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Research Paper



In vitro cytotoxic activity of aqueous extract of *Delonix elata* (L.) gamble (fabaceae) leaves on mcf-7 & hep g-2 cell lines

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Abstract

To investigate, *in vitro* cytotoxic effect of aqueous extract of *Delonix elata* leaves belongs to family Fabaceae. The dried *Delonix elata* leaf extract was used to determine the qualitative preliminary phytochemical analysis and *in vitro* cytotoxic assay against breast cancer (MCF-7) & Hepatic cancer (HepG2) cells by using MTT assay method. Phytochemical evaluation revealed the presence of flavonoids, tannins, phenolic compounds, steroids and saponins and this result was supported by IR data, which exhibits anticancer activity against breast and hepatic cancer cell lines. So the current investigation showed that *Delonix elata* leaf extract possesses the dose dependent cytotoxic activity against breast and hepatic cancer cell lines.

Keywords: *Delonix elata, Aqueous Extract, Flavonids, MCF-7 & Hep G2 Cell Line.*

1. Introduction

Cancer is the major disorder, which increases morbidity and mortality in both male and females (Yu et al., 2006; Jemal et al., 2010). Chemotherapy, Radiotherapy and Surgery were used for the treatment of cancers. Even though different therapies exists, the incidence of cancer deaths increased overwhelmingly over a few decades, because they were found to have limited survivability with potential toxic effects on cells (Fadeyi et al., 2013; Mathi et al., 2014). Hence, there is a need for development of effective targets against cancer cells with minimal toxic effects on normal cells (Cragg et al., 2006). Furthermore, anti-cancer regimens evaluation largely depends on the appropriate pre-clinical experimental models, but a slender success was identified in the field of preclinical models like solid tumors. A new era is involved to study the cancer cell biology and for the development of novel cytotoxic agents with clinical correlation i.e. human cancer derived cell lines (Gottesman et al., 2012; Damia et al., 2009). Currently, 60% of the cytotoxic treatment is related to natural plant products, which directly interfere with cell functions.

Delonix elata (L.) gamble belonging to the family fabaceae (leguminosae). Literature reports on medicinal uses of *Delonix elata* leaf extract revealed the pharmacological actions like anti-inflammatory activity (Shah et al., 2009), larvicidal activity against *A. stephensi* and *A. aegypti*, antioxidant activity (Hoskori et al., 2013; Bharathi et al., 2014). Plant extract contains chemical constituents like alkaloids, tannins, triterpenoids, steroids, flavonoids and glycosides (Hoskeri et al., 2013). Our previous study on *Delonix regia* showed cytotoxic activity against different cancer cell lines (Ranjit PM et al., 2014). Hence, the present study has been carried out to evaluate the cytotoxic effect of aqueous leaf extract of *Delonix elata* on MCF-7 (Breast cancer cell line) and HepG-2(a human liver carcinoma cell line) by using MTT based assay.

2. Materials and methods

2.1. Plant material

Delonix elata leaves were collected from Acharya Nagarjuna University campus, Nagarjuna Nagar, Guntur. The collected leaves were identified, confirmed and authenticated by Dr. Satyanarayana raju, Botanist, Acharaya Nagarjuna University, Guntur.

2.2. Preparation of extracts

The shade-dried powder of leaves was cut into small pieces, powdered and the crude drug is boiled directly with distilled water at 60-70º c for 3hrs. Then it is cooled and filtered. The extract was concentrated under reduced pressure and stored in vacuum desiccator and performed phytochemical analysis.

2.3. Qualitative preliminary phytochemical analysis

Preliminary qualitative phytochemical screening of *Delonix elata* leaf extract shows a positive test for reducing sugars, steroids, flavonoids, saponins, tannins and glycosides.

2.4. FTIR analysis of the extract

Extract was scanned using infrared range 4000-400 cm^{-1} by Fourier Transform infrared spectrophotometer Alpha (BRUKER, USA).

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The spectral data obtained was compared with standard chart to identify the functional groups present in extract.

2.5. *In vitro* cytotoxic activity

2.5.1. Cell culture

Human cancer cell lines used in this study were procured from National Centre for Cell Science, Pune. All cells were grown in Minimal essential medium (MEM, GIBCO) supplemented with 4.5 g/L glucose, 2 mM L-glutamine and 5% fetal bovine serum (FBS) (growth medium) at 37°C in 5% CO2 incubator.

