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Evaluation of Antioxidant and Cytotoxic Potency of *Thespesia populnea* Leaf Extract

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Abstract: A large tree called *Thespesia populnea* (Malvaceae) is found in the tropical and coastal forests of India. In different parts of the plant, *T. populnea* has been shown to have antifertility, antibacterial, anti-inflammatory, antioxidant, purgative, and hepatoprotective properties. The goal of the current investigation was to find out how *T. populnea* leaves affected the HCT15 colorectal cancer cell line. All the *in vitro* experiments were conducted using aqueous and ethanol extracts. *Thespesia populnea* L. was subjected to a preliminary phytochemical study, which found the presence of alkaloids, flavonoids, saponin, tannin, proteins, anthraquinone, polyphenol, and carbohydrates in both extracts with the exception of terpenoids and glycosides. The aqueous extract had the greatest total phenolic and flavonoid content, with 168.04 mg of GAE per g, 34.22 mg per g, and 4.81 mg per g, respectively. It also exhibited the highest DPPH radical scavenging activity, at 81.82 per cent. This research demonstrated that *T. populnea* leaf is a potential source of antioxidants. These extracts were examined for their capacity to kill colorectal cancer cells in a test tube (HCT15). Aqueous extract has a high extractive percentage of 55.56 per cent at 100 µg/ml. All of the investigated cell lines had a dose-dependent viability inhibition from both aqueous and ethanol extract.

Keywords: *Thespesia populnea*, Antioxidant, Anticytotoxic, DPPH, Flavonoids, Alkaloids, Saponin

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Introduction

To treat any sickness that affects people, nature has provided a wide range of treatments. Because they can treat conditions that pose a serious

danger to life, plants are naturally fortunate. As a source of medicine, plants have been utilised since the beginning of civilization. Plants are now the

starting point for the production of pharmaceuticals, acting as natural models for the development of new drugs as well as phytomedicines for the treatment of disease (Iwu, 1993). A cell grows uncontrolled and spreads throughout the body in the sickness known as cancer, which ultimately results in the death of the host. Even in developing nations, the number one cause of morbidity and death is shifting from other diseases to cancer due to changes in dietary practises, exposure to numerous chemicals and radiation, and the availability of effective treatments for many infectious diseases (Notani, 2001). 60% of presently used anticancer drugs are derived from natural sources, including medicinal plants, demonstrating the significance of medicinal plants in the development of successful anticancer therapies (Chandran *et al.*, 2015). Plant-derived secondary metabolites have promise as nutraceuticals for the treatment of a number of illnesses, including cancer (Hafidh *et al.*, 2014).

Thespesia populnea (L.) is a small to medium-sized Malvaceae tree with a pan-tropical range that is generally found in coastal areas. It is also frequently referred to as the "Indian tulip tree." In Kerala's coastal lowlands and midlands, *T. populnea* is a common tree that is commonly grown for its several benefits in backyard gardens and other agroforestry systems (Chandran *et al.*, 2014). It has long been believed to possess medicinal qualities including hepatoprotective, purgative, anti-inflammatory, antifertility, and anti-inflammatory effects. Additionally, the medicinal benefits of *T. populnea*, including its antibacterial, antifertility, and antinociceptive properties, have been reported (Vasudevan and Parle, 2006). The goal of the current investigation was to find out how *T. populnea* leaves affected the HCT15 colorectal cancer cell line.

Materials and Methods

All of the chemicals used in the extraction procedure were purchased from SD Fine Chemicals, Mumbai, India, and were of analytical quality.

Plant material collection and identification:

Thespesia populnea fresh leaves were harvested from Ozhugarai, Tamil Nadu, India.

Preparation of Extracts:

Aqueous extract:

100 ml of distilled water was used to soak around 10 g of powder. The water-soaked powder was subjected to 20 min of intense magnetic swirling at 80°C. The filtrate was then collected and kept at 4°C for subsequent processing after filtration using Whatman filter paper.

Extract of ethanol:

Using the Soxhlet equipment, 200 ml ethanol was extracted 18 times with 10 g of dried leaf powder. The extract was concentrated in a rotating vacuum evaporator for 3 h at temperatures between 30 and 400 °C. Vacuum desiccators were used to dry the concentrates, and the dried extract was kept at -20°C.

Screening of Qualitative Phytochemicals:

To determine the secondary metabolites contained in the ethanolic and aqueous extracts of *Thespesia populnea* leaf extracts, preliminary qualitative phytochemical analysis was conducted.

Screening for Quantitative Phytochemicals:

In order to be utilised for the subsequent analysis, the extracts were dissolved in their appropriate solvents.

