.292	0.117	0.007	0.438
	0.42	0.377	0.281	0.138	0.008	0.421
	0.415	0.361	0.291	0.107	0.007	0.431
Average	**0.421**	**0.375**	**0.288**	**0.120667**	**0.007333**	**0.43**

Table 5: MTT assay - % Cell Inhibitionand IC 50 of methanolic extract of *Jatropha curcus*

Concentration(μg)	MTT Assay (% Cell Inhibition)	IC 50	R^2
18.75	2.093023		
37.5	12.7907	98.18 μg/ml	0.9939
75	33.02326		
150	71.93798		
300	98.29457		

Table 6 : MTT assay methanolic extract of *Jatropha gossippifolia*

Concentration	18.75 μg	37.5 μg	75 μg	150 μg	300 μg	Cont μg
ABS	0.424	0.374	0.282	0.178	0.015	0.438
	0.429	0.415	0.276	0.186	0.01	0.421
	0.435	0.391	0.279	0.174	0.009	0.431
Average	0.429	0.393	0.279	0.179	0.01133	0.43

Table 7: MTT assay - % Cell Inhibitionand IC 50 of methanolic extract of *Jatropha gossippifolia*

Concentration(μg)	% Cell Inhibition	IC 50	R^2
18.75	0.155039		
37.5	8.527132	110.6 μg/ml	0.9766
75	35.11628		
150	58.29457		
300	97.36434		

Figure 9 shows the *Jatropha gossipifolia* separation on the mobile phase which a mixture of methanol – acetonitril –water (60:20:20:v/v/v) under other chromatographic conditions showed high performance in the separation of the flavonoid compounds.

Around 55 samples were collected from the ethanolic extract of *Jatropha curcus,* 49 samples from the methanolic extract of *Jatropha curcus,* 39 samples for ethanolic extract of *Jatropha gossipifolia,* 37 samples from methanolic extract of *Jatropha gossipifolia*.

Nazeema ABone

Date/Time: 2011-02-05,3:20:52 PM
Data File: C:\N2000\hashim\Sample0001.org

Analyst: Hashim
Date/Time: 2011-02-07,8:52:37 PM
Quantification: Area/Area%

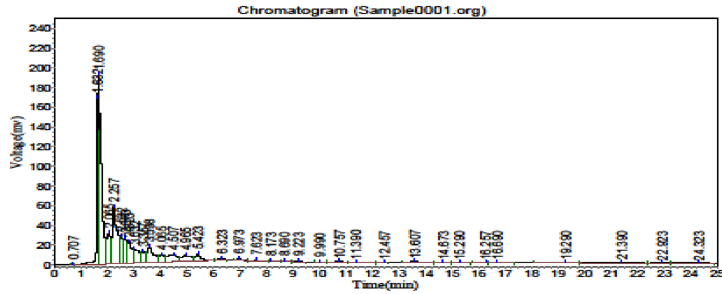
Type of Instrument: LC

Gradient:High Presure

Detector:UV
Wavelength(nm):254nm

Sample Description:
Methanol:Water
50:50
flowrate:1.0ml/min
Sample

Column:Grace
Part no:5144984
Dimensions:25cmx4.6mm,5-microns



Results

Peak No.	Peak ID	Ret Time	Height	Area	Conc.
1		0.707	156.455	452.700	0.0088
2		1.632	171368.719	584515.813	11.3284
3		1.690	192780.797	1505581.875	29.1794
4		2.065	30993.527	343274.688	6.6529
5		2.257	57659.613	740725.563	14.3559
6		2.532	26976.084	177002.328	3.4304
7		2.673	25281.539	207636.188	4.0242
8		2.823	21598.432	243788.078	4.7248
9		3.032	13278.394	204701.047	3.9673
10		3.357	10996.495	118340.359	2.2935
11		3.598	15206.211	270859.469	5.2495
12		4.065	6808.767	102628.594	1.9890

Fig. 7: HPLC of methanolic *J. curcus*

Nazeema EF-three

Company:
Date/Time: 2011-02-05,4:55:16 PM
Data File: C:\N2000\hashim\Sample0002.org

