

# EVALUATION OF ANTIBACTERIAL ACTIVITY, PHENOL AND FLAVONOID CONTENTS OF *THESPESIA POPULNEA* FLOWER EXTRACTS

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## ABSTRACT

Flavonoids which were reported as having many pharmacological activities, antimicrobial, antioxidant, cytotoxic, chemoprevention activities and they possess strong antiproliferative effects related to inhibition of cell cycle progression and apoptosis induction. On the basis of this *Thespesia populnea* (L.) Sol. Ex Correa (Family-Malvaceae) was selected and it is having the major composition of flavonoids and the antibacterial activity of methanolic extract of *Thespesia populnea* flowers was investigated by agar well diffusion method. Furthermore our phytochemical studies indicated that methanolic extract of *Thespesia populnea* flowers contains flavonoids, alkaloids, tannins and anthroquinone glycosides. Moreover the individual components were identified by thin layer chromatography and  $R_f$  value was compared with standard flavonoid quercetin. The total phenolic and flavonoid content studies were also quantified. The bacteria used for antibacterial study were *Shigella flexneri* (NCIM 4924), *Rhodococcus terrae* (NCIM 5126), *Escherichiae coli* (ATCC 11775), *Streptococcus faecalis* (NCIB 2406), *Klebsiella pneumoniae* (ATCC 13883), *Brevibacterium luteum* (NCIM 2923), *Micrococcus flavum* (NCIM 2376), *Proteus mirabilis* (NCIB 8268), *Bacillus licheniformis* (NCIM 2468), *Micrococcus luteus* (ATCC 2984), *Flavobacterium devorans* (NCIM 2581), *Shigella sonnei* (ATCC 29930), *Shigella boydii* (ATCC 8700) and *Shigella dysenteriae* (ATCC 13313). According to our results in the lowest tested concentration of 62.5µg/ml and 125µg/ml 7.2% of the plant extract were active, 5% active in the concentration of 250µg/ml, 75.7% active in the concentration of 500µg/ml and 92.8% active at the concentration of 1000µg/ml in a dose dependent manner.

**Keywords:** *Thespesia populnea*, antibacterial activity, agar well diffusion, malvaceae, methanol extract.

## INTRODUCTION

In India herbal medicines have been the basis of treatment and cure for various diseases or physiological conditions in traditional methods practiced such as ayurveda, unani and siddha. Although reports of antibacterial activity of indigenous plants have been evaluated. Phenolics like flavonoids and tannins are widely distributed in plant kingdom, vegetables, flowers etc. For centuries preparations containing flavonoids as active constituents have been used to treat human diseases and in anti-infective research. With this concept the antimicrobial screening results of *Thespesia populnea* (L.) Sol. Ex Correa (Family-Malvaceae) was selected for our study along with references to their traditional use was emphasized, what interesting is quiet often a particular plant may be used for different diseases for example the decoctions of *Thespesia populnea* is considered to be used in the treatment of cutaneous infections, skin and liver diseases (Shirwaikarkumar *et al.*, 1995).

*Thespesia populnea* is evergreen shrubby tree, commonly to 13m (40ft) tall, with young branches minutely brown scaly. Leaves alternate, simple, with petioles 5-10cm (2-4in) long. Flowers showy, hibiscus like, single at upper

leaf axils, to 8cm (3in) across: corolla yellow with a red center, turning maroon by nightfall, stamens united in to a column shorter than petals (Vasudevan *et al.*, 2007). The bark, leaves, and flowers are useful in cutaneous infections such as scabies, psoriasis, eczema, ringworm and guinea worm (Elmo *et al.*, 1986). The bark and flowers possess astringent, hepatoprotective and antioxidant activities.

*Thespesia populnea* may be confused with another naturalized exotic, sea hibiscus (*Hibiscus tiliaceus* L), but its leaves wider, with dense star shaped hairs on lower surfaces, and with the endangered Florida native, wild cotton (*Gossypium hirsutum* L), but its leaves opposite. Other Malvaceae family members in Florida rarely reach tree structure (Wilkinson *et al.*, 2003).

