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Original Research Paper

ANTI-OXIDANT AND ANTI-INFLAMMATORY ACTIVITY OF *THESPESIA POPULNEA* (FLOWERS)

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ABSTRACT

Thespesia populnea is also called as Indian tulip tree, poovarasu, is tropically distributed plant. In the present study of ethanolic extract of flowers of *Thespesia populnea* was screened for anti-inflammatory and antioxidant activities. Anti-inflammatory activity of ethanolic extract was determined by HRBC and Albumin denaturation method. Anti-oxidant activity of ethanolic extract was determined by DPPH assay and ABTS method. The results of the study suggest that the ethanolic extract possesses anti-oxidant and anti-inflammatory activity.

Keywords: *Thespesia populnea*, Antioxidant activity, Anti-inflammatory activity, HRBC method, Albumin denaturation, DPPH, ABTS assay.

INTRODUCTION

Therapeutically active principles are extracted from all parts of the plant body, but the concentration of these components varies from part to part. Normally, parts known to contain the highest concentration of the principles are preferred to therapeutic purposes and it can either be the leaves, stems, barks, roots, bulks, rhizomes, woods, flowers, fruits or the seeds.¹ The literature review revealed that antimicrobial, analgesic, antifertility and antibacterial, anti-inflammatory, antioxidant, purgative and hepatoprotective activities. In the indigenous system of medicine, the paste of the fruits, leaves and roots of *Thespesia populnea* is applied locally for their anti-inflammatory effects on swollen joints.² Gossypol was found to be the major component of *Thespesia populnea* which is responsible for anti-inflammatory and antifertility effects in rats as well as in human beings.³ The flowers contained kaempferol, kaempferol-7-glucoside and gossypetin, The fruit kernels were reported to contain β -sitosterol, ceryl alcohol and a yellow pigment, thespesin.⁴ The plant is traditionally claimed to possess useful medicinal properties^{5,6} such as antifertility, anti-inflammatory, antioxidant, purgative and hepatoprotective⁷ activities and its bark, leaves and flowers are useful in cutaneous infections such as scabies, psoriasis, eczema, ring worms, guinea worm,⁸ anti-inflammatory for poultice as a folk medicine etc. In addition to these *T. populnea* has been scientifically proved to possess medicinal properties such as antibacterial, antifertility, and antinociceptive activities,⁹ as also in the treatment of Alzheimer's disorder,¹⁰ for its memory enhancing activity, antioxidant and hepatoprotective activity,¹¹ anti-psoriatic activity,¹² diuretic activity¹³ and wound healing activity.¹⁴

MATERIALS AND METHODS

Collection of Plant Materials

Fresh flowers of *Thespesia populnea* were collected from O. Koothur Village, Ariyalur district, Tamil Nadu, India, during the month of October and identified by Head, PG & Research Department of Botany, Periyar E.V.R. College, Trichy, Tamil Nadu.

Flower Extraction

2 kg of fresh flowers were soaked with 90% ethanol at room temperature (25°-30°C). After 72 hrs the ethanolic extract was filtered. This extract was concentrated in vacuum and the dry powder obtained was dissolved in DMSO to get required concentrations and were used for screening anti oxidant and anti-inflammatory activities.

IN VITRO ANTIOXIDANT ACTIVITY

DPPH Assay Method

The DPPH free radical is reduced to a corresponding hydrazine when it reacts with hydrogen donors. The DPPH radical is purple in colour and upon reaction with hydrogen donor changes to yellow colour. It is a decoloration assay, which is evaluated by the addition of the antioxidant to a DPPH solution in ethanol or methanol and the decrease in absorbance was measured at 490nm¹⁵.

Reagents

A. 2,2-Diphenyl 1-picryl hydrazyl solution (DPPH, 100µM):

22 mg of DPPH was accurately weighed and dissolved in 100 ml of methanol. From this stock solution, 18 ml was taken and diluted to 100 ml using methanol to obtain 100 µM DPPH solution.

B. Preparation of test solutions:

21 mg of the solid obtained from ethanolic extract was dissolved in distilled DMSO to obtain a solution of 21 mg/ml concentration. This solution was serially diluted to obtain lower concentrations.

C. Preparation of standard solutions:

10 mg each of ascorbic acid and rutin were weighed separately and dissolved in 1 ml of Dimethyl sulfoxide (DMSO) to get 10 mg/ml concentrations. These solutions were serially diluted with DMSO to get lower concentrations.

D. Procedure

The assay was carried out in a 96 well microtitre plate. To 200µl of DPPH solution, 10µl of each of the test sample or the standard solution was added separately in wells of the microtitre plate. The final concentration of the test and standard solutions used were 1000, 500, 125 and 31.25 µg/ml. The plates were incubated at 37° C for 30 min and the absorbance of each solution was measured at 490 nm, using a microplate reader.

