



EVALUATION OF IN VITRO ANTI-INFLAMMATORY ACTION OF *THESPESIA POPULNEA* (Linn.)

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ABSTRACT

The major objective of current work is to standardize the raw material such as leaves of *Thespesia populnea* L by pharmacognostical analysis and to evaluate the effect of flavonoid rich fraction of leaf extract of *Thespesia populnea* L for its , anti-inflammatory activity. The current study was aimed at evaluation of pharmacognostical, phytochemical and biological activities of flavonoid rich fraction of *Thespesia populnea* Linn. (Malvaceae). Flavonoids are the major plant phenolic compounds and the most studied phytochemicals. This includes five major classes different by their specific chemical structure, namely flavones, flavones, flavonols, flavonols and anthocyanidins. Flavonoids constitute one of the most characteristic classes of compounds in higher plants. Hence the current study had been aimed at exploring the possible biological activities of plant under investigation. The anti-inflammatory action are determined by HRBC MEMBRANE STABILIZATION METHOD.

KEYWORDS:

INTRODUCTION

A number of drugs from plant sources are known to cause anti-inflammatory effects and used in conditions like rheumatoid arthritis, gout, dysmenorrheal etc. Inflammation may occur due to mechanical causes, infection or autoimmune diseases. Inflammation is the reaction of living tissues to injury, infection or irritation. Bacterial infections cause an increased number of neutrophils, which produce an oxidative burst at the site of microbial invasion. The uncontrolled release of reactive oxygen species is assumed to be responsible for certain pathological conditions as heart attacks, septic shocks and rheumatoid arthritis.

Lipoxygenases (Los) are a family of iron containing enzymes that catalyze the deoxygenating of polyunsaturated fatty acids in lipids containing a *cis, cis*-1, 4-pentadiene structure. They convert arachidonic acid, a component of membrane phospholipids, into pro-inflammatory mediators called leukotrienes which are a group of highly potent molecules having diverse biological actions are increasingly implicated in a variety of disease states including asthma, chronic obstructive pulmonary disease (COPD), cancer, osteoporosis and atherosclerosis. Recently, there has been considerable interest in the development of LO inhibitors for therapeutic indications. Although, anti-inflammatory drugs are used extensively, prolonged consumption of

these medications is usually coupled with numerous side effects. Therefore, there is a need to explore alternative strategies to lower the formation of inflammatory mediations with the help of natural dietary products.

AIM AND SCOPE OF THE WORK

The current study was aimed at evaluation of pharmacognostical, phytochemical and biological activities of flavonoid rich fraction of *Thespesia populnea* Linn. (Malvaceae). Flavonoids are the major plant phenolic compounds and the most studied phytochemicals. This includes five major classes different by their specific chemical structure, namely flavones, flavones, flavonols, flavonols and anthocyanidins. Flavonoids constitute one of the most characteristic classes of compounds in higher plants. Hence the current study had been aimed at exploring the possible biological activities of plant under investigation

PLANT PROFILE

***Thespesia Populnea* (Linn.) Soland ex Correa**

Synonym : *Hibiscus populnea* Linn.

Family : Malvaceae

The plant yields kaempferol and its glycosides, herbacetin and its glucoside, populneol, populnin, populnetin, quercetin, rutin, gossipetin, (\pm) gossypol, β -sitosterol and its glucoside, lupeol, lupenone, alkanes,

myricylalcohol, calycopterin, sequiterpenoidal quinines viz; thespesone, mansonones C,D,E and F, amino acids and carbohydrates.

Plan of work

1. Collection and authentication of plant material
2. Pharmacognostical study
3. Physicochemical evaluation
4. Phytochemical investigation
 - Extraction of plant material
 - Preliminary phytochemical screening
 - Determination of total phenolic content
 - Preparation of flavonoid rich fraction.
5. Evaluation of biological activities
 - Anti-inflammatory action

Physicochemical Determination

1. Determination of total ash value
2. Determination of acid insoluble ash value
3. Determination of water soluble ash value
4. Determination of alcohol extractive value
5. Determination of water soluble extractive value.

Parameters	Values (% w/w)
Total ash	8.63
Water soluble ash (% w/w)	6.15
Acid insoluble ash (% w/w)	1.57
Water soluble extractive value	12.46
Alcohol soluble extractive value	18.48

EXTRACTION OF PLANT MATERIAL

The fresh leaves were collected, dried and pulverized using a mechanical grinder and preserved in an airtight container for further use. The dried leaf powder was extracted with 95% ethanol by cold maceration method and kept for 48 hrs and were filtered, concentrated.

PRELIMINARY PHYTOCHEMICAL SCREENING

The different qualitative chemical tests can be performed for establishing profile of given extract for its chemical composition. The above tests were performed on extracts.

Test	Ethanol extract
Alkaloids	+
Carbohydrates	+
Glycosides	-
Saponins	+
Proteins and amino acids	+
Phytosterols	+
Terpenes	+
Fixed oils	-
Phenols	+
Flavonoids	+
Tannins	+
Gums and mucilage	+
Volatile oils	-

(+) Present

(-) Absent

DETERMINATION OF *IN VITRO* ANTI-INFLAMMATORY ACTIVITY

HRBC MEMBRANE STABILIZATION METHOD

The human red blood cell membrane stabilization method was used for this study. The blood was collected from healthy volunteer who was 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% sodium chloride in 100ml water) 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 (250, 500 and 1000 mcg/ml) using distilled HRBC suspension were added. It is incubated at 37°C for 20min. The hemoglobin content of the supernatant solution was estimated spectrophotometrically at 560nm. Diclofenac (50mcg/ml) was used as reference standard and a control was prepared omitting the extracts.

$$\text{Inhibition} = \frac{\text{Control} - \text{Test}}{\text{Control}} \times 100$$

The *in vitro* anti-inflammatory activity was carried out by HRBC membrane stabilization method and the percentage inhibition of test was compared with that of the standard at 1000µg/ml.

Table 5: HRBC Membrane Stabilization Method.

Sl. No.	Concentration (µg/ml)	Flavonoid rich fraction
		% Inhibition
1.	1000	63.91±0.96***
2.	500	46.23±1.36***
3.	250	27.96±1.01***
4.	Std (Diclofenac, 50µ/ml)	71.92±0.44

Values are expressed as mean ± SEM, ***P<0.0001vs std-control (grp-4).

Symbol represents the statistical significance done by t-test.

CONCLUSION

The present study had shown that the flavonoid rich fraction of *Thespesia populnea* to possess a good *in vitro* anti-inflammatory activity. This might be due to the antioxidants principles present in the extract which compete with oxygen to react with nitric oxide thereby inhibiting the generation of nitrite. However the presence of phenolic compounds such as tannins and flavonoids would have played a major role in exhibiting the biological activity. Hence the plant can further be explored and could be used in the drug development for treating oxidative stress induced diseases and conditions including diabetes, cardiovascular diseases, inflammatory conditions, cancer and aging.

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