2.5.2. MTT assay

In vitro cytotoxic effect of aqueous leaf extract of *D.elata* was evaluated by using MTT assay, which was developed by Mosmann (Mosmann et al., 1983; Yousefi et al., 2014). 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% CO_2 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 (6.25,12.5, 25,50,100 and 200µg/ml) in triplicates to achieve a final volume of 100 µl and then cultured for 48hr. The compound was prepared as 1.0 mg/ml concentration stock solutions in PBS. Culture medium and solvent are used as controls. Each well then received 5 µl of fresh MTT (0.5mg/ml in PBS) followed by incubation for 2hr at 37°C. The supernatant growth medium was removed from the wells and replaced with 100 µl of DMSO to solubilize the colored formazan product. After 30 min incubation, the absorbance (OD) of the culture plate was read at a wavelength of 572 nm on an ELISA reader, Anthos 2020 spectrophotometer. The percent cell viability is calculated by the formula

% Viability = Corrected OD of sample /Control OD ×100

% Inhibition = 100 - %viability.

2.6. Statistical analysis

Data were represented as Mean. $^{*}P < 0.05$ was considered as significant when compared with tamoxifen (one way ANOVA) by using Graph pad prism 5 version.

3. 3. Results

3.1. Phytochemical analysis

Aqueous leaf extract of *Delonix elata* showed the presence of reducing sugars, steroids, flavonoids, saponins, tannins and glycosides. FTIR spectra of extract showed phenolic O-H stretching vibration at 3241.86 cm^{-1}, alkane at 2918 cm^{-1}, Aromatic at 1554.99 cm^{-1}, nitro group at 1398.82cm^{-1} and ether at 1041.60cm^{-1} (Table 1& Fig.1).

Table 1: FTIR Peaks and Functional Groups of Extract

Functional Groups	FTIR peak values(cm^{-1})
Acohol/Phenol (OH)	3241.86
Alkane	2918.96, 2850.57
Aromatic	1554.99
Nitro group	1398.82
Ether	1041.60

3.2. *In vitro* cytotoxic assay

Tables 2&3 indicates the % inhibition of cell viability of the extract and the standard against MCF-7 (breast cancer) & Hep-G2 (liver cancer) cell lines. Table 4 represents the IC_{50} values of both the standard and extract. The plant extract showed dose dependent *in vitro* cytotoxic effect against MCF-7 & Hep-G2 cell lines (% inhibition of cell growth) of different concentrations 6.25, 12.5,25,50,100 and 200 µg/ml are 3.8, 14.6, 22.6, 42.2, 54.7 and 61%, and 2.8, 7.9, 13.3, 21.5, 35.7 and 57.5 %, respectively. The IC_{50} concentration (µg/ml) of extract is 4.64µg/ml against MCF-7 and 164.23 µg/ml against Hep-G2 cell lines, when compared to standard tamoxifen for MCF-7 (9.15 µg/ml) and 5-fluorouracil for Hep-G2 (11.95 µg/ml), IC_{50} values of test samples are shown in Table 4. Fig.6 indicates the images of cell death.

Table 2: Percent Inhibition of Plant Extract on MCF-7 Cell Line

Conc (ug/ml)	% Cell Inhibition of Tamoxifen	% Cell Inhibition *D.elata*
6.25	17.7	3.8
12.5	54.5	14.6
25	69.1	22.6
50	84	42.2
100	95.1	54.7
200	99.6	61

