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In vitro Cytotoxic Activity of Hydro Ethanolic Extract of *Delonix regia* (Bojer ex. Hook.) Flowers on Cancer Cell Lines

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Authors' contributions

This work was carried out in collaboration between all authors. Author MRP designed the study, wrote the protocol, and wrote the first draft of the manuscript. Author TJ involved and managed lab work and author PKK performed the statistical analysis. Both Authors ACY and GG managed the analyses of the study. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: To evaluate the *In vitro* cytotoxic effects of the hydro ethanolic extract (HEE) of the *Delonix regia* flowers against different cell lines.
Methodology: The dried *Delonix regia* flowers are subjected to soxhlet extraction by using 70% ethanol. The dried extract was used to determine the qualitative preliminary phytochemical analysis, total phenolic and flavonoid content. The cytotoxic property of the extract was determined by using MTT assay against breast cancer (MCF-7), cervix (HeLa), brain and colon cancer cells. Tamoxifen is used as a **s**tandard for all the cell lines.
Results: Qualitative phytochemical tests of extract HEE showed the presence of sugars, flavonoid, tannins, phenolic compounds, steroids and saponins. The percentage of phenolic and flavonoid content was determined as 31.42 mg/g, 29.22 mg/g respectively. The cytotoxic activity of the extract showed, IC_{50} concentrations (µg/ml) against MCF-7

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(breast), carcinoma of cervix HeLa cells, carcinoma of the brain, and carcinoma of colon cells against tamoxifen are 141.6 ± 0.08, 223.7 ± 2.16, 173.9 ± 0.7, 168.33 ± 0.04 respectively.
Conclusions: The experimental data clearly demonstrate the hydro ethanolic extract (HEE) showed cytotoxic properties against human cancer cells.

Keywords: Delonix regia, Hydro ethanolic extract (HEE), Flavonoids, MCF-7 Cell line.

1. INTRODUCTION

Delonix regia (*Bojer ex.Hook*.) (Family: *Caesalpiniaceae*) commonly known as Krishnachura, Tabachine, Malinche and Gulmohar. It is usually grown as an ornamental tree, and given the name 'Royal Poinciana' or 'Flamboyant'. [1], best growth flowering and of this plant is observed when it is in sun location [2]. Prior studies were reported about *Delonix regia* flowers showed anti-inflammatory, analgesic and antimicrobial properties [3].The literature survey reveals that the flowers of *Delonix regia* contain flavonoids and flavanoid glycosides [4]. Other reports are also revels the presences of carotenoides, tannins, saponins, steroids, alkaloids and β-sitosterol [5]. Adje et al. 2008 reported about anthocyanin characterization of the plant *D. regia* flower anthers are a rich source of zeaxanthin [6]. Other report explains, fractionation of the ethanolic extract of the flowers of *Delonix regia* led to the isolation of three sterols, namely, stigmasterol (1.54 %), β-sitosterol and its 3-O-glucoside, a triterpene, namely, ursolic acid and four flavonoids: quercetin, quercitrin, isoquercitrin and rutin in addition to the amino acid L- azeditine-2-carboxylic acid [7].

The aim of the present research is, to determine the preliminary phytochemical constituents, total phenolic, flavonoid contents and cytotoxic activities of hydro-ethanolic (70%) extract of *Delonix regia* flowers on different cell lines.

2. MATERIALS AND METHODS

2.1 Collection of Plant Material

Delonix regia flowers were collected in the month of June from the area Gurunanak colony, Vijayawada. The collected flowers were authenticated by botanist Dr. K. Madhava Chetty, Asst. Professor, Dept. of Botany, Sri Venkateswara University, Tirupati. Herbarium specimen was deposited in the Department of Pharmacognosy, NRI College of pharmacy, with specimen no.NRI/COL/P.COG/DR1 (Flowers).

