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Cytotoxicity activity of kidney cell line (PK-15) using ethanol extract of *Justicia gendarussa* burm f leaves

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Abstract
The study of Cytotoxicity was widely used as a fundamental research and for paving way for drug discovery and to screen libraries for toxic compounds. In this study well known medicinal herbs *Justicia gendarussa* Burm f. plant leaves ethanolic extract was examined for their cytotoxicity activity by using PK-15 cells. This experiment was done by utilizing the procedure through cell viability assay. Thisassaycan be varied as the redox potential of the cell population and the integrity of cell membranes or with the activity of cellular enzymes such as esterase's. This plant has significant effect on cytotoxicity activity on pk15 cell and the concentration ranges of about 25 - 500µg/ml were used in this study. According to results obtained it was understood that the ethanolic extract of the leaves of *Justicia gendarussa* Burm f. plant could be a possible source of herbal medicine for cancer-related ailments.

Keywords: *Justicia gendarussa* burm f, ethanol extract, PK-15 cells, cytotoxicity

Introduction
There are many plants which has been unexplored in the field of medicine or science. Because of this, continuous approaches are going on to isolate various therapeutically active compounds from different plants. Many active drugs were derived from different medicinal plants, and the process is going on [1]. Traditional herbal medicines are naturally occurring plant-derived substances with no industrial processing that are being used to treat illness within local or regional healing practices [2].
The *Justicia gendarussa* Burm. f is a medicinal plant which comes under Acanthaceae family and found in many countries like Indonesia, India, and Malaysia. In traditional medicinal system, it has been used for a variety of disease condition which include, such as amenorrhea, stomach troubles, haemoptysis, cough and asthma etc. This plant also possess some pharmacological actions such as antimicrobial, anti-inflammatory, antipyretic and anti-cancerous effects [3, 4, 5]. Use of this plant based remedies is also wide spread in many countries and numerous pharmaceuticals are resultant from plant compounds [6].
Several assays are used to determine the effect of a drug on cells propagated *in vitro*. They range from simple assays that measure cell viability after drug exposure. This cytotoxic assay measures the percentage of the cell population capable of giving rise to clones, and measures the effect of the compound on the proliferating fraction of the population [7].
The aim of thisstudyis to investigate the cytotoxic activity of the leaves of *J. Gendarussa Burm. f* extracted with ethanol using PK15 cell. To our knowledge, there is no scientific report pertaining to the cytotoxic activities of *J. gendarussa* ethanolic leaf extracts against PK-15 cells. Therefore, in this study, we report for the first time regarding the cyto toxic effect of the crude ethanolic extracts of *J. gendarussa* against PK-15 cells.

Materials and Methods
Collection and identification of the plant
Plant leaves were collected in natural environment and were washed thoroughly. This plant was authenticated by (specimen - SJCBOT2183) by Dr. S. Soosairaj, Assistant Professor, Department of Botany, St. Joseph's College, Tiruchirappalli. District, Tamilnadu, India. Fresh *J. gendarussa* plant leaves were washed and shade dried. Then ground into fine powder. Then 50g leaf powder of *Justicia gendarussa* (Burm) f. were soaked in 500ml of ethanol. Then kept in orbital shaker for 48h at room temperature. After 48h, the mixture was filter through a clean muslin cloth. The filtrate again filtered by using a Whatman no. 1 filter paper and then the extracts were concentrated and dried in a rotary evaporator at 37ºC till a sticky mass was obtained. After evaporation, the dried extracts were stored at 4ºC until further use [8-11].

Qualitative Phytochemical analysis
The extract was qualitatively screened for various constituents (alkaloids, saponins, tannins, sterol, flavonoids, terpenoids, glycosides, simple sugars) using standard protocol [12-14].

a. **Test for Carbohydrates:** To about 1 ml ethanol extract of the plant about 5 ml of Benedict's reagent was added and was boiled for 5 minutes. Appearance of bluish green showed the presence of carbohydrates.
b. **Test for Glycosides:** About1 ml concentrated sulphuric acid was added to 1 ml ethanol extract of the plant. Fehling's solution was added to this test solution. A black red precipitate was formed indicating the presence of glycosides.
c. **Test for Alkaloids:** To 1 ml of the plant extract about 2 ml of 2N hydrochloric acid and Mayer's reagent (Potassium mercuric iodide solution) were added. Formation of turbid white precipitate shows the presence of alkaloids.

d. Test for Flavonoid: Few drops of 1% NH_3 was added to 1 ml ethanol extract of the plant. Observation of intense yellow colour indicates the presence of flavonoid compounds.

e. Test for Tannins: 2 ml of 5% $FeCl_3$ was added to 1 ml ethanol extract of the plant. Appearance of blue-black precipitate indicated the presence of tannins compounds.