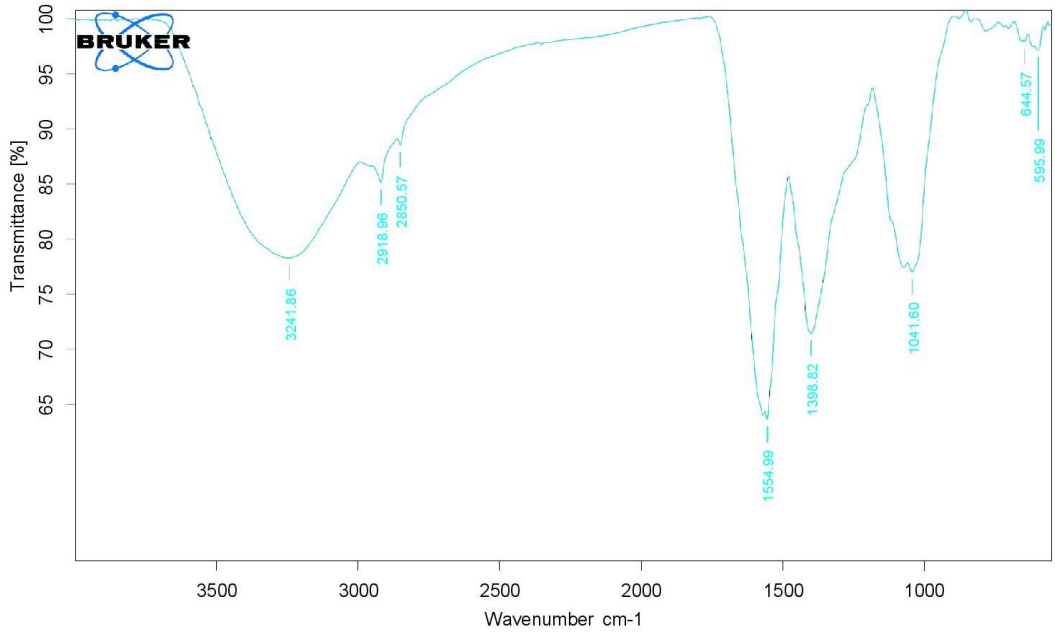


Fig. 1: FTIR Spectra of Delonix Elata Leaf Eextract

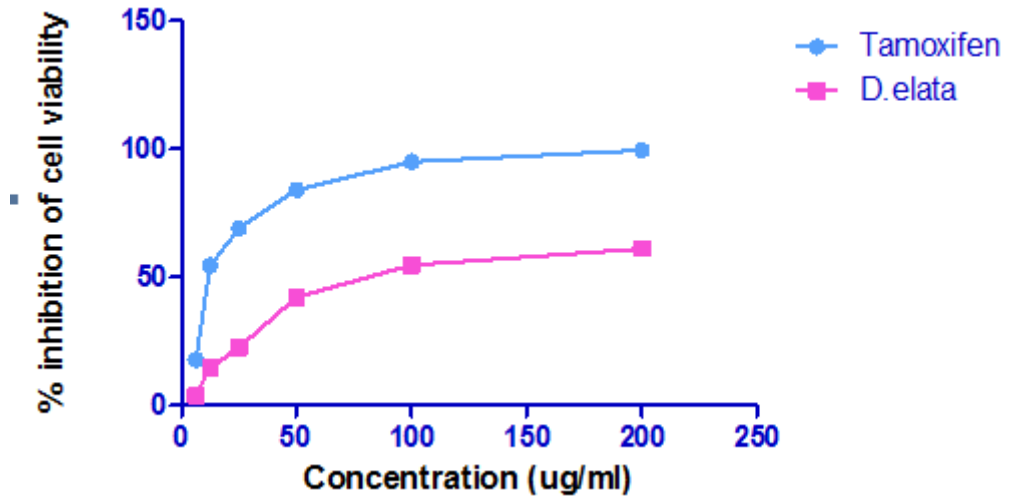


Fig. 2: Anticancer Activity of *Delonix elata* on MCF-7 Cell Line

Table 3: Effect of Plant Extract on Hep-G2 Cell Line

Conc (ug/ml)	% Cell Inhibition of 5-fluoro uracil	% Cell Inhibition of *D.elata*
6.25	17.7	2.8
12.5	54.5	7.9
25	62.6	13.3
50	70.8	21.5
100	79	35.7
200	88.8	57.5