2.2 Preparation of Extracts and Qualitative Preliminary Phytochemical Analysis

The shade-dried powder of flowers was subjected to extraction in soxhlet extractor with 70% ethanol (Hydro-ethanolic) for 48 hours (extract yield: 13.5%) and extract is collected. The collected extract is evaporated to dryness using a rotary evaporator; the dry residue was stored in desiccators until use. Further, a qualitative preliminary phytochemical analysis was performed. Preliminary phytochemical screening of extract by using standard methods for identification of reducing sugars [8], protein [9], fats[9], resins[10], tannins[10], steroids[10], alkaloids[10],flavonoids[11],saponins [8], and phenols[12].

2.3 Determination of Total Phenolic Content

The concentration of phenolic content present in the hydro ethanolic extract (HEE) was determined by folin-ciocalteu's phenol reagent (FCR) [13-14]. 1 ml of the solution (contains 1 mg) of the hydro ethanolic extract (HEE) was added to 46 ml of distilled water and 1 ml of FCR, and mixed thoroughly. After 3 min, 3 ml of sodium carbonate (2%) was added to the mixture and shaken. A blue color was developed in each tube because the phenols undergo a complex redox reaction with phosphomolyobdic acid in folin ciocalteau reagent in alkaline medium which resulted in a blue colored complex, molybdenum blue. The solutions were warmed for 1minute, cooled at room temperature. The absorbance was measured at 650 nm using known concentrations of catechol by using ELICO SL 244 double beam UV-Visible spectrophotometer. The concentrations of phenols in the test samples were calculated from the calibration plot and expressed as mg catechol equivalent of phenol per gram of sample.

2.4 Determination of Total Flavonoid Content

To determine the total flavonoidal content, stock solution of extract (HEE 10mg/ml) was prepared with ethanol to a suitable concentration for analysis for the determination of flavonoid content [14]. Aliquots of each extract (HEE) was pipetted out in a series of test tubes and volume was made up to 0.5ml with distilled water; sodium nitrate (5%: 0.3ml) was added to each tube & incubated for 5min. at room temperature; Aluminium chloride solution (10%; 0.06ml) was added and incubated for 5 min, at room temperature; Sodium hydroxide (1M; 0.25ml) was added and total volume was made to 1ml with distilled water. Absorbance was measured at 510 nm against a reagent blank (Quercetin) using ELICO SL 244 double beam UV-Visible spectrophotometer and concentration of flavonoids in the test sample was determined and expressed as mg equivalent per gram of sample.

2.5 *In vitro* Anticancer Activity

2.5.1 Cell culture

Carcinoma of breast cancer [Michigan Cancer Foundation-7 (MCF-7], cervix (HeLa), brain (U87MG) and colon (SW480) human cancer cell lines are used in this study were procured from National Centre for Cell Science, Pune. All cancer cells were maintained in Dulbecco's modified essential medium (DMEM) supplemented with grown in Minimal essential medium (MEM, GIBCO) supplemented with 4.5 g/L glucose, 2 mM L-glutamine, antibiotics (50U/mL of Benzyl pencillin, 50µg/mL of Streptomycin and 50µg/mL of Amphotericin-B) and 5% fetal bovine serum (FBS) (growth medium) at 37°C in 5% CO_2 incubator.

2.5.2 MTT assay

The MTT assay developed by Mosmann [15] was modified [16] and used to determine the inhibitory effects of test compounds on cell growth *In vitro*. In brief, the trypsinized cells from T-25 flask were seeded in each well of 96-well flat-bottomed tissue culture plate not the same concentration but minimum of 5000 cells per well were seeded l 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 extract (8, 16, 32, 64, 128 and 256 µg/ml) in triplicates to achieve a final volume of 100 µl and then cultured for 48 hours. The compound was prepared as 1.0 mg/ml concentration stock solutions in PBS. 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. Tamoxifen is taken as positive control in order to compare IC50 of extract against standard drug used. Culture medium and solvent used as negative controls. After 30 min incubation, the absorbance (OD) of the culture plate was read at a wavelength of 570 nm on an ELISA reader, Anthos 2020 spectrophotometer.

2.6 Statistical Analysis

Data were represented as Mean ± SD. and IC50 values calculated were considered as significant when compared with tamoxifen (t- test) by using Graph pad prism 5 version.