EVALUATION OF TOTAL ANTIOXIDANT CAPACITY OF THE EXTRACT

The total antioxidant capacity was determined by phosphormolybdenum method and is based on the reduction of Mo (VI) to Mo (V) by the antioxidant compounds and the formation of a green Mo (V) complex which has the maximal absorption at 695 nm.

Preparation of Test and Standard Solutions

Weighed accurately 55 mg each of the sample and the standard ascorbic acid and dissolved in 5 ml of DMSO. The lower dilutions were made serially with DMSO.

Procedure

An aliquot of 0.1 ml of the sample solution containing a reducing species in DMSO was combined in an Eppendorff tube with 1 ml of reagent solution (0.6 mM Sulphuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The tubes were capped and incubated in water bath at 95 °C for 90 min. The samples were cooled to room temperature, and the absorbance of each solution was measured at 695 nm. The total antioxidant capacity was expressed as mM equivalent of ascorbic acid (Mojca *et al.*, 2005).

Total antioxidant activity = 153.5 µg/ml

ABTS Radical Scavenging Activity

ABTS radical scavenging activity was performed as described by Re *et al.*, 1999 with a slight modification. 7.0 mM ABTS in 14.7 mM ammonium peroxy-disulphate was prepared in 5.0 ml distilled water. The mixture was allowed to stand at room temperature for 24 hours. The resulting blue green ABTS radical solution was further diluted such that its absorbance is 0.70 ± 0.020 at 734 nm. Various concentrations of the sample solution dissolved in ethanol (20.0 µl) were added to 980.0 µl of ABTS radical solution and the mixture was incubated in darkness for 10 min. The decrease in absorbance was read at 734 nm. A test tube containing 20.0 µl of ethanol and processed as described above served as the control tube. Different concentrations of ascorbic acid were used as reference compound.

ANTI-INFLAMMATORY ACTIVITY

The Human Red Blood Cell (HRBC) Membrane Stabilization Method

The method as prescribed (Gopalkrishnan *et al.*, 2009; Sakat *et al.*, 2010) was adopted with some modifications. The blood was collected from healthy human volunteer who had not taken any NSAIDS for 2 weeks prior to the experiment and mixed with equal volume of Alsever solution (2 % dextrose, 0.8 % sodium citrate, 0.5 % citric acid and 0.42 % NaCl) and centrifuged at 3,000 rpm. The packed cells were washed with isosaline and a 10 % suspension was made. Various concentrations of extracts were prepared in mg/ml using distilled water and to each concentration, 1 ml of phosphate buffer, 2 ml hypo saline and 0.5 ml of HRBC suspension were added. It was incubated at 37 °C for 30 minutes and centrifuged at 3,000 rpm for 20 minutes and the hemoglobin content of the supernatant solution was estimated spectrophotometrically at 560 nm. Diclofenac (100 Jg/ml) was used as reference standard and a control was prepared by omitting the extracts. The experiments were performed in triplicates and mean values of the three were considered. The percentage (%) of HRBC membrane stabilization or protection calculated using the following formula^{16,17}

Percentage of protection (%) = (100 - OD of drug treated sample/OD of Control) X 100

Albumin Denaturation Method

The method as prescribed (Sakat *et al.*, 2010) was followed with modifications. The reaction mixture was consisting of test extracts and 1% solution of bovine albumin fraction. pH of the reaction mixture was adjusted using small amount of HCl. The sample extracts were incubated at 37°C for 20 minutes and then heated to 51°C for 20 minutes. After cooling the samples the turbidity was measured spectrophotometrically at 660 nm. Diclofenac sodium was taken as a standard drug. The experiment was performed in triplicates and the mean value of the three was considered. Percent inhibition of protein denaturation was calculated as follows^{18,19}

Percentage of inhibition (%) = (OD of Control- OD of Sample/ OD of Control) X 100

RESULTS AND DISCUSSION

Isolation of pure, pharmacologically active constituents from plants remains a lengthy and tiresome process. For this reason, it is crucial to have methods available which eradicate unnecessary separation procedures. Chemical screening is thus performed to allow localization and targeted separation of new or useful constituents with potential activities. Strong occurrence of flavonoids in extract has been shown to possess potent anti-inflammatory properties. Analgesic and anti inflammatory effects have been observed in flavonoids. The results strongly suggest anti-inflammatory effects and anti oxidant effects by percentage of inhibitions are explained in the table1, 2, 3, 4 and also by graphical representation1, 2, 3and 4.