Analyst: Hashim
Date/Time: 2011-02-07,9:02:53 PM
Quantification: Area/Area%

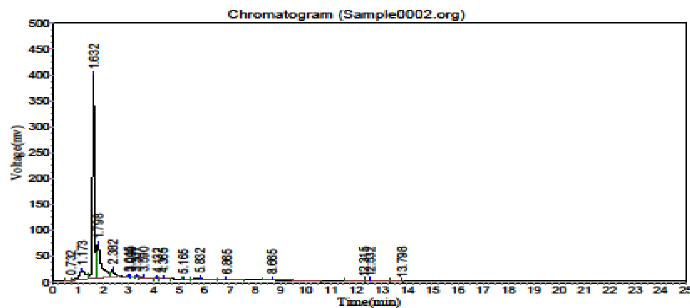
Type of Instrument: LC

Gradient:High Presure

Detector:UV
Wavelength(nm):254nm

Sample Description:
Methanol:Water
50:50
flowrate:1.0ml/min
Sample

Column:Grace
Part no:5144984
Dimensions:25cmx4.6mm,5-microns



Results

Peak No.	Peak ID	Ret Time	Height	Area	Conc.
1		0.732	53.308	197.850	0.0056
2		1.173	16996.449	299664.781	8.4104
3		1.632	397299.063	2233997.250	62.6997
4		1.798	64803.461	830190.625	23.3002
5		2.382	13152.874	127304.219	3.5729
6		3.015	456.207	2189.354	0.0614
7		3.098	719.966	6237.121	0.1751
8		3.307	853.862	5112.019	0.1435
9		3.590	1247.046	4180.000	0.1173
10		4.132	97.706	609.017	0.0171
11		4.365	552.000	8049.870	0.2259
12		5.165	79.364	166.500	0.0047

Fig. 8: HPLC of methanolic *J. gossipifolia*

Anticancer activity of the isolated active principle in ethanolic and methanolic extracts of *Jatropha curcus* and *Jatropha gossipifolia*

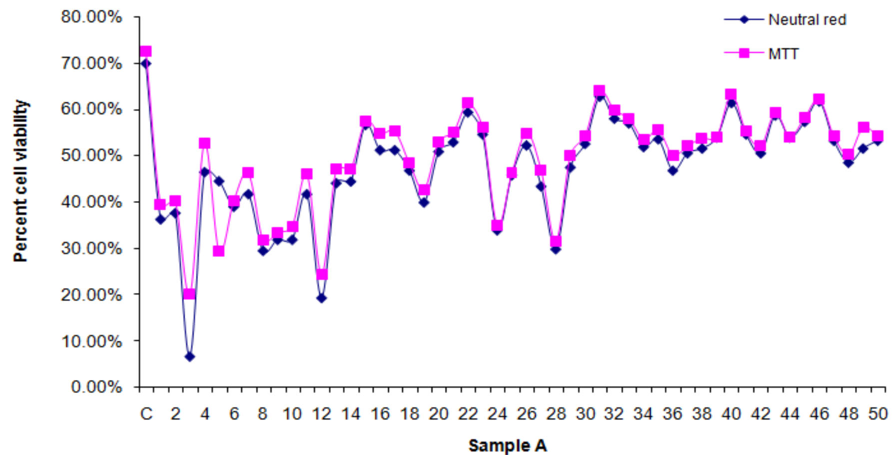


Fig. 9 : Total cell viability assay Ethanolic extract of *Jatropha curcus*

The third isolate from HPLC of the ethanolic extract of *Jatropha curcus* was found to have the lowest cell viability using MTT and Neutral red assay. The active principles responsible for the anticarcinogenic property can be isolated from this particular aliquot of *Jatropha curcus*

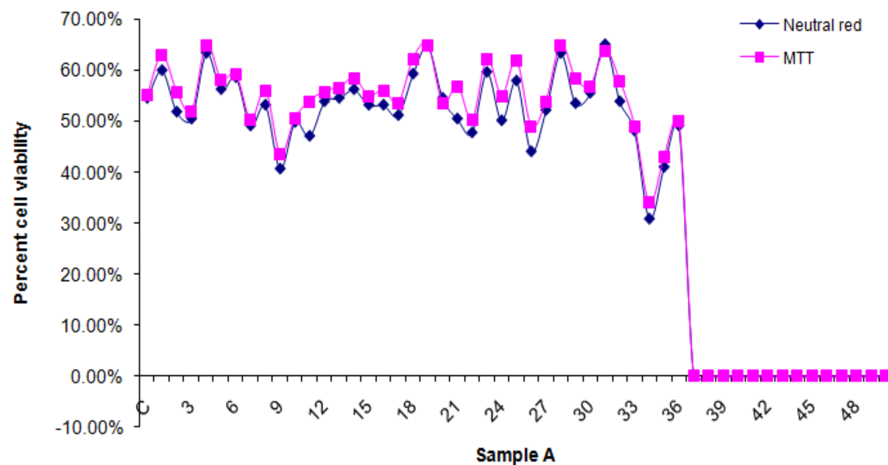


Fig. 10 : Total cell viability assay Methanolic extract of *Jatropha curcus*

The thirty fifth isolate from HPLC of the methanolic extract of *Jatropha curcus* was found to have the lowest cell viability using MTT and Neutral red assay. The active principles responsible for the anticarcinogenic property can be isolated from this particular aliquot of *Jatropha curcus*