f. Test for Saponins: 5 ml ethanol extract and 5 ml de-ionised distilled water was placed in a test tube, and was shaken vigorously. Appearance of foam that lasted for 15 minutes indicates the presence of saponins.

g. Test for Steroids: 2 ml of ethanol extract of the plant was dissolved in 2 ml of chloroform and concentrated sulphuric acid was added along the sides of the test tube. The upper layer turned red and sulphuric acid layer showed yellow colour with green fluorescence indicating the presence of steroids.

h. Test for Terpenoids: 2 ml of ethanol extract of the plant was dissolved in 2 ml of chloroform and was evaporated to dryness. 2 ml of concentrated sulphuric acid was added to this mixture which forms a layer of reddish brown colour indicating the presence of Terpenoids.

Cell culture and MTT assay

Cytotoxicity testing was performed using 3 - (4, 5-dimethylthiazol-2-yl) - 2, 5 diphenyl tetrazolium bromide (MTT, Sigma) according to the method reported in previous studies. The Kidney cells (PK-15) were plated separately using 96 well plates with the concentration of 1×10^5cells/well in DMEM media with 1X Antibiotic Antimycotic Solution and 10% fetal bovine serum (Himedia, India) in CO_2 incubator at 37˚C with 5% CO_2. The cells were washed with 200 µl of 1X PBS, then the cells were treated with various test concentration ofethanolic extract of the *Justicia gendarussa* (Burm) plant in serum free media and was incubated for 24 h. The medium was aspirated from cells at the end of the treatment period. 0.5mg/mL MTT prepared in 1X PBS was added and incubated at 37˚C for 4 h using CO_2 incubator. Following the incubation period, the medium containing MTT was discarded from the cells and washed using 200 µl of PBS. The formed crystals were dissolved with 100 µl of DMSO and thoroughly mixed. The color intensity was evaluated at 570nm. The formazan dye turns to purple blue color. The absorbance was measured at 570 nm using microplate reader [15].

The cytotoxicity was recorded as the drug concentration causing 50 % growth inhibition of cell lines (IC_{50} value) using the formula given below [16].

% cell viability= Asample (mean) / Acontrol (mean)×100 %

Where (A) - absorbance

Statistical Analysis

Statistical analyses were performed ANOVA by using SPSS v25 Statistical software to examine the difference betweenthe control and the plant extracts with different concentrations to the cell line.

Results and Discussion

Qualitative Phytochemical analysis

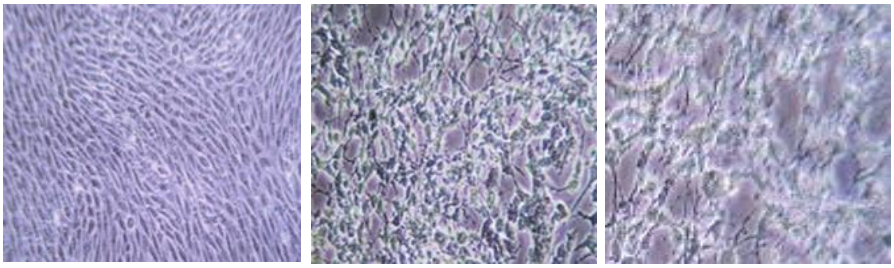
The Phytoconstituents present in the ethanolic extract of the *Justicia gendarussa* (Burm) plant leaves were shown in the Table 1. The ethanol extracts of the plant leaves were tested positive for Carbohydrates, Glycosides, Alkaloids, Flavonoids, Tannins, phenols, Saponins and Steroids. This preliminary phytochemical analysis will be helpful in finding chemical constituents in the plant material that may lead to their quantitative estimation and also in locating the source of pharmacologically active chemical compounds. The present of chemical constituents might of great use be useful for pharmacognostic studies and standardization of herbal drugs used currently in folk medicine. They also provide therapeutic diagnostic tools for scientists who wish to evaluate herbal medicines obtained from indigenous resources [17].

Table 1: Phytochemical compounds of the ethanolic extract of *Justicia gendarussa* (Burm) plant leaves

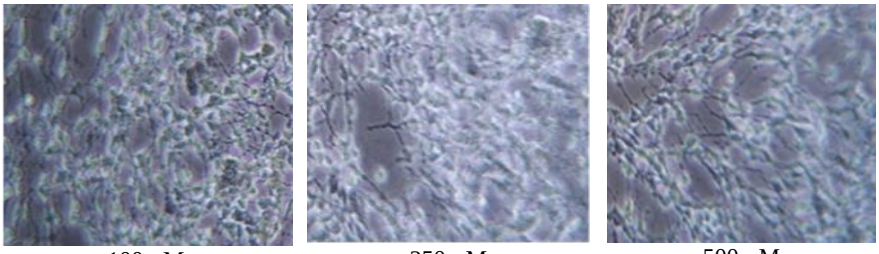
S. No	Phytoconstituents	Ethanol
1	Carbohydrates	++
2	Glycosides	++
3	Alkaloids	++
4	Flavonoids	+
5	Tannins	+
6	Phenols	++
7	Saponins	+
8	Steroids	++
9	Terpenoids	++

