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PHYTOCHEMICAL ANALYSIS AND IN VITRO ANTI-CANCER ACTIVITY OF FRUIT EXTRACT OF *THESPESIA POPULNEA* AGAINST HELA AND K562 CANCER CELL LINES

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ABSTRACT

Aim: The purpose of the study was to determine the phyto-chemical constituents and anti-cancer activities of hydro-alcoholic fruit extract of *Thespesia populnea* on K562 (leukaemia cancer) and HeLa(cervical cancer) cell lines. **Methods:** Fruit sample of *Thespesia populnea* was subjected to extraction method with increasing polarity from mintroleum, Ethyl acetate, methanol and hydro-alcohol solvents. The high percentage extractive was found in Hydro-alcohol with soxhlet extraction. Phytochemical constituents of the plant, *Thespesia populnea* were calculated using different solvents based on polarity (chloroform, ethyl acetate, hydro-alcohol(70:30)(methanol:water) and water). Qualitative analysis of extract was done revealing the presence of flavonoids, phenols, alkaloids, sterols, tannins and reducing sugars. The extract was tested for cytotoxic activities against cancer cell lines, HeLa(cervical cancer) and K562(leukaemia). The hydro-alcoholic extract of fruits is found to be cytotoxic to the cell lines of K562 and HeLa *invitro* with IC50 values 2886 and 2284 respectively.

KEYWORDS: Anticancer, Phyto-chemical, *Thespesia populnea*, K562 and HELA.

1. INTRODUCTION

Cancer is one of the diseases that occur in both developed and developing countries cause of high mortality rate. Most widely used method of treatment for cancer is chemotherapy and

major defect is the toxicity that is the caused to the normal cells due to the inability of cancer cells, Natural compounds are selective against cancer cells. Although many drugs are there in active development and many are in clinical trials. There is emergent need to develop much more effective and less toxic drugs. It has been reported that 50% of all drugs in clinical use are derived from natural products like vincristine, podophyllotoxin, paclitaxel and camptothecin. Mostly flavanoids and polyphenolic compounds are having anticancer activity from natural origin.

K562 were the first immortalized myelogenous leukemia line to be established. K562 cells are erythro-leukemia type. The cells are non-adherent and rounded are positive for the bcr:abl fusion gene and bear some proteomic resemblance to both undifferentiated granulocytes and erythrocytes. Two sub lines are available which express MHC class-1 A2 and A3. K562 cells are part of the NCI-60 cancer cell line panel used by National Cancer Institute. HeLa is a cell type is an immortal cell line used in scientific research. It is the oldest and most commonly used human cell line. The line was derived from cervical cancer cells from Henrietta Lacks. The cell line was found to remarkably durable and prolific which warrants its exclusive use in scientific research.

Thespesia populnea plant belonging to malvaceae has been reported to have many pharmacological effects. *Thespesia populnea* fruit extract was found to be having wound healing, antipyretic, anti-inflammatory, anti-oxidant, anti-diabetic, anti-psoriatic, anti-bacterial, anti-noceceptive, Alzheimers disease, dermatitis, anti-hyperglycemic and anti-implantation. Leaves extract is found to be having antipyretic, anti-inflammatory, anti-hyperglycemic, anti-cancer, and anti-ulcer activity. The bark, flowers, root extract is also possessing hypoglycemic, Immunomodulatory, Memory enhancing activity, Hypoglycemic, antipyretic and antioxidant activity. The cytotoxic activity was reported on the flowers, leaves and heartwood parts of plant extract but not on fruits. Seeds are noticed to have anti-hyperglycemic, anti-microbial and anti-oxidant activity. The past phytochemical history was revealing that the plant consists of alkaloids, terpenoids, tannins, saponins, polyphenols, flavanoids, carbohydrates and sterols.

2. MATERIAL AND METHODS

2.1 Collection and authentication of plant

Thespesia populnea was collected from Visakhapatnam Eastren Ghats of Andhra Pradesh in December 2017. The plant was identified and authenticated by Dr. BodaihPadal, Taxonomist,

Department of Botany, Andhra University, Visakhapatanam. Fresh fruits were collected and washed with water, air dried, cut into small pieces and dried in shade till they became brittle, then blended to a fine powder by mechanical blending mill. The powder was stored in airtight containers in dark and dry place for further use.