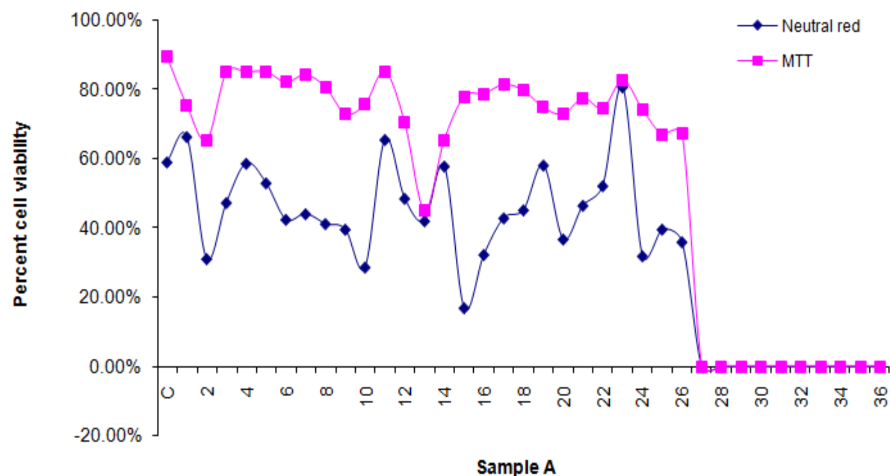


Fig. 11: Total cell viability assay Ethanolic extract of *Jatropha gossipifolia*

The fourteenth isolate from HPLC of the ethanolic extract of *Jatropha gossipifolia* was found to have the lowest cell viability using MTT and Neutral red assay. The active principles responsible for the anticarcinogenic property can be isolated from this particular aliquot of *Jatropha gossippifolia*

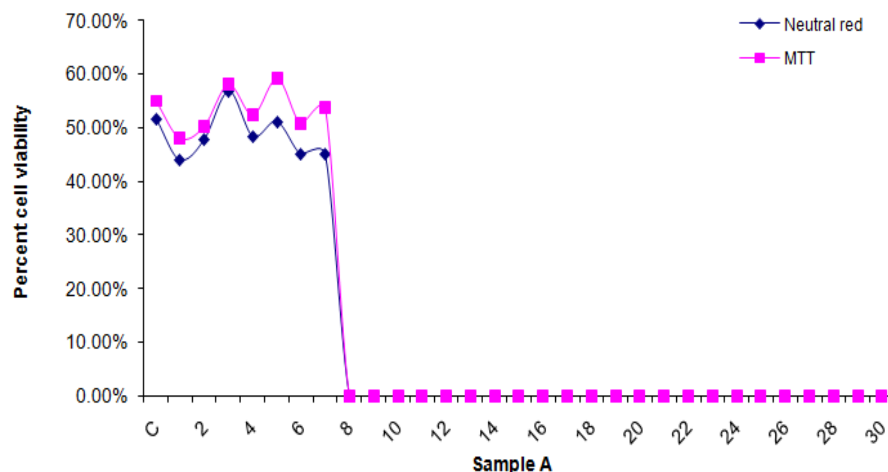


Fig. 12: Total cell viability assay Methanolic extract of *Jatropha gossipifolia*

The second isolate from HPLC of the ethanolic extract of *Jatropha gossipifolia* was found to have the lowest cell viability using MTT and Neutral red assay

CONCLUSION

Based on the above results, the ethanolic and methanolic extracts of *Jatropha curcus* and *Jatropha gossypifolia* showed that the four aliquots have the highest anticarcinogenic property. It has also proved its ability to quench the free radicals. The cytotoxicity of ethanolic and methanolic extracts of *Jatropha curcus* and *Jatropha gossypifolia* can be studied on the expression of apoptotic regulator molecules.Therefore, the potential growth inhibitory activity of the isolated aliquots from ethanolic and methanolic extracts of *Jatropha curcus* and *Jatropha gossypifolia* should be considered for further development as a cancer chemopreventive or chemotherapeutic agent against cervical cancer.

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