3. RESULTS AND DISCUSSION

Qualitative phytochemical tests of extract HEE showed the presence of reducing sugar, flavonoid, tannins, phenolic compounds, steroids and saponins. The total phenolic and flavonoid content was determined as 31.42 mg/g (R^2 value: 0.998) and 29.22 mg/g (R^2 value: 0.997) in HEE respectively. Based upon this total phenolic and flavonoid content, we are design the in vitro cytotoxic activity. These contents were determined to know the any difference in quantity of flavonoid and phenolic contents with respective to geographical condition. Phenols and its congeners are known to cytotoxicity on various cancer cell lines and induce caspase-mediated apoptosis activity [17,18]. The results listed in the Table 1,2, 3, and 4, showed that percentage of inhibition and Ic_{50} concentrations (µg/ml) against MCF-7 (breast), carcinoma of cervix HeLa cells, carcinoma of the brain, and carcinoma of colon cells against tamoxifen and HEE respectively. Figs. 1,2,3, and 4 showed the comparison of % inhibition of HEE *vs* standard against different against various cancer cell lines. Table 5 showed the IC_{50} values both standard and test sample (HEE) against MCF-7 (breast), carcinoma of cervix HeLa cells, carcinoma of the brain, and carcinoma of the colon cells. When comparison with standard tamoxifen, IC_{50} values of extract (HEE) are showed lower. The results are expressed as mean±S.D (IC_{50}) and statistical difference was tested by using Student's t-test. A difference in the mean *P* value <0.01 was considered as significant. Fig.5 showed the comparison of IC50 values of HEE and Standard against different cell line.

The antitumor activity of tamoxifen through inhibits protein kinase C, facilitate apoptosis in cancer cell, generation of oxidative stress resulting in thiol depletion and activation of the transcriptional factor NF-kappaβ. Novotny et al., reviews the application of tamoxifen in various cancers like melanoma, small cell lung carcinoma, pancreatic, other endocrine and soft tissue cancers [19]. Many clinical studies explain the tamoxifen application in various kinds of malignant diseases [20]. Tamoxifen is clinically used for treatment of breast cancer, so it was used as a standard against MCF-7 cancer cell lines. According to a review of literates, tamoxifen was used as a standard for other cell lines mentioned above. Previous studies have also reported the presence of anthocyanin glycosides [21], carbohydrates, steroids, flavonoids, tannins, phenolic compounds and other constituents of hydro ethanolic extract *Delonix regia* flowers[22] Many reports suggested that flavonoids have exhibited antineoplastic activity and antioxidant activity [23]. Other reports suggested that, anthocyanins extract fractions showed *in vitro* anticancer properties [24].In a similar way, hydro ethanolic extract (HEE) of *D.regia* flowers showed *In vitro* cytotoxic activity may be due to the presence of flavonoid and anthocyanin content. Previous reports also explain the presence of these constituents in glycosidal form, increases the water solubility [25]. In this experimental study we were used 70% ethanol as solvent. Aly et al reported that

fractionation of the ethanolic extract, it's non-polar and flavonoid rich fraction showed cytotoxic activities against human liver cancer cell line. This is due to the presence of flavonoids and their efficient free radical scavenging properties may explain this liver protection ability. So ethanolic extract of the *D.regia* flower may used against liver cancer and liver toxicity mediated by chlorinated agents [7].

Table 1. Showed the mean ± S.D of % inhibition of both standard tamoxifen and test (HEE) on MCF-7 cells

Conc. µg/ml	Standard	% Inhibition	Test HEE
8	26.26±0.78		6.68±0.05
16	29.4±0.43		6.58±0.10
32	31.26±0.82		27.87±0.02
64	39.56±0.40		39.65±0.02
128	61.33±0.09		48.68±0.02
256	74.13±0.46		56.73±0.01

Table 2. Showed the mean±S.D of % inhibition of both standard tamoxifen and test (HEE) on cervix HeLa cell line

Conc. µg/ml	Standard	% Inhibition	Test HEE
8	29.38±0.34		2.59±0.59
16	36.61±0.26		18.88±0.32
32	51.79±0.49		24.39±0.50
64	61.35±0.18		33.68±0.50
128	75.65±0.57		39.46±0.10
256	78.45±0.15		50.62±0.11