2.2 Preparation of plant extract

About 300g dried fruit powder was coarsely powdered and subjected to extraction by Soxhlet extractor. The extraction was done with different solvents in their increasing order of polarity such as mintroleum, Ethyl acetate, methanol and hydro-alcoholic(70:30). Each time fresh plant material was taken and later extracted with other solvents. All the extracts were concentrated by a rotary evaporator and the left-over solvent was evaporated to dryness using a sand bath. The amount of extract obtained was found to be more in hydro-alcohol solvent.

2.3 Phytochemical analysis

The crude fruit powder of *Thespesia populnea* was extracted using hydro-alcohol and other organic solvents to ensure the extraction of polar and non polar constituents. Qualitatively tested for different phytochemical constituents namely alkaloids, flavanoids, glycosides, polyphenols, saponins, terpinoids, carbohyrdrates, sterols by following the standard screening tests.

2.4 Chemicals

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide(MTT), FBS(Gibco, Invitrogen) Cat No-10270106, Antibiotic-Antimycotic 100X solution (Thermofisher Scientific)-Cat No-15240062, Phosphate buffer, Di-methyl sulfoxide, Paclitaxel.

2.5 Culturing of cell lines

The cell lines K562(Leukemia) and HeLa(Cervical cancer) were procured from NCCS, Pune. The cells were seeded at a density of approximately 5×10^3 cells/well in a 96-well flat-bottom micro-plate and maintained at 37^0 C in 95% humidity and 5% CO_2 overnight.

SL NO	CELL LINES	MEDIA
1	K562(Leukemia)	DMEM with low glucose(Cat No-11965-092)
2	HELA(Cervical Cancer)	DMEM with low glucose (Cat No-11965-092)

2.6 Treatment groups

K562, HeLa cells lines were treated with *Thespesia populnea* hydro-alcoholic fruit extract of desired concentration which were prepared in dimethyl sulfoxide prior to the experiment. The reactant mixtures were diluted with media and cells were treated with different concentration ranges of the extract (500,250,125,62.5,32.75 µg/mL) and incubated for 48hours, respectively which was the optimal treatment time of the extracts in each of the cell lines. The cells in the well were washed twice with phosphate buffer solution, and 20µL of the MTT staining solution(5mg/ml in phosphate buffer solution) was added to each well and plate was incubated at 37^0C. After 4hrs, 100µL of di-methyl sulfoxide was added to each well to dissolve the formazan crystals, and absorbance was recorded with a 570nm using micro plate reader the results were analyzed in triplicate and the percentage was calculated.

2.7 Statistical Analysis

The results were expressed as the mean±standard deviation. Descriptive statistics was used to analyze the mean, standard deviation, variation and level of significance between groups. When $p \leq 0.05$ and $p \leq 0.01$, it was considered statistically significant for analysis of percent inhibition of cell growth.

3. RESULTS

3.1 Total yield of crude extract

The total yield of crude extracts from *Thespesia populnea* fruits by using the solvents, namely, mintroleum, ethyl-acetate, methanol, and hydro-alcoholic were 32gm, 41gm, 57gm, 69gm respectively, with reference to the air dried plant material.

3.2 Phytochemical analysis

Table 1: Preliminary phytochemical screening of *Thespisia populnea.*

Constituents	Chloroform	Ethyl acetate	Hydro alcohol	Water
Alkaloids	+	+	+	+
Steroids	+	+	+	+
Coumarins	_	_	_	_
Tannins	+	+	+	+
Phenols	_	+	+	+
Flavonoids	_	+	+	+
Saponins	+	_	_	_
Carbohydrate	+	+	+	+
Glycosides	+	_	_	_

"-" indicates Absent, "+" indicates Presence

3.3 Effect of *Thespesia populnea* hydroalcoholic fruit extract on K562(Leukemia) and HeLa(Cervical cancer) cell lines.

The result of MTT assay revealed that the hydro-alcoholic fruit extract of *Thespesia populnea* decreased the viability of all the cells but to different extent. Cytotoxicity effect induced by the extract is more similar to the effect induced by many chemotherapeutic cancer agents including morphological changes and shrinkage of cells leading to the death of cell lines. The IC50 values of the hydro-alcoholic fruit extract of *Thespesia populnea* on both cell lines are included in the below mentioned table.