CONCLUSION

In conclusion, the present study has confirmed that DPPH assay, Total anti-oxidant and ABTS have showed a strong antioxidant activity and also the human red blood cell (HRBC) membrane stabilization, Inhibition of albumin denaturation indicated the anti-inflammatory activity. The present investigations have demonstrated a strong connection between the anti-inflammatory and antioxidant activities of *Thespesia populnea* flowers. The prevention of oxidative damage to tissue could therefore be one of the mechanisms responsible for the anti-inflammatory effect shown by both the cultivars of this plant. The medical use of *Thespesia populnea* as a useful remedy in arthritic disorders could possibly be because of its excellent anti-inflammatory and antioxidant potential. The isolation, purification and mechanism of action of phenolic and flavonoidal components and other components of *Thespesia populnea* flowers are of interest for further investigation and shall be carried out in future studies.

Table 1: DPPH assay activity of ethanolic extract of flowers of *Thespesia populnea*

S. No.	Concentration ($\mu\text{g/ml}$)	% CTC ₅₀ Cytotoxicity ($\mu\text{g/ml}$)	CTC ₅₀
1	1000	85.49	95.27 $\mu\text{g/ml}$
2	500	72.13	
3	125	54.75	
4	31.25	45.17	

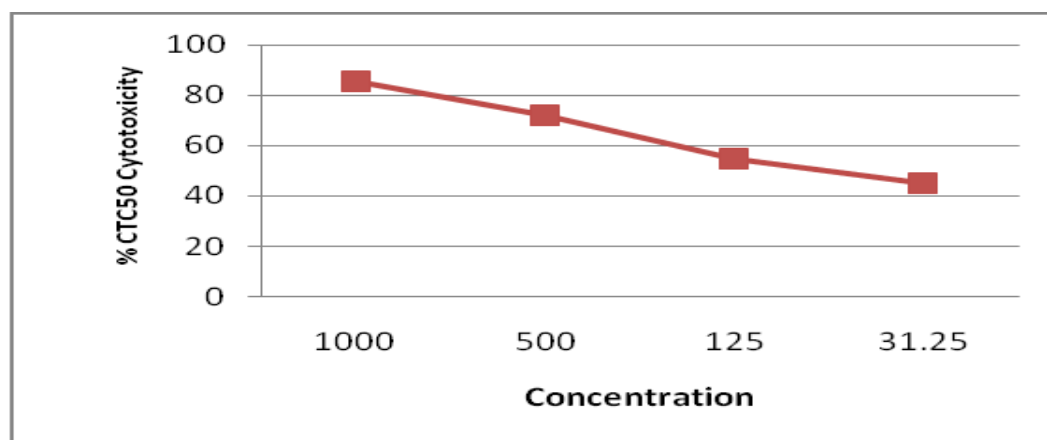


Figure 1: Graphical representation of DPPH activity of ethanolic extract of flowers of *Thespesia populnea*

Table 2: ABTS assay activity of ethanolic extract of flowers of *Thespesia populnea*

S. No.	Concentration ($\mu\text{g/ml}$)	% CTC ₅₀ Cytotoxicity ($\mu\text{g/ml}$)	CTC ₅₀
1	1000	92.15	65.16
2	500	83.01	
3	125	64.93	
4	31.25	46.12	

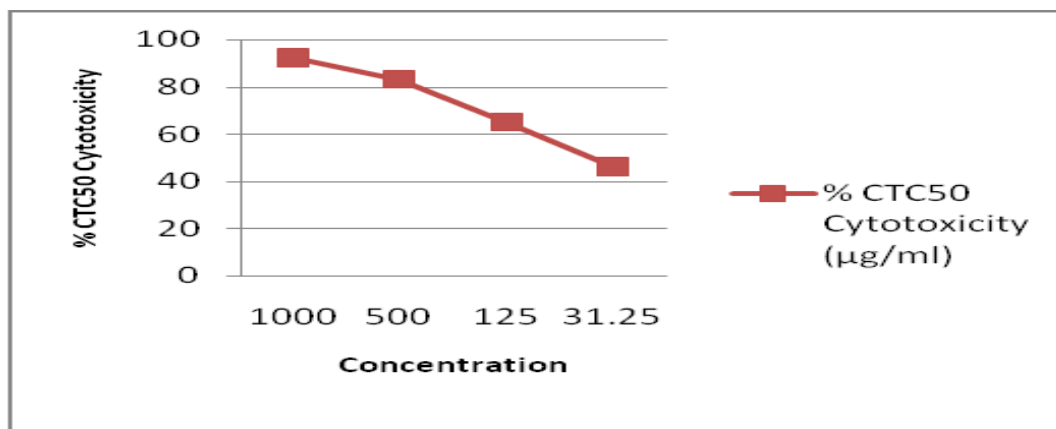


Figure 2: Graphical representation of ABTS radical scavenging activity of ethanolic extract of *Thespesia populnea*

