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CHARACTERIZATION OF *THEPESIA POPULNEA (LINN.)* FOR ITS INVITRO ANTI OXIDANT ACTIVITY

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ABSTRACT

The anti-oxidative properties of flavonoids are due to several different mechanisms, such as scavenging of free radicals, chelation of metal ions, such as iron and copper, and inhibition of enzymes responsible for free radical generation. Depending on their structure, flavonoids are able to scavenge practically all known ROS. Oxygen consumption inherent in cell growth leads to the generation of a series of reactive oxygen species (ROS). They are continuously produced by the body's normal use of oxygen such as respiration and some cell-mediated immune functions. ROS include free radicals such as superoxide anion radicals (O^2), hydroxyl radicals (OH) and non-free radical species such as hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O_2$). ROS are continuously produced during normal physiologic events and can easily initiate the peroxidation of membrane lipids, leading to the accumulation of lipid peroxides. ROS are also capable of damaging crucial biomolecules such as nucleic acids, lipids, proteins and carbohydrates and may cause DNA damage that can lead to mutations. If ROS are not effectively scavenged by cellular constituents, they lead to disease conditions. ROS have been implicated in more than 100 diseases. The in-vitro anti oxidant activity is determined by NITRO OXIDE ACAVENGING METHOD.

KEYWORDS:

INTRODUCTION

Tyrosinase is responsible for enzymatic browning is plants, and it may cause undesirable changes in color, flavor and nutritive value of plant-derived foods and beverages. This enzyme is a copper containing enzyme which is mainly involved in melanin biosynthesis. Tyrosinase catalyses melanin biosynthesis in human skin and epidermal hyper pigmentation may cause various dermatological disorders, such as melasma, freckles and age spots. In mammals, tyrosinase is responsible for pigmentation of the skin, eyes and hair. In plants, it causes undesired enzymatic browning of farm products, such as bruised or cut fruits and vegetables, which subsequently leads to a significant decrease in nutritional and market values. In insects, the enzyme is essential for the sclerotization of the exoskeleton, wound healing and parasite encapsulation. It is apparent that inhibitors have been used as depigmenting agents for the treatment or prevention of pigmentation disorders. Tyrosinase, present in melanosome granules within the melanocyte, catalyzes the formation of melanin, the brown or black polymeric pigment in skin, melanomas hair, and eyes.

Recently, safe and effective tyrosinase inhibitors have become inportat for their potential applications in improving food quality. Furthermore, tyrosinase inhibitors are also important in cosmetic application for skin-whitening effects. Since plants are a rich source of bioactive schemicals, and are mostly free of harmful side effects, there is an increasing interest in using them as a source of natural tyrosinase inhibitors.

Plant Profile

Thespesia Populnea (Linn.) Soland ex Correa
Synonym: Hibiscus popuknea Linn.
Family: Malvaccae

Chemical constituents

The plant yields kaempferol and its glycosides, herbacetin and its glucoside, populneol, populnin, populnetin, quercetin, rutin, gossipetin, (±) 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.

Aim and Scope of Present Work

The current study was aimed at evaluation of pharmacognostical, phytochemical and biological activities of flavonoid rich fraction of Thespesia Linn. (Malvaceae). Flavonoids are the major plant phenolic compounds and the most studied phythochemicals. 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.

Objectives of the Present Study

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-oxidant activity against free radicals, anti-inflammatroy and tyrosinase enzyme inhibition activity which would help in development of anti-hyperpigmentation agent.

Plan of Work

1. Collection and authentification of plant material
2. Pharmacognostical study
3. Physiochemical 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-oxidant activity

Collection and Identification of Plant Material

The fresh leaves of *Thespesia populnea* were collected from Kerala (Kollam district, Punalur) and was taxonomically identified and authenticated by Dr. P. Jayaraman, Director, National institute of Herbal Science (PARC), Chennai.

Physiochemical Properties

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

Preliminary Phytochemical Screening

The different qualitative chemical tests can be performed for establishing profile of given extract for its chemical composition. The following tests may be performed on extracts to detect various phytoconstituents present in them.

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

(+) Present
(-) Absent

6 Determination of Total Phenolic Content

Sl. No.	Concentration	Absorbance at 765nm
	Gallic acid (µg/ml)	
1.	100	0.2014
2.	200	0.4228
3.	300	0.7251
4.	400	1.0044
5.	500	1.2310
	Flavonoid rich fraction	
1.	Unknown concentration	0.2010

Determination in Vitro Anti-Oxidant Activity
Nitro Oxide Acavenging Method

Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions. This can be determined by the use of the Griess reagent Illosvoy reaction 2ml of 10mm sodium nitroprusside in 0.5ml phosphate buffer saline (pH 7.4) was mixed with 0.5ml of extract at various concentrations and the mixture incubated at 25^0C for 1500 mins. From the incubated at room temperature for 5min. finally, 0.1ml naphthyal ethylenediamine dihydrochloride (0.1% w/v) was mixed and incubated at room temperature for 30min. the absorbance at 546nm was measured with spectrophotometer. The nitric oxide scavenging activity was calculated according to the following equation [73]

$$\text{percentage Inhibition} = \frac{\text{Aborbance of control} - \text{Abdorbance of Test}}{\text{Absorbance of Control}} X\ 100$$

The in vitro antioxident for the extract was performed by nitric oxide scavenging method and the percentage inhibition of test was compared with the standard. Resuts are given in tabel no. 4 and fig. 16:

Table 4: Nitric Oxide Free Radical Scavenging Activity.

Sl. No.	Cone µg/ml	% Inhibition	
		Standard (Acrobic Acid)	Flavonoid rich fraction
1	1000	87.35±0.52	73.15±0.35***
2	500	85.24±0.18	68.41±27***
3	250	82.47±0.39	61.83±0.49***
4	125	69.32±0.47	56.23±0.51***
5	62.5	64.57±0.51	49.89±0.32

Values are expressed as mean±SEM., ***P<0.0001
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 antioxidant, in in vitro ant-inflammatory and tyrosinase inhibitory activities. 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 the explored and could be used in the drug development for treating oxidative stress unduced diseases and conditions including diabetes, cardiovascular diseases, inflammatory conditions, cancer and ageing. The phytochemical screening performed using ethanolic extract of plant material showed the presence of alkaloids, phytosterols and polar compounds such as phenolic compounds. The extract was also found be rich in phenolic compounds. The biological evaluation performed showed the extract to possess a good antioxidant Activity.

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