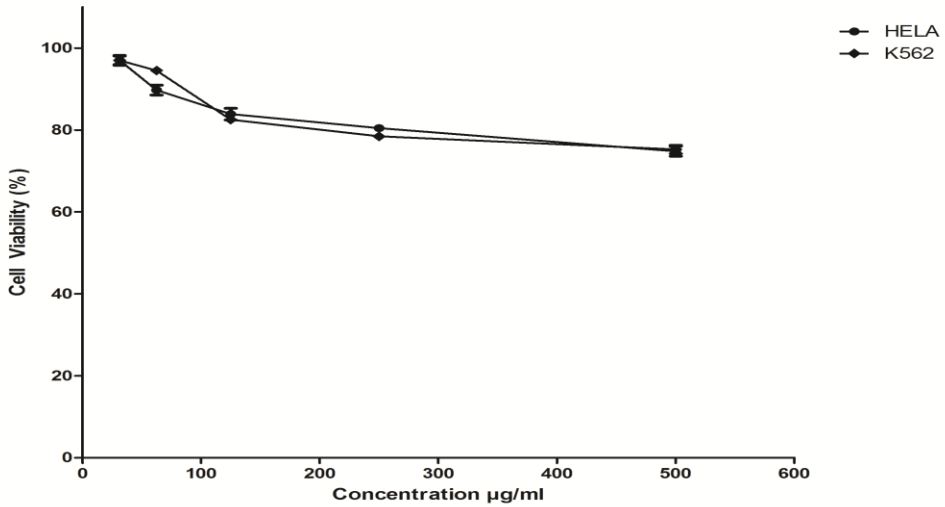
Table 2: Standard Drug(Paclitaxel).

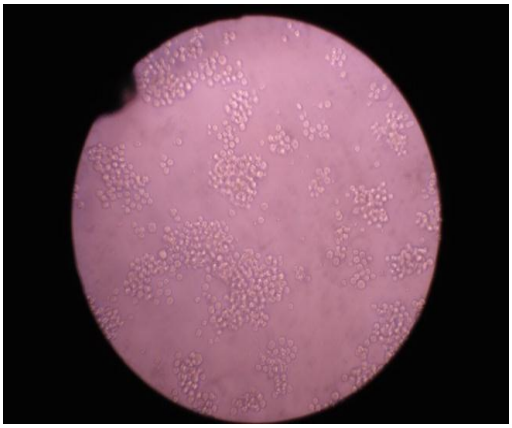
Cell lines	IC50	
	µM	µg
K562	0.29	247.63
HELA	0.23	196.98

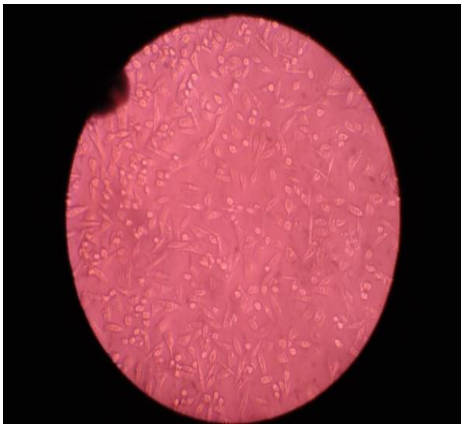
Table 3: Ic50 Value OF Compounds IN µG/ML

Compound	HeLa	K562
Thespesia populnea plant extract	2886	2283

	Plant Extract (Mean Cell Viability)	
Conc(µL/mL)	HELA	K562
500	74.798	75.259
250	80.444	78.468
125	83.871	82.505
62.5	89.718	94.513
32.75	96.976	96.998
NC	100	