Table 3: The human red blood cell (HRBC) membrane stabilization activity of ethanolic extract of flowers of *Thespesia populnea*

S. No.	Concentration (µg/ml)	% of Inhibition
		Membrane Stabilization Mean ± S. E. M (S-I)
1	100	18.47±0.49
2	200	32.86±1.05
3	400	42.97±1.18
4	600	64.84±1.26
5	800	76.18±1.73

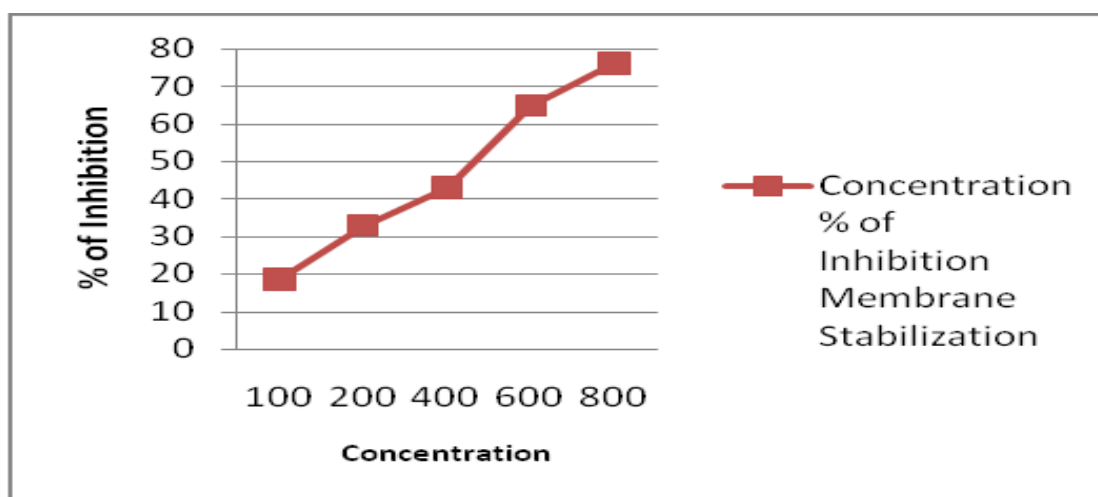
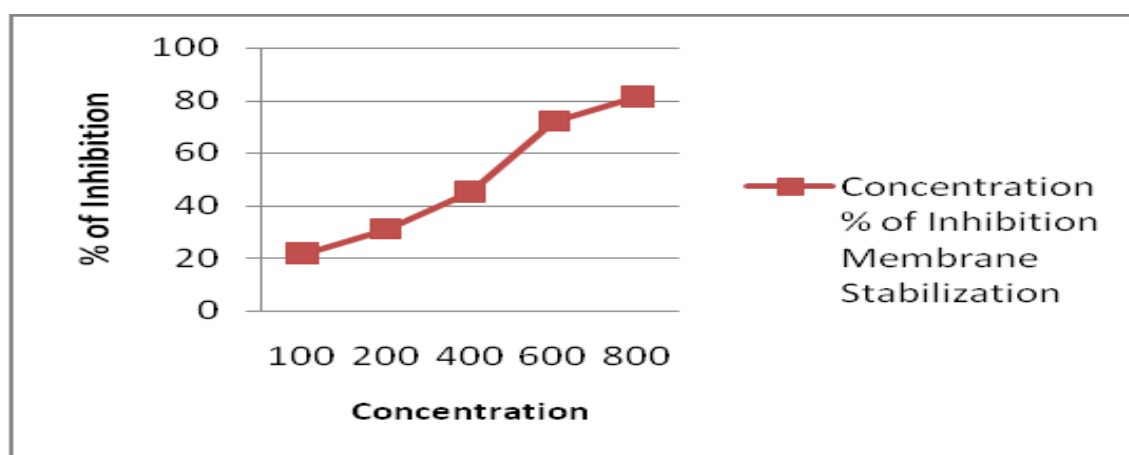


Figure 3: Graphical representation of human red blood cell (HRBC) membrane stabilization activity of ethanolic extract of flowers of *Thespesia populnea*

Table 4: The Inhibition of Albumin Denaturation activity of ethanolic extract of flowers of *Thespesia populnea*

S. No.	Concentration ($\mu\text{g/ml}$)	% of Inhibition
		Membrane Stabilization Mean \pm S.E.M(S-I)
1	100	21.47 \pm 0.48
2	200	30.96 \pm 1.57
3	400	45.16 \pm 1.29
4	600	72.05 \pm 1.84
5	800	81.39 \pm 1.59

**Figure 4:** Graphical representation of Inhibition of Albumin Denaturation activity of ethanolic extract of flowers of *Thespesia populnea*

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