The development of antimicrobial agents for clinical use has brought unquestionable benefit to individuals and society. Infectious diseases that were formerly often fatal became curable (Shirwaikarkumar *et al.*, 1995). However, mankind is now confronted with new re-emerging infections for which no effective treatments are available. In contrast, to other types of medication, antibiotics ultimately lose their effectiveness as they are used

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overtime and resistant strains of bacteria develop (Jeannette Day *et al.*, 2002). One example is that of gram positive, methicillin resistant *Staphylococcus aureus* strains. Incidence figures in some hospitals have shown that more than 40% of *Staphylococcus aureus* strains are now resistant to methicillin. There is thus an urgent need to identify novel, active chemo types as lead for drug development. Natural products could play a crucial role in meeting this demand of drugs approved between 1983 and 1994 by either the united states Food and Drug Administration (FDA) or comparable entities in other countries, Drugs of natural origin predominated (78%) in the area of antibacterial research (Padmaja *et al.*, 1993).

Many plants derived from nature possess antimicrobial and insecticidal activities. The interest in these plants is increasing because of finding safer microbicides in combination with the need of preventing environmental degradation. For centuries preparations containing flavonoids as the principal physiologically active constituents have been used to treat Human Diseases. Increasingly, this class of natural products is becoming the subject of anti-infective research and many groups have isolated and identified the structures of flavonoids possessing antimicrobial and cytotoxic activities (Cragg *et al.*, 1997).

Reports of activity in the field of antibacterial flavonoid research are widely conflicting, probably owing to their inter and intra assay variation in susceptibility testing (Leven *et al.*, 1979). However, several high quality investigations have examined the relationship between flavonoid structure and antibacterial activity and these are in close agreement. Most of the medicinal plants have identified and used for treatment of human diseases are well documented (Iqbal Ahmed *et al.*, 1998).

## MATERIALS AND METHODS

### *Plant materials*

The plant materials (flowers) were collected during April-May 2006 from tropical areas of Western Ghats regions of Erode and Nagercoil and then shade dried at room temperature. The plant materials were identified by G.S.R.Murthy, Joint Director at Botanical survey of India (BSI) Coimbatore, India and a voucher specimen (SC 5/23) was deposited in Herbarium of Laboratory of Botany, Coimbatore, Tamilnadu, India.

### *Preparation of crude extract*

The powdered plant materials (10gms) were extracted with 100ml of methanol for 1hr on an ultrasonic bath. The extract was filtered the filtrate was evaporated in vacuo at 45°C and then lyophilized. The extracts were prepared according to the polarity (Olukayo *et al.*, 1993).

### *Preliminary phytochemical screening*

The preliminary phytochemical screening of *Thespesia populnea* was carried out for the decoction of various

Phytoconstituents using standard procedure (Evans, 1996). The following solvents were used for the study, petroleum ether, chloroform, ethyl acetate, methanol, ethanol and water. The methanolic extract was found to contain more flavonoids. The preliminary phytochemical screening of methanolic extract reveals the presence of alkaloids, flavonoids, tannins, triterpenes, gums and mucilage.

### *Chemicals*

Ciprofloxacin (10 µg/ml) discs, Molten Mueller Hinton (MH) Agar and Nutrient agar medium were obtained from Hi-media laboratories, Mumbai. All other chemicals were of analytical grade and obtained locally.

### *Test microorganisms*

The test microorganisms used were *Shigella sonnei* (ATCC 29930), *Escherichiae coli* (ATCC 11229), *Streptococcus faecalis* (ATCC 8043), *Shigella boydii* (ATCC 8700), *Rhodococcus terrae* (NCIM 5126), *Micrococcus flavum* (NCIM 2984), *Flavobacterium devorans* (NCIM 2581), *Proteus mirabilis* (NCIB 8268), *Brevibacterium leuteum* (ATCC 15830), *Bacillus licheniformis* (NCIM 2468), *Shigella dysenteriae* (ATCC 13313), *Klebseilla pneumoniae* (ATCC 11229), *Micrococcus leuteus* (ATCC 9341) , *Shigella flexneri* (NCIM 4924).