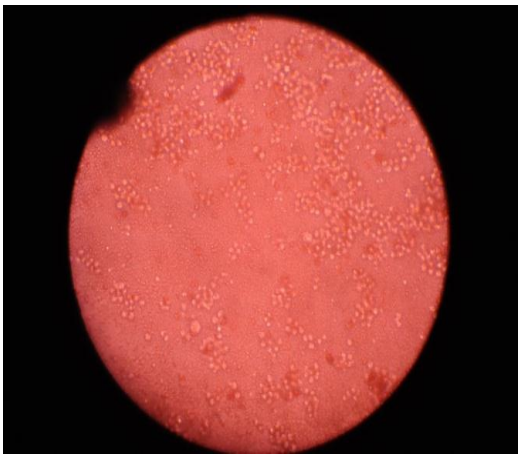


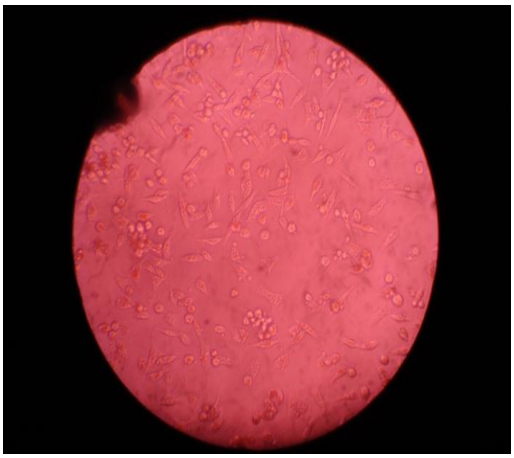




A.K62 Cell Line **B. Hela Cell Line**

Fig 1: Control (Paclitaxel) Tearted.





C.K562 Cell Line **D. Hela Cell Line**

Fig 2: Extract Treated.

4. DISCUSSION

In recent years, the use of herbal medicine in cancer treatment has received increasing attention due to their varied phyto-metabolic contents with multiple biological activities. The plants collected from Eastern Ghats of Southern India was identified according to the taxonomical characters as *Thespesia populnea* and analyzed for the presence of phytochemicals with four solvent extracts. Preliminary phytochemical analysis revealed the presence of secondary metabolites in selected extracts of the plant. These secondary metabolites are reported to have many biological and therapeutic properties. Phyto constituents were identified, phenols and flavanoids were detected in the alcohol and hydro-alcohol extracts presence of alkaloids steroids and carbohydrates in all extracts, coumarins are absent in all extracts. The studied phytochemicals of fruits in hydro-alcohol extract were pharmaceutically useful compounds. Among the different phytochemicals phenolic

compounds have gained attention of different areas of applications such as in pharmaceutical, health, food and cosmetic industry. These compounds are wide spread in the plant kingdom as part of our daily diet and are attractive as natural antioxidants.

The evaluation of the anti-cancer activity of plant extracts is essential for sage treatment. It enables identification of the intrinsic toxicity of the plant and the effects of acute overdose. The MTT assay is used in screening the crude extracts as well as in the isolated compounds to assess the toxicity. It could also provide an indication of possible cytotoxic properties of the tested plant extracts. MTT assay is based on the reduction of MTT by mitochondrial dehydrogenase by purple formazan product. It is frequently used as an invitro model system to measure the cytotoxic effects of variety of toxic substances and plant extracts against the cancer cell lines. Invitro cytotoxicity test using K562 and HeLa cancer cell lines were performed to screen potential toxic compounds that effect the basic cellular functions and morphology. The extract of *Thespesia populnea* invitro growth inhibition effects on the two cancer cell lines with concentrations like 500,250, 125, 62.5, 32.75 respectively. The 500µg/ml was showing maximum effect producing percentage growth inhibition. The results showed that the extract is most potent with IC50 value of 2886µg/ml and 2283µg/ml for HeLa and K562 cell lines respectively. The results also confirmed the effect induced by the extract and standard drug in cancerous cells. Therefore, the inhibition of cell growth by *Thespesia populnea* extract is due to the presence many polyphenolic and flavanoid compounds in the hydro-alcoholic extract.

From the results, it was observed that the plant *Thespesia populnea* contains a wide variety of secondary metabolites that hold strong anticancer potential. *Invivo* animal models can be put forward an attempt to carry out trails for further study.

5. CONFLICT OF INTEREST

The authors declare that they have no competing interests.

6. CONCLUSION

In the present study highest percentage extractive was observed in methanolic extracts of *Thespesia populnea.* Higher cytotoxic activity was observed in hydro-alcohol extracts in *Thespesia populnea* in both K562 and HeLa cell lines. Thus the test plant exhibited potent cytotoxic activity.

7. ACKNOWLEDGEMENT

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