Table 3. Showed the mean ± S.D of % inhibition of both standard tamoxifen and test (HEE) on Brain cell line

Conc. µg/ml	Standard	% Inhibition	Test HEE
8	22.52±0.18		7.10±0.03
16	35.05±0.19		14.85±0.06
32	47.07±0.53		16.92±0.02
64	60.01±0.83		30.14±0.02
128	73.29±1.07		46.58±0.16
256	82.87±0.28		55.70±0.18

Table 4. Showed the mean±S.D of % inhibition of both standard tamoxifen and test (HEE) on colon cell line

Conc. µg/ml	Standard	% Inhibition	Test HEE
8	23.29±0.74		7.10±0.03
16	29.37±0.98		17.89±0.07
32	54.19±0.50		28.20±0.01
64	58.67±0.22		38.52±0.02
128	71.72±0.12		42.75±0.02
256	81.76±0.32		55.49±0.02

Table 5. Showed mean±S.D of IC_{50} values of standard and test (HEE). Statistical difference was tested by using Student's t-test. A difference in the mean *P* value <0.01 was considered as significant

Type of cell line	Standard	Test HEE
MCF-7 cells	73.48±0.71	141.6±0.08***
HeLa cell line	30.44±0.05	223.7±2.16***
Brain cell line	36.1±0.18	173.9±0.7***
Colon cell line	36.29±0.22	168.33±0.04***

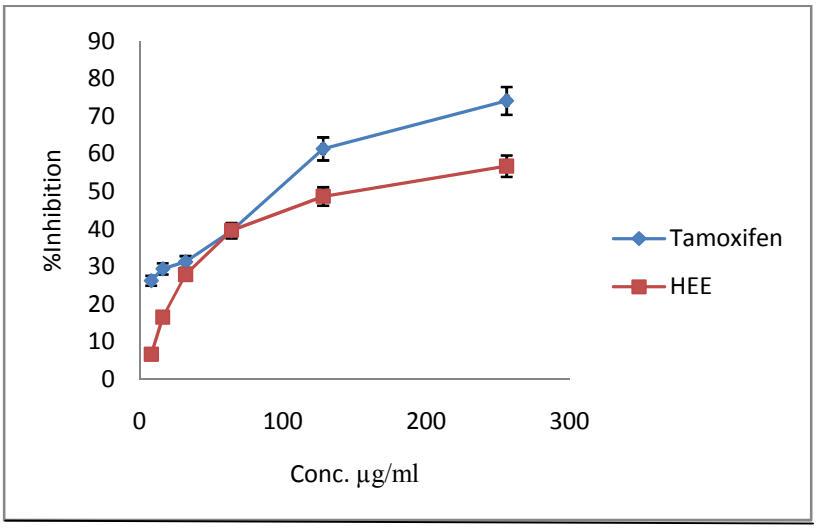

Fig. 1. Comparison of % inhibition of Standard tamoxifen vs HEE against MCF-7 cell lines

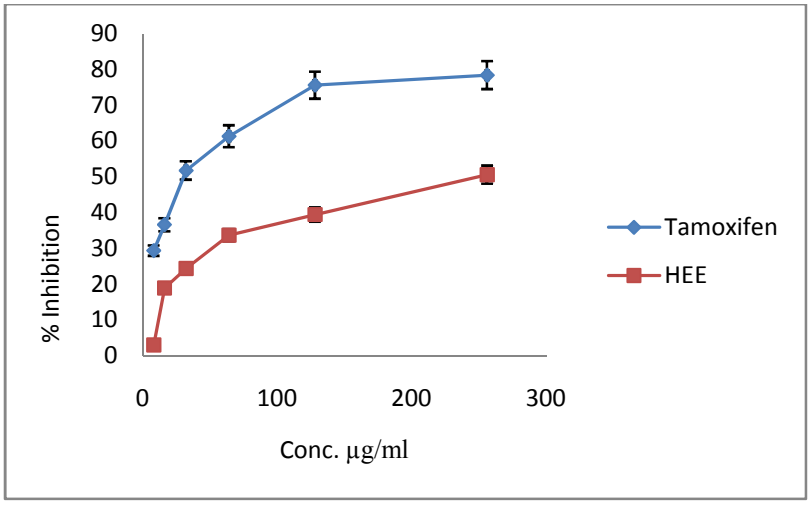

Fig. 2. Comparison of % inhibition of standard tamoxifen vs HEE against Hela cell line