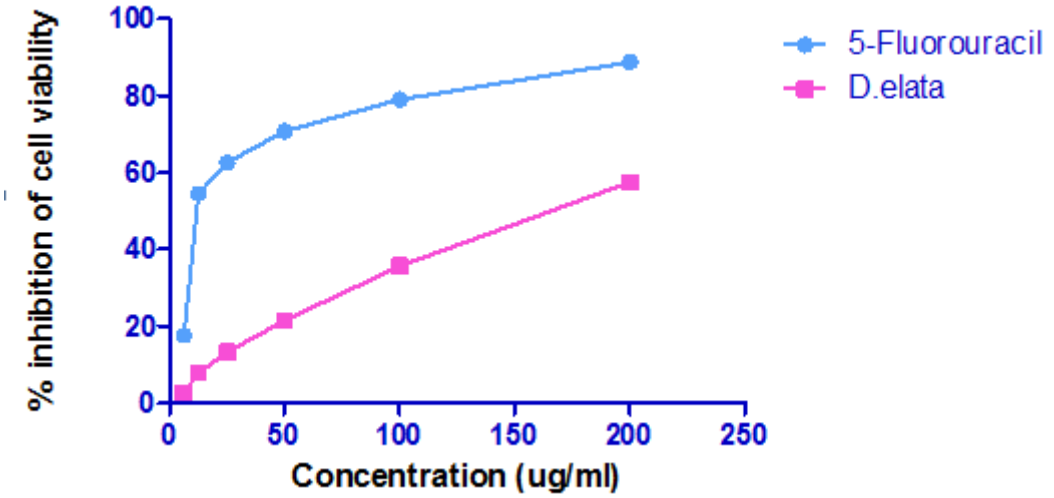


Fig. 3: Anticancer Activity of *Delonix elata* on Hep G2 Cell Line

Table 4: Ic_{50} Values of Plant Extract on Mcf-7 & Hep-G2 Cell Lines

Cell line	IC_{50} Values (µg/ml)	
	Standard	*D.elata*
MCF-7 (Tamoxifen)	9.15	94.64
Hep-G2 (5-Fluorouracil)	11.95	164.23

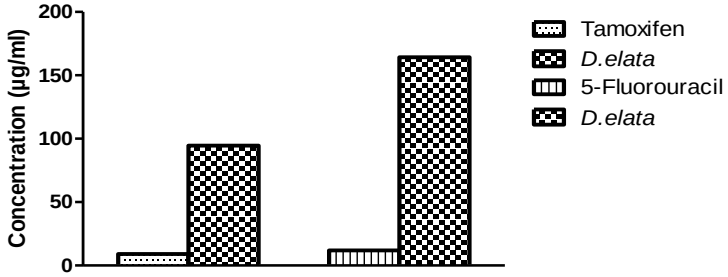
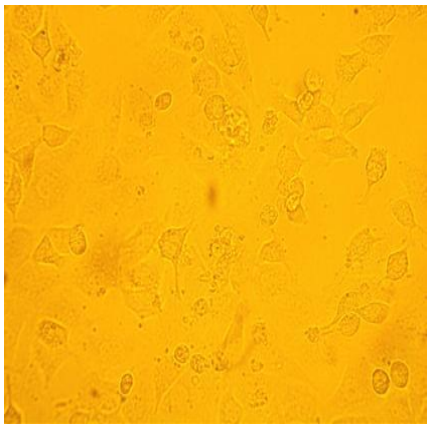
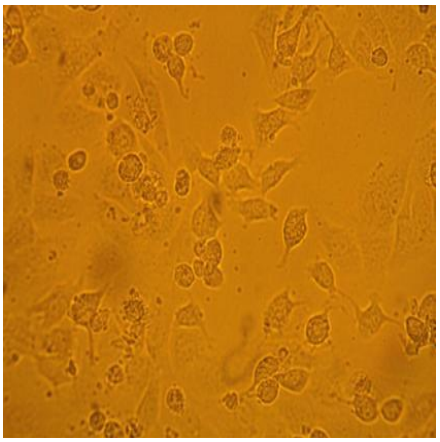


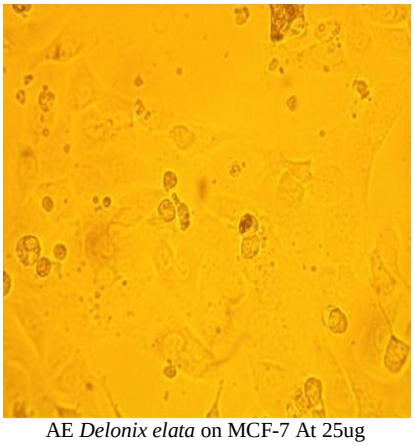
Fig. 4: IC_{50} Values of Reference Drugs and *Delonix elata*



