

PHYTOCHEMICAL CHARACTERIZATION OF LEAF EXTRACTS OF *THESPESIA POPULNEA L.*

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Article Received on
29 August 2014,

Revised on 24 Sept 2014,
Accepted on 19 Oct 2014

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ABSTRACT

Thespesia populnea L. is a medicinally important plant which is used to treat different types of diseases, especially skin diseases. The present research work is aimed to screen the phytochemicals from leaf extracts of *Thespesia populnea*. Leaf powder of *Thespesia* was dissolved in different solvents viz., water, methanol, chloroform, acetone, ethyl acetate and petroleum ether and subjected to screen for secondary metabolites. Results revealed the presence of saponins, steroids, phenols, flavonoids, terpenoids, alkaloids, cardiac glycosides, reducing sugars, tannins. In the present investigation, the water and methanol extracts yielded more number of phytochemicals than the chloroform,

acetone, and ethyl acetate. Apart from these the petroleum ether extracts showed very less amount of phytochemical constituents. We hope that the present study will be helpful for the phytochemists and pharmacologists for the identification of new compounds.

KEY WORDS: Leaf extract, *Thespesia populnea L.*, Secondary metabolites.

1. INTRODUCTION

Since ancient times, people have been exploring the nature particularly plants in search of new drugs. This has resulted in the use of large number of medicinal plants with curative properties to treat various diseases (R. Verpoorte, 1998). Nearly 80% of the world's population relies on traditional medicines for their primary health care, most of which involves the use of plant extracts (B.Sandya, S.Thomas, W.Isabel et al, 2006). In India, almost 95% of prescriptions are plant based in the traditional systems of Unani, Ayurveda,

Homeopathy and Siddha (G.V. Satyavati, 1987). The study of the plants continues principally for the discovery of novel secondary metabolites. Phytochemical screening is very important in identifying new source of therapeutically and industrially valuable compounds like steroids, alkaloids, flavonoids, phenols, tannins and terpenoids (A.J. Akindele, 2007). *Thespesia populnea L.* (milo) is a large tree belongs to the family *Malvaceae*, found in tropical regions and coastal forests of India. Various parts of this plant are found to be useful with valuable medicinal properties. Leaves are applied externally on swollen joints, to cure anti-inflammatory effects and also used for skin diseases, hepatitis, jaundice, ulcers, wounds, psoriasis, scabies, urinary tract infections, diabetes, cholera, cough, asthma and guinea worm infections (Anonymous, 1995). The fruits of the plant are used to cure diabetes in Ayurveda (T. Satyanarayana and T. Saritha *et al*, 2004). The bark and flowers acts as astringent, hepatoprotective and antioxidant (R. Iavarasan and M. Vasudevan, 2003).

2. MATERIAL AND METHODS

The healthy and disease free mature leaves of plant of *Thespesia populnea L.* material were collected from Thirumala hills, Chittoor district, Andhra Pradesh, India in the month of December, 2013. The collected leaf material was washed thoroughly under running tap water, shade dried in open air separately. Powder of the leaf is obtained by grinding them mechanically. About 10g dried powder of the leaf were soaked separately in 200 ml of different solvents like water, methanol, chloroform, acetone, ethyl acetate, and petroleum ether in conical flasks and then subjected to agitation on a rotary magnetic shaker for about 72 hours. After three days the leaf extracts were filtered with No 42 whatman filter paper separately. Concentrated extracts was preserved in sterilized air tight labeled bottles and preserved in refrigerator at 4°C until required for further use. The extract was filtered under reduced pressure using rotary flash evaporator and for further preliminary phytochemical tests. Different tests were conducted for the identification of each phytochemical constituent by using the methods adopted by Edeogal *et al* (2005) and B.Thamilmalai selvi *et al* (2011).

2.1. Preliminary Phytochemical Tests

1. **Test for Saponins:** Saponins are tested by boiling 5ml of extract in 10ml of distilled water in a test tube and are shaken vigorously for about 30 seconds. The test tube is allowed to settle for half an hour. Formation of froth indicates the presence of saponins.
2. **Test for steroids:** For testing the presence of steroids 1ml extract was dissolved in 10ml of chloroform and equal volume of concentrated sulphuric acid was added from the walls

of the test tube. Appearance of red colour in the upper layer and yellow with green fluorescence indicates the presence of steroids.

3. **Test for phenols:** Phenols are tested by adding 2ml of ferric chloride solution to 2ml of plant extract. Appearance of bluish green colour solution indicates the presence of phenols.
4. **Test for flavonoids:** To one ml of the extract, a few drops of dilute sodium hydroxide are added. An intense yellow colour is produced in the plant extract, which became colorless on addition of few drops of dilute acid. This indicates the presence of flavonoids.
5. **Test for terpenoids:** 1ml of the extract was dissolved in 1ml of chloroform:1ml of acetic anhydride was added following the addition of 2ml of concentrated sulphuric acid. Formation of reddish colour indicates the presence of terpenoids.
6. **Test for alkaloids:** To the 5ml of extract 5ml of 2N HCL is added and boiled and then the mixture is filtered. To the filtrate a few drops of Mayer's reagent is added. A cream colour precipitate was produced immediately indicating the presence of alkaloids.
7. **Test for cardiac glycosides:** To 1ml of extract glacial acetic acid, few drops of ferric chloride and then finally concentrated sulphuric acid were added from the walls of the test tube. Appearance of the reddish brown at the junction of two layers and the bluish green colour in the upper layer indicates the presence of cardiac glycosides.
8. **Test for reducing sugars:** 1ml of extract was added 5 to 10 drops of Fehling's solution. Mixture was then subjected to boiling for 15 minutes. Appearance of brick red precipitate indicates the presence of reducing sugars.
9. **Test for tannins:** Tannins are tested by adding a few drops of 1% lead acetate to 5 ml of plant extract. Appearance of yellow precipitate indicates the presence of tannins.