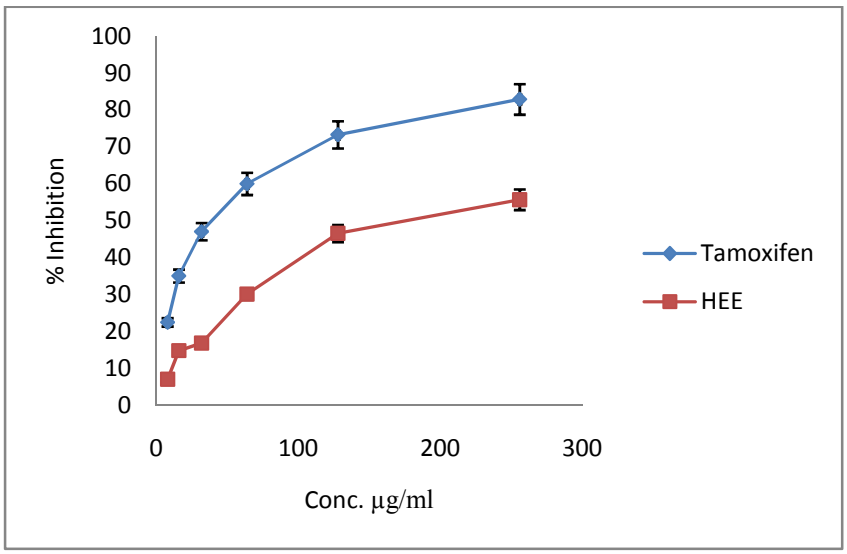

Fig. 3. Comparison of % inhibition of standard tamoxifen vs HEE against Brain cell line

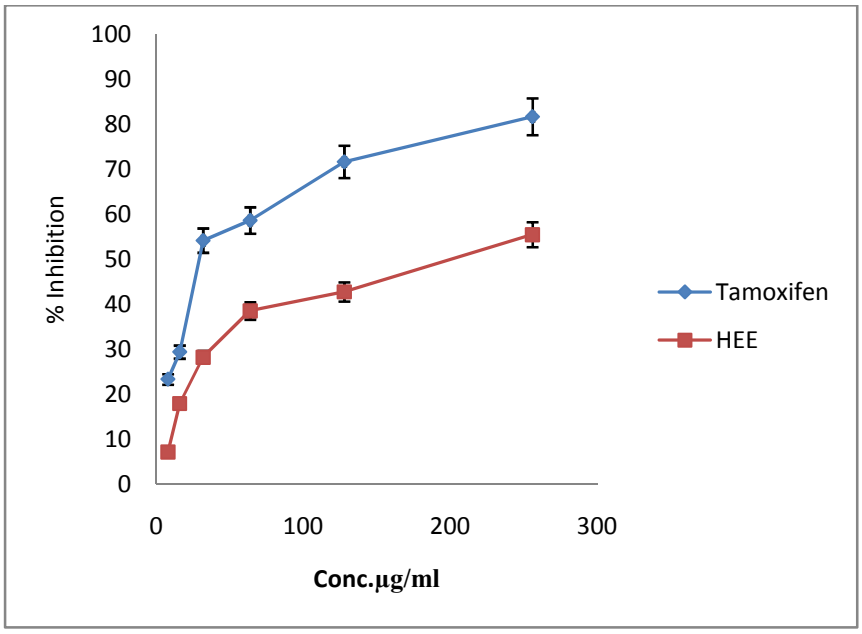

Fig. 4. Comparison of % inhibition of standard tamoxifen vs HEE against Colon Cell line