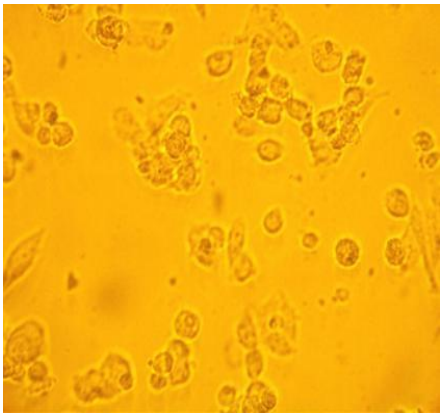
AE *Delonix elata* on MCF-7 At 6.25ug



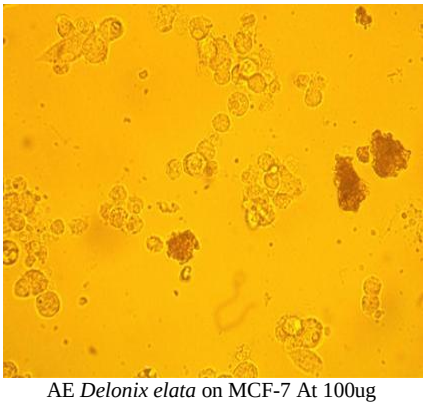
AE *Delonix elata* on MCF-7 At 12ug



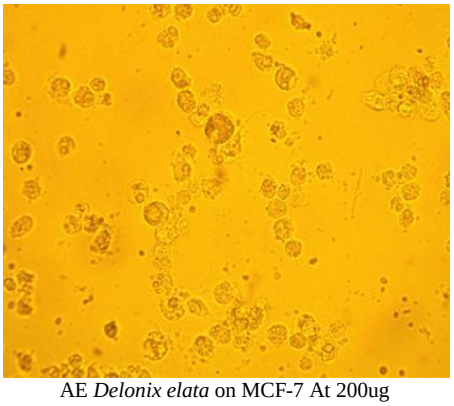
AE *Delonix elata* on MCF-7 At 25ug



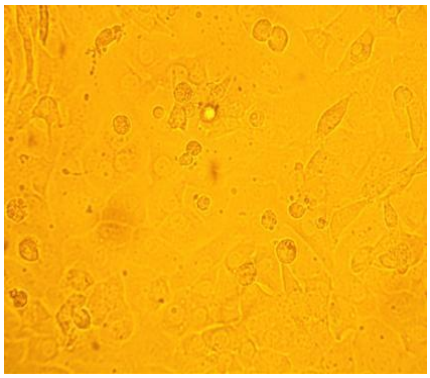
AE *Delonix elata* on MCF-7 At 50ug



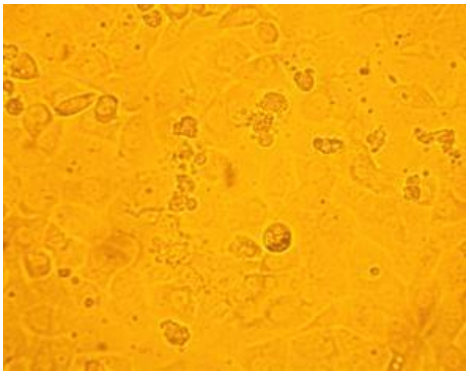
AE *Delonix elata* on MCF-7 At 100ug



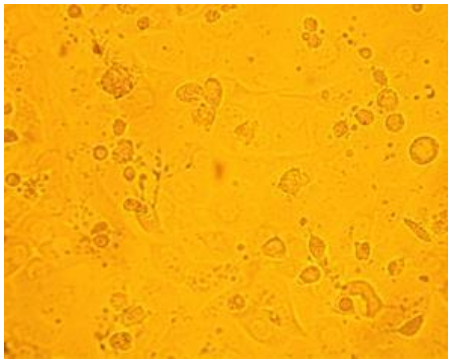
AE *Delonix elata* on MCF-7 At 200ug



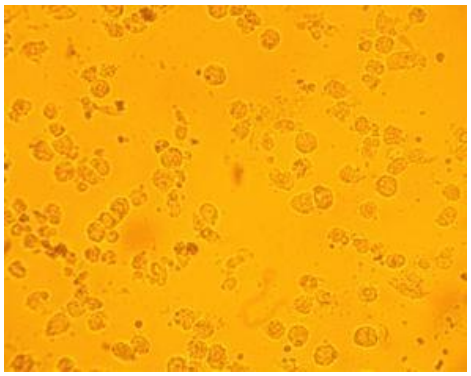
AE *Delonix elata* on Hep-G2 At 6.25ug



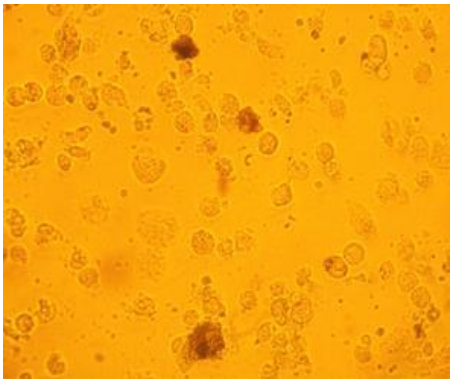
AE *Delonix elata* on Hep-G2 At 12 Ug



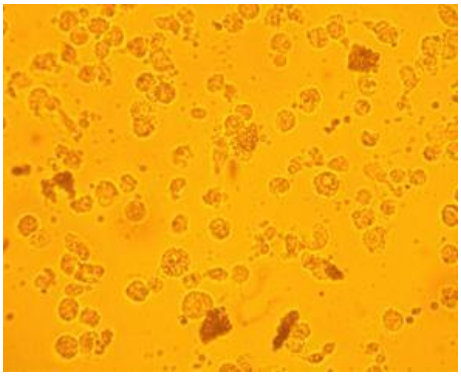
AE *Delonix elata* on Hep-G2 At 25ug



AE *Delonix elata* on Hep-G2 At 50ug



AE *Delonix elata* on Hep-G2 At 100ug



AE *Delonix elata* on Hep-G2 At 200ug

Fig 5: Effect of *Delonix elata* on MCF-7 & Hepg2 Cell Lines Images

4. Discussion

Phytomedicine is an ancient form of health care known to human being. Phytoprinciples in medicinal plants such as alkaloids, steroids, tannins and flavonoids are used to cure various human ailments like diabetes, obesity, inflammation associated diseases, cardiovascular diseases, cancer (Agarwal et al., 2011). These phytoprinciples differ from plant to plant due to vast biodiversity. Recent studies on plant extracts reported the presence of flavonoids, tannins, saponins and glycosides are responsible for cytotoxicity against cancer cell lines. Several *in vitro* cytotoxic reports on cell lines revealed the importance of chemical constituents as potent cytotoxic agents against different cancer cell lines (Dai et al., 2010; Machana et al., 2012, Russo et al., 2010)