Calculation of the Phenol Content in Total (TPC):

Folin Ciocalteu reagent was used to calculate the TPC of plant extract. Folin Ciocalteu reagent (1:10 diluted with distilled water), 5 ml of plant extract (0.5 ml of 1:10 g/ml), and 4 ml of Na₂CO₃ were combined (1 M). A UV-visible spectrophotometer was used to test the mixture's absorbance at 760 nm after it had stood for 15 min (Shimadzu, UV 2450). The standard solution was made by mixing methanol and water (50:50, v/v) with gallic acid (50–250 mg per litre). Gallic acid equivalents (GAE) were used to represent the total phenolic content in mg/g of sample (McDonald *et al.*, 2001).

Total Flavonoid Content is Approximated (TFC):

Chang *et al.* (2002) described AlCl_3 colorimetric technique which was used in this study to quantify the total flavonoid content (TFC). Briefly, 1.5 ml of 95% alcohol, 0.1 ml of 10% AlCl_3 , 0.1 ml of 1 M $\text{CH}_3\text{CO}_2\text{K}$, and 2.8 ml of deionized water were combined with 0.5 ml of 1:10 g/ml plant extract. The reaction mixture was measured at 415 nm using UV-visible spectrophotometer (Shimadzu, UV-2450) against a blank of deionized water after 40 min incubation at 37°C. A range of concentrations (12.5-100 g/ml) of quercetin was utilised as the standard. The amount of flavonoids was reported as mg/g of quercetin equivalents (QE) (Chang *et al.*, 2002).

Testing for Antioxidant Activity:

The change in optical density of the target was used to gauge the scavenging activity of the plant extracts. We evaluated 2,2 diphenyl-1-picrylhydrazyl (DPPH) using the technique described by Gregory *et al.* (1996). The different concentrations of plant extract, ranging from 25 to 150 $\mu\text{g/ml}$, were diluted with methanol and combined with 200 μl of 0.1 mM DPPH. They were well combined, incubated at 37 °C for 30 min, and the degenerate colour of DPPH was measured at 517 nm using ascorbic acid as a reference point (Koleva *et al.*, 2002).

$$\text{DPPH \%} = (\text{Absorbance test} - \text{Absorbance control}) / (\text{Absorbance test} \times 100)$$

Cancer cell line treatment using plant extract:

The crude extracts were applied to the cell lines, and then 1 ml of MEM containing sodium pyruvate and 10% FCS was added. The dissolved aqueous extracts of *T. populnea* were then filtered using a Whatman syringe filter (0.2 μm pore size). 1 ml of stock included the filtered crude extracts. In the 96 well plates, 100 μl of diluted *T. Populnea* aqueous extracts were added. The 96 well plates were studied 72 h after being placed in a 5 per cent CO_2 incubator at 36 °C.

Test for cytotoxicity of plant extracts (MTT Assay):

Using a colorimetric assay that measures the reduction of yellow-3-(4,5Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) by mitochondrial succinate dehydrogenase enzyme described by Mosmann (1983), the cell cytotoxicity of *T. populnea* leaf and flower extracts on colon cancer cell lines (HCT15) was determined. The amount of formazan directly relates to the number of active cells and the percentage of inhibition. A simple colorimetric test was used to measure the color's intensity. A multi-well scanning spectrophotometer (ELISA reader) was used to measure colour intensity (Talib and Mahasneh, 2010).

Results and Discussion

Plant Phytochemical Screening at an Early Stage:

Thespesia populnea L. was subjected to a preliminary phytochemical study, which showed the presence of alkaloids, flavonoids, saponin, tannin, proteins, anthraquinone, polyphenol, and carbohydrates in both extracts with the exception of terpenoids and glycosides (Table 1).

The first screening of leaf extract for phytochemicals revealed the presence of compounds considered to be active medicinal chemical agents. *Thespesia populnea* leaves contained significant medicinal phytochemicals as flavonoids, alkaloids, terpenoids, reducing sugar, tannins, and glycosides; these secondary metabolites were in charge of the plant's antioxidant, antibacterial, anticancer, and pharmacological potential.

Table 2 and Figure 1 display a quantitative assessment of significant secondary metabolites. Plant material from *Thespesia populnea* has more phenol than flavonoid. One of the widely distributed polyphenolic secondary metabolites in plants, flavonoids have a number of well-known properties, including the ability to block both oxidative and hydrolytic enzymes as well as operate as an anti-inflammatory and free radical scavenger. Due to their potential benefits for

Table 1: Preliminary Phytochemical constituents of *Thespesia populnea* leaf extracts

S. No.	PHYTOCHEMICALS	AQUEOUS EXTRACT	ETHANOL EXTRACT
1	Alkaloids	+	+
2	Flavonoids	+	+
3	Carbohydrate	+	+
4	Saponin	+	+
5	Tannin	+	+
6	Terpenoids	-	-
7	Proteins	+	+
8	Anthraquinone	+	+
9	Polyphenol	+	+
10	Glycosides	-	-