Cytotoxic Activity

Cytotoxicity activity was assessed by the IC_{50} values obtained by presenting 50% of cells inhibition by the ethanolic extract of *Justicia Gendarussa* Burm f. plant. Fig 1 shows the morphological changes of PK-15 cells using the concentrations 25-500µg/ml of ethanolic extracts of *J. gendarussa* which is compared to cells without any treatment. IC_{50} values of ethanolic extracts of *J. Gendarussa*were (<100-500µg/ml) considered as cytotoxic and 25-50 µg/ml were found to exhibit weak cytotoxic effect. More than 500 µg/ml were not considered to be cytotoxic due to the higher percentage viability of the cells [18, 19].



Control 25 µM 50 µM



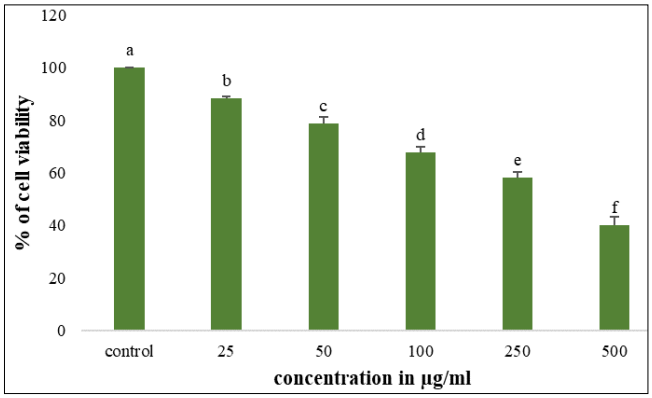
100 µM 250 µM 500 µM

Fig 1: Cell viability assay of PK-15 cells morphological changes in Ethanol Extract of *Justicia Gendarussa* Burm f. plant observed and comparing with control

MTT assay is a rapid and highly accurate colorimetric approach that widely used to check the cell cytotoxicity for the development of new drug. It measures the cell membrane integrity by determining mitochondrial activity through enzymatic reaction on the reduction of MTT to formazan. Percentage cell viability of cell lines were carried out by using formazan dye exclusion technique. The ethanolic extract treatment on the cells in trypan blue exclusion assay produced almost similar results as in the MTT assay. The significant reduction in the number of viable cells got increased from 100 µg/ml in the MTT assay (Fig. 1) to 500 µg/ml in the try pan blue exclusion assay (Fig. 2). These studies result are tabulated in Table 2 and are graphically represent in Fig 2. The figure shows the significant difference between the cell viabilitydue to ethanolic extract of *J. gendarussa* burm f. plant and the control [20, 21].

Table 2: Percentage of pk-15 cell viability of due to Ethanolic Extract of *Justicia Gendarussa* Burm f. plant

Concentration(µg/ml)	% of cell viability		
25	87.7	88.3	89.1
50	81.2	76.1	78.9
100	69.0	65.5	69.5
250	59.9	58.6	55.6
500	43.4	39.2	37.6
Control	100	100	100



Results are presented in mean ± SD P<0.05 compared with control

Fig 2: Percentage of live and dead cell as per Trypan blue exclusion method

The increase might be explained by the staining procedure used in cell counting for trypan blue exclusion assay. This resultobtained with the ethanolic extract of *Justicia Gendarussa Burm f.* plant has significant effect on cell growth inhibition. According to the previous report, the active compounds particularly flavonoid contents in *J. gendarussa* leaf extracts might contribute the cytotoxicity activity against cancer cells and these trends are in accordance with previous studies [22, 18, 24].

Results of this study make us understand that ethanolic extract of *Justicia Gendarussa* Burm f. plant has potential cytotoxic activity on PK-15 cells. This indicates the presence of cytotoxic compounds in these extracts.

Conclusion

The present study reveals that the cytotoxicity activity of ethanolic extracts of *J. gendarussa* plant in *vitro* in dose dependant manner.This may be due to the fact thatthe ethanolic extract of *Justicia Gendarussa* Burm f. plant is a source of secondary compounds and as previously described in literature it could be an important source of bioactive molecules for pharmaceutical applications. Hence, it is suggested that further studies with ethanolic extract of *Justicia Gendarussa Burm f.* against cell lines has to be conducted in order to attribute the cytotoxic result brought by them due to a specific compound. Further studies could help in the identification of the specific compound, which would be a break through facilitating the utilization of the plant for medicinal applications.

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Conflict of Interest

The author(s) declare that they have no conflict of interest.

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