Delonix elata leaves and stem bark contains quercetin, rutin (Senthilkumar et al., 2014; Pradeepa Krishnappa et al., 2014). FTIR data of *Delonix elata* leaf extract showed O-H phenolic stretching vibration at 3241.81 $cm^{-}1$, this is supported by the querctin standard FTIR data by Chourasiya et al., in 2012, reported the presence of phenolic group at 3411 cm^{-1} as quercetin. These results indicate the presence of flavonoid quercetin in leaf extract. *In vitro* studies on polyphenols like flavonoids, tannins, and saponins suggested that they exhibit cell growth arrest at different cell cycle phases: G1, S, S-G2 and G2 by direct and indirect ways like down regulating cyclins and cyclin dependent kinases (CDK), inducing the expression of p21, p27 and p53 genes respectively by acting as prooxidants (Gerhauser et al., 2008; Thomasset et al., 2009; Lala et al., 2006; Ding et al., 2006).

The cytotoxic activity of *Delonix elata* was determined by using MCF-7 and HepG2 cell lines. The results indicate the presence of polyphenols and the aqueous leaf extract had shown dose dependent effect on MCF-7 & HepG2 cell lines proliferation, but minimal inhibitory effect was observed, when compared to standard tamoxifen for MCF-7 & 5-Fluorouracil for HepG2.

5. Conclusion

Present study report, indicates that *Delonix elata* leaf extract showed cytotoxic activity on MCF-7 and HepG2 cell lines. Further work is essential to explore the compounds which are responsible for cytotoxic action and their mechanism of actions.

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