+ Indicates present; - Indicates Absent

Table 2: Quantitative analysis of phytochemical constituents of *Thespesia populnea* leaf extract

EXTRACT	TOTAL PHENOLIC CONTENT (mg GAE/g of plant extract)	TOTAL FLAVONOID CONTENT (mg QE/g of plant extract)
AQUEOUS	168.04 ± 14.90	34.22 ± 4.81
ETHANOL	94.92 ± 7.45	14.78 ± 4.81

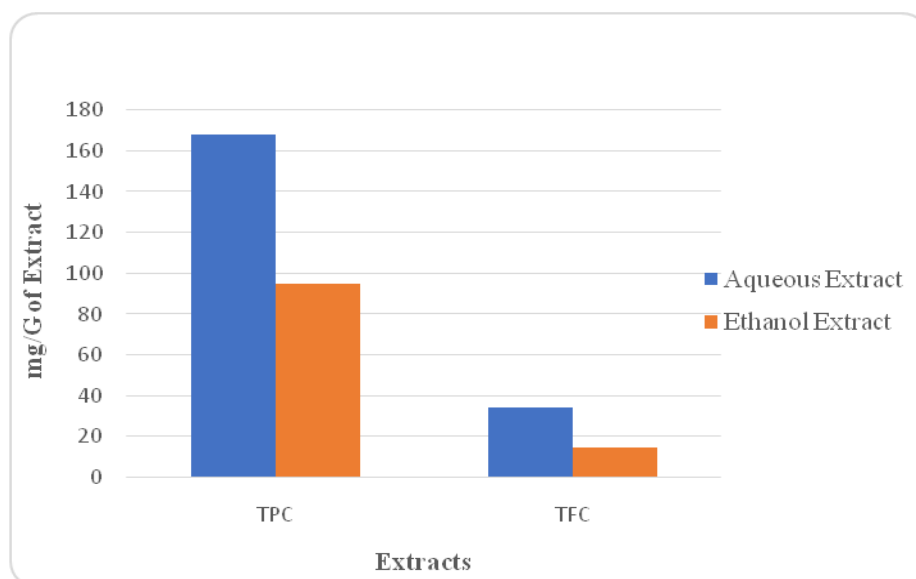


Fig. 1: Quantitative analysis of phytochemical constituents of *Thespesia populnea* leaf extract. TPC= total Phenolic content; TFC= Total Flavonoid content.

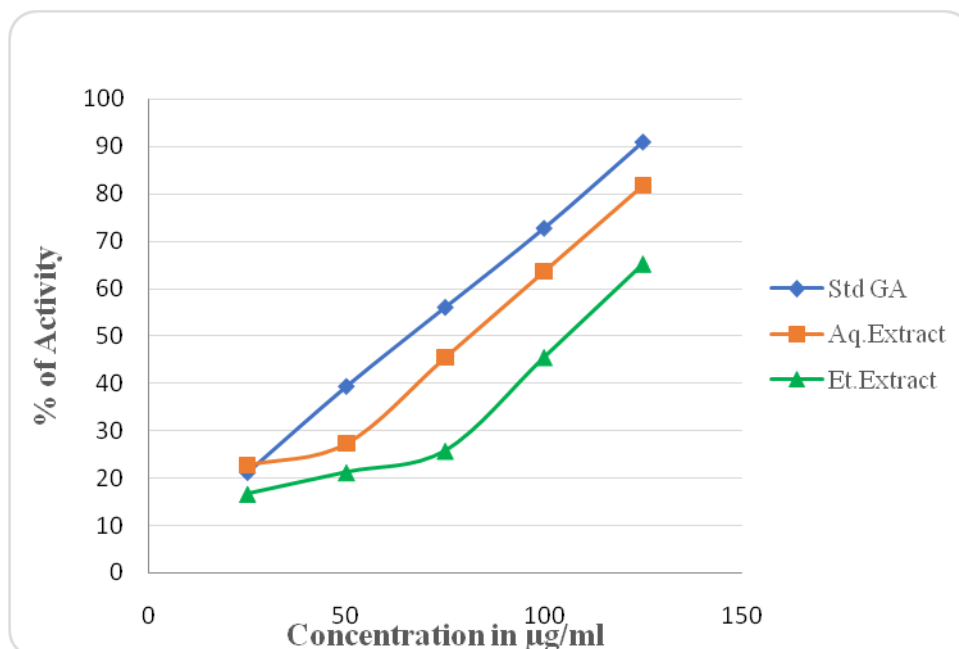


Fig. 2: Free radical – scavenging activity of *Thespesia populnea* leaf extract.

human health, dietary polyphenols are of highest interest (Syed and Ganapasam, 2017).