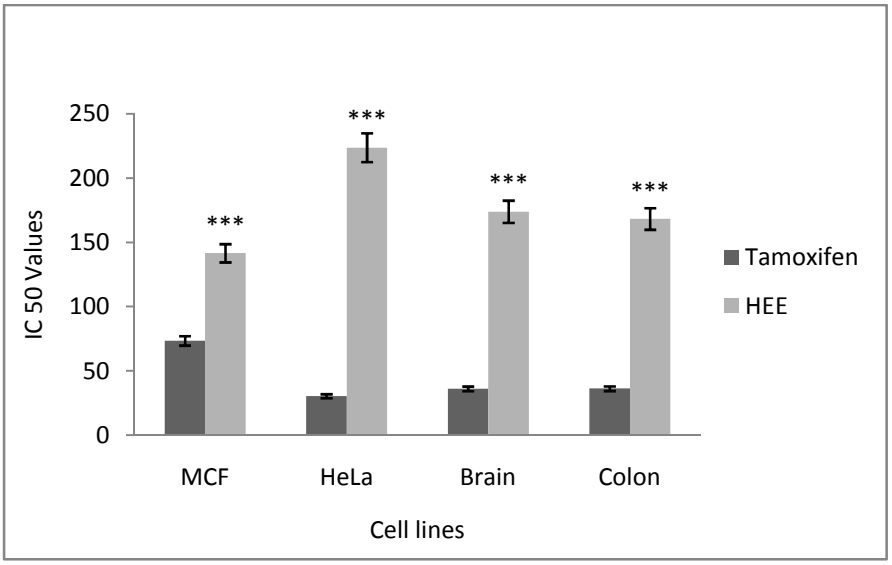

Fig. 5. Comparison of IC 50 Values of HEE vs standard against different cell line

4. CONCLUSION

The reported hydro ethanolic extract showed *in vitro* cytotoxic properties may be due to presence of flavonoid content. The IC_{50} concentration of extract showed lower activity when compared with standard tamoxifen; this may be due to its crude nature. This investigation has helped to identify the compounds present in the flowers of *D. regia*. HEE showed cytotoxic action on MCF-7 cell line (IC_{50} concentration µg/ml; 141.6 ± 0.08) when compared with other cell line, this may be due to the presence of quercetin and rutin. Previous research studies explained that both quercetin and rutin showed excellent anticancer properties against breast cancer. So these flowers were used as source of antitumor agents and flavonoids. Further studies are required to identify, which chemical constituents is responsible for *in vitro* cytotoxic activity of ethanolic extracts and establish the molecular mechanism of action with regard to their cytotoxic activity.

CONSENT

Not applicable.