3. RESULTS AND DISCUSSION

Table 1: Phytochemical Screening Test of Leaf Extracts of *Thespesia Populnea L.*

S. No	Phytochemicals	Water	Methanol	Chloroform	Acetone	Ethyl acetate	Pet ether
1	Saponins	+	+	-	+	+	++
2	Steroids	+	+	+	+	+	-
3	Phenols	-	+++	+	+	+	-
4	Flavonoids	+	+	+	-	-	+
5	Terpenoids	+	+	-	-	+	-
6	Alkaloids	+	+	-	-	-	-
7	Cardiac glycosides	-	-	-	+	-	+
8	Reducing sugars	+	-	+	-	-	-
9	Tannins	+	-	-	-	-	-

(+++)= **strongly present** (+) = **poorly present**

(++)= **moderately present** (-) = **absent**

Table 1 indicate the presence of phytochemicals saponins, steroids, phenols, flavonoids, terpenoids, alkaloids, cardiac glycosides, reducing sugars and tannins found in all the used solvents. The phytochemical screening and qualitative estimation of *Thespesia populnea* leaf extracts showed that the leaves are rich in saponins, steroids and moderately presence of flavonoids, phenols. Terpenoids are present only in aqueous, methanol, and ethyl acetate extracts. Alkaloids are found in aqueous and methanolic extracts followed by cardiac glycosides presence in the extracts of acetone, and petroleum ether. Reducing sugars are only present in aqueous and chloroform and absent in all other solvent extracts. Constituents like tannins are only positive in aqueous and showed negative results in methanol, chloroform, acetone, ethyl acetate, and pet-ether extracts.

The result shows that most of the phytochemicals which are of saponins, steroids, phenols, flavonoids, terpenoids, alkaloids, cardiac glycosides, reducing sugars and tannins. *Thespesia populnea* leaf extracts solubilised abundantly in water, ethyl acetate, acetone and methanol solvents. Most of the extracts showed the similar properties to the screening tests. Saponins are only absent in chloroform and strongly present in petroleum ether extract. Traditionally saponins have been extensively used as detergents, as pesticides, and molluscides, in addition to their industrial application as foaming and surface active agents and also have beneficial health effects (J.Shi, K.Arunasalam, et al 2004). Steroids are only absent in pet-ether and present in all the solvents like water, chloroform, methanol, acetone, ethyl acetate. These are very valuable constituents and also important in pharmacy due to their relationship with sex hormones (R. Santhi, G. Lakshmi, et al 2011). Phenolic compounds are of great importance

they can protect the human body from the oxidative stress which may cause many diseases, including cancer, cardiovascular problems and ageing (K. Robards, P.D. Prenzler, 1999). These compounds are strongly found in methanolic extract and absent in aqueous, petroleum ether solvents. The presence of flavonoids in aqueous, methanol, chloroform, pet ether, absent in acetone and ethyl acetate extracts. Terpenoids are attributed for analgesic and anti-inflammatory activities and flavonoids are shown to possess many useful properties, including anti-inflammatory, oestrogenic, enzyme inhibition, antimicrobial, antiallergic, antioxidant, vascular and cytotoxic antitumour activity (B. Havesteen et al 1990). Aqueous, methanolic extracts are shown to yield alkaloid compounds. These are produced by large variety of organisms including bacteria, fungi, plants and animals; and are part of the group of natural products; some alkaloids have a bitter and are toxic to other organisms (V.K. Gupta et al, 2010). Glycosides, flavonoids, alkaloids have hypoglycemic activities (B. Oliver, 1980). Aqueous and chloroform extracts showed the presence of reducing sugars and absent in all other extracts. Tannins contribute the property of astringent activity, healing of wounds and inflamed mucous membrane (D.E. Okwu, C. Josiah, 2006), as well as useful to inhibit the growth of many fungi, yeasts, bacteria, and viruses (K.T. Chung, T.Y. Wong, et al 1998). Saponins, steroids, phenols, flavonoids are found to be rich in leaf extracts, where as terpenoids, alkaloids, cardiac glycosides, reducing sugars, tannins are found to be less amount compare to above said phytochemicals. However the presence of bioactive compounds indicates the medicinal value of *Thespesia*. The present research results attempt to screen the importance of phytochemical properties in leaves of *Thespesia populnea L.* to improve the health status of people and also use in industrial, pharmaceutical, and nutraceutical products of commercial importance. The present study also helps in choosing the suitable solvent keeping in view their interest of bioactive compounds.

4. CONCLUSION

From the literature of ancient history, *Thespesia populnea* is widely used in traditional medicine. Medicinal value of this plant used to combat and cure various ailments because it is rich in secondary metabolites. The anti-inflammatory, anticancer, antioxidant, cardiovascular problems, ageing, anti allergic, and anti microbial activities of this plant can be attributed to their high saponins, steroids, phenols, flavonoids and terpenoids. Exploitation of these pharmacological properties involves further investigation and identification of these active compounds by applying of techniques like extraction, purification, separation, characterization and structural identification of specific compounds.

5. ACKNOWLEDGEMENT

1. Authors are highly acknowledged to UGC Govt.of India (RFSMS-FELLOWSHIP) for funding this project.

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