Additionally, phenolics have a major role in oxidative stability and antibacterial defence in diets. Flavonoids are recognised to have significant effects on human nutrition and health and to be potential antioxidants. The scavenging or chelating processes are the methods through which flavonoids work (Kolar *et al.*, 2017).

Free-radical scavenging activity in the DPPH:

Thespesia populnea leaf extract was found to contain a potent DPPH radical scavenger. Aqueous extracts had the strongest DPPH radical scavenging capabilities (Fig. 2). Using DPPH radicals as antiradicals, several organic compounds and plant materials have been examined for their potential to scavenge free radicals. Higher antioxidant capacity in the corresponding extract is directly connected with stronger DPPH reductions (Andina and Musfirah, 2017). This study implies that compounds in the plant extract have the capacity to give a free radical a hydrogen atom, causing it to become unstable. It was shown that these herbal extracts had strong anti-radical activities, which might be

useful for treating pathological tissue damage caused by radicals. The presence of phenolic phytochemicals like flavonoids that were discovered to be present in the extract may be responsible for this antioxidant effect.

Anti-Cancer activity:

The colon cancer cell line (HCT15) was treated with various drug concentrations ranging from 10 to 100 µg/ml for 72 h of incubation in order to evaluate the cytotoxic effect of the sequentially prepared extracts of *T. populnea* leaf extract on the cells. Cell viability was assessed using the MTT assay (Fig. 3). All of the investigated cell lines had a dose-dependent viability inhibition from both aqueous and ethanol extract (Andina and Musfirah, 2017). Andina and Musfirah (2017) have separately isolated the heartwood and wood of *T. populnea* using dichloromethane, and found that these extracts had a potent cytotoxic impact on a number of cancer cell lines, including MCF-7, HeLa, HT-29, and KB cells. The results further substantiated the impact that both the extract and conventional medicine had on cancerous cells. The fact that *Thespesia populnea* leaf extract inhibits cell growth is thus explained by the presence of

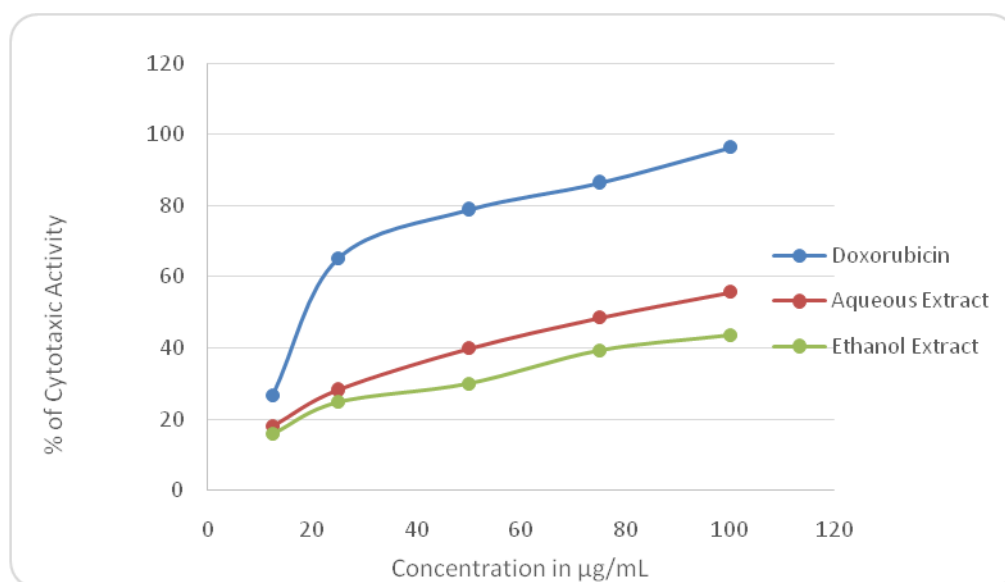


Fig. 3: Cytotoxic activity of various extract of *Thespesia populnea* leaf extract.

several polyphenolic and flavonoid components in the extract. *Thespesia populnea* is a plant that has been shown to have several secondary metabolites that have potent anticancer activities. Attempts may be made to carry out tests for more study utilising in vivo animal models.

Conclusion

In this study *Thespesia populnea* L. leaf extract exhibited powerful anti-oxidant and cytotoxic capabilities. The results of the current investigation partially support the claim made by traditional healers that the leaf extract is advantageous since the aqueous extract of *Thespesia populnea* L. has more apoptotic activity than the ethanolic extract. If the structural identities and properties of any of the compounds are discovered, there could be possibilities for future anticancer drug development.

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