ETHICAL APPROVAL

The authors hereby declare that all experiments have been examined and approved by the Appropriate ethics committee and have been performed in accordance with the ethical Standards laid down in the 1964 Declaration of Helsinki.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Md. Mahafuzur Rahman, Md. Nazmul Hasan , Asish Kumar Das , Md. Tozammal Hossain, Rownak Jahan, Mst. Afsana Khatun, Mohammed Rahmatullah. Effect of *Delonix regia* leaf extract on glucose tolerance in glucose induced hyperglycemic mice. Afr J Tradit Complement Altern Med. 2011:8(1):34-36.
2. Edward F, Dennis G, Watson. Delonix Regia- royal Poinciana. Face sheet ST-228, a series of Environmental Institute of food and Agricultural Sciences, University of Florida; 1993.
3. Rajabhau S Shiramane, Karnakumar V Biradar, Basavaraj V Chivde, Shambhulingayya HM and Veerana goud. *In-Vivo* antidiarrhoeal activity of ethanolic extract of *Delonix regia* flowers in experimental induced diarrhoea in wistar albino rats. International journal of research in pharmacy and chemistry. 2011:1(3).
4. Y Ragasana, Joy G.Hofilena. Antimicrobial coumarin derivative form Delonix regia. The manila journal of Science. 2011:7-11.
5. Shanmukhai, Harshil Patel, Jignesh Patel, Riyazunnisa. Quantification of total Phenol and flavonoid content of Delonix regia Flowers. International Journal of ChemTech Research. 2011:3(1).
6. Adje F, Lozano YF, Meudec E, Lozano P, Adima A, Gaydou EM. Anthocyanin characterization of pilot plant water extracts of *Delonix regia* flowers. Molecules. 2008; 13:1238-1245.
7. Aly M, El-sayed, Shahira M, Ezzat, Maha M. Salama and Amany A. Sleem, Hepatoprotective and cytotoxic activities of *Delonix regia* flowers extracts. Pharmacognosy journal. 2011;3(19):49-56.
8. Brain KR. and Tuner TD, The practical evaluation of phytopharmaceuticals. Wright Scientectica Publishers, Bristol; 1975.
9. Debela A, Manual for Phytochemical Screening of Medicinal Plants. Ethiopian Health and Nutrition Research Institute, Addis Ababa, Ethiopia; 2002.
10. Cuilel I, Methodology for the analysis of vegetables and drugs. Chemical Industry Division, NNIDO Romania; 1994.
11. Sofowora A. Medicinal plants and traditional medicine in Africa. Spectrum books limited, Ibadan, Nigeria; 1993.
12. William C Evan. Trease and Evans Pharmacognosy.16th Indian edition: Elsevier limited; 2009.
13. Maryam Zahin, Farrukh Aqil and Iqbal Ahmad. The *in vitro* antioxidant activity and total phenolic content of four indian medicinal plants. International Journal of Pharmacy and Pharmaceutical Sciences. 2009:1(1).
14. Hanmukha I, Harshil Patel, Jignesh Patel, Riyazunnisa. Quantification of Total Phenol and Flavonoid Content of *Delonix regia* Flowers. International Journal of ChemTech Research. 2011:3(1):280-283.
15. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J. Immunol. Methods. 1983;16;65(1-2):55-63.

16. Pusapati Madan Ranjit*, Krishna priya M, Silpa P, nagalakshmi V, Anjali M, Girish K, and YA Chowdary. In vitro cytotoxic activities of *Calotropis procera* latex and flower extracts against MCF-7 and Hela cell line cultures. Int J Pharm Pharm Sci. 2012:4(1):66-70.
17. Owen RW, Giacosa A, Hull WE, Haubner R, Spiegelhalder B, Bartsch H. The antioxidant/anticancer potential of phenolic compounds isolated from olive oil. Eur J Cancer. 2000;3:6(10):1235-47.
18. Nandi S, Vracko M, Bagchi MC. Anticancer activity of selected phenolic compounds: QSAR studies using ridge regression and neural networks. Chem Biol Drug Des. 2007;70(5):424-36.
19. Novotny L, Rauko P, Vachalkova A, Peterson-Biggs M. Tamoxifen in cancer therapy. Neoplasma. 2000; 47:1.
20. NCI PDQ Clinical trial Search. Bethesda, USA, National Cancer Institute. www.cancer.gov.
21. Felix Adje, Yves F Lozano, Emmanuelle Meudec, Paul Lozano, Augustin Adima, Georges Agbo Nzi and Emile M. Gaydou. Anthocyanin Characterization of Pilot Plant Water Extracts of *Delonix regia* Flowers. Molecules. 2008;13:1238-1245.
22. Maria jancy Rani P, Kannan PSM, Kumaravel S. Screening of antioxidant activity, total phenolics and gas chromatograph and mass spectrometer (GC-MS) study of *Delonix regia.* African Journal of Biochemistry Research. 2011;5(12):341-347.
23. Tapas AR, Sakarkar DM, Kakde RB. Flavonoids as Nutraceuticals: A Review. Tropical Journal of Pharmaceutical Research, September 2008:7(3):1089-1099.
24. Dumitrita Rugina , Zorita Sconta, Loredana Leopold, Adela Pintea, Andrea Bunea, and Carmen Socaciu. Antioxidant Activities of chokeberry extracts and the cytotoxic action of their anthocyanin fraction on HeLa human cervical tumor cells. J Med Food. 2012; 15(8):700–706.
25. Alan Crozier, Michael N, Clifford, Hiroshi Ashihara. Plant secondary metabolites Occurrence, Structure and Role in the human diet. Blackwell Publishing Ltd.; 2006.

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