### *Total phenolic constituents study*

Total phenols were determined by Folin Ciocalteu reagent (McDonald *et al.*, 2001). A dilute extract of each plant (0.5ml of 1:10g/ml) or Gallic acid (Std. phenolic compound) was mixed with Folin Ciocalteu reagent (5ml, 1:10 diluted with distilled water) and aqueous Na<sub>2</sub>CO<sub>3</sub> (4ml, 1M). The mixtures were allowed to stand for 15 minutes and the total phenols were determined by colorimetry at 765nm. The standard curve was prepared using 1, 10,100 and 200 mg/l solutions of Gallic acid in methanol: water (50:50, v/v). Total phenol values are expressed in terms of Gallic acid equivalent (mg/g of dry mass), which is a common reference compound (fig. 1).

### *Total flavonoids determination*

Aluminum chloride colorimetric method was used for flavonoids determination (Chang *et al.*, 2002). Each plant extract (0.5 ml of 1:10 g/ml) in methanol were separately mixed with 1.5 ml of methanol, 0.1ml of 10% Aluminium chloride, 0.1 ml of 1M potassium acetate and 2.8ml of distilled water. It remained at room temperature for 30 minutes. The absorbance of the reaction mixture was measured at 415nm. The calibration curve was prepared by preparing quercetin solutions at concentrations 12.5 to 100 µg/ml in methanol.

### *Antibacterial study (plate hole diffusion method)*

The plate hole diffusion assay was used to determine the growth inhibition of bacteria by plant extracts. Bacteria were maintained at 4°C on nutrient agar plates before use.

Nutrient agar was prepared and 25ml of each was poured in to sterile universals. The universals with the broth were inoculated with different species of bacterias and incubated at 37°C overnight (Hook *et al.*, 1995). A total of 25ml of molten Muller Hinton (MH) agar (OXOID) held at 40°C was poured in to sterile universals maintained at 40°C in a water bath (Lopez *et al.*, 2001). Each universal was inoculated with 0.2ml of different bacterial species mixed well, transferred in to sterile petri dishes and allows to set. Using a sterile cork-borer 6mm diameter, four holes per plate were made in to the set agar containing the bacterial culture. A total of 0.2ml of plant extracts were poured in to the wells and one containing distilled water, the plates were kept in incubator overnight and the zone diameter was then recorded if greater than 6mm (Jussi-Pekka *et al.*, 2002).

## RESULTS

### Preliminary phytochemical screening

The preliminary phytochemical screening reveals the presence of Flavonoids Alkaloids, Tannins and Anthraquinone glycosides. The results were shown in table 2.

**Table 1:** Flavonoids and Phenol content in *Thespesia populnea*

Plant name	Flavonoids (mg/g)	Phenol (mg/g)
<i>Thespesia populnea</i>	25.05±0.18*	31.2±4*

\*Each value in the table was obtained by calculating the average of three experiments ± standard deviation.

**Table 2:** Preliminary Phytochemical Screening

Plant name	Extract fractions	Flavonoids	Tannins	Alkaloids	Anthroquinone glycosides	Steroids
<i>Thespesia populnea</i>	a	+	+	+	+	-
	b	+	+	+	+	-
	c	+	++	++	++	-
	d	+++	+	+	++	-
	e	++	+	+	+	-
	f	+	+	+	+	-
Chemical tests		Shinoda test	Phlonata nins test	Wagners reagent	Salkowskii test	Extract + 10ml of CHCL <sub>3</sub> + equal volume of Conc.H <sub>2</sub> SO <sub>4</sub>
Observations		Effervescence dark brown solution turns red	Red precipitate was formed	Yellow colour was formed instead of brown precipitate	Reddish brown colour at interphase	Upper layer turns red & sulphuric acid layer showed yellow with green fluorescence

a-Petroleum ether, b- Chloroform, c - Ethyl acetate, d- Methanol, e- Ethanol, f- Water.  
 - =Absent, + =Trace amounts, ++ =Presence, +++ = High.

### Antibacterial study

From the phytochemical screening, the methanolic extract showed high amount of flavonoids when compared to others, so we selected the methanolic extract of *Thespesia populnea* flowers for antibacterial screening. The results of the total phenolic constituent and flavonoids study was 31.2 ± 4, 25.05 ± 0.18 respectively (table 1). The fourteen bacterias were used for antibacterial screening. Various concen-trations of methanolic extract were used (1000µg/ml, 500µg/ml, 250µg/ml, and 62.5µg/ml) to test the antibacterial activity. From the results of antibacterial screening, 7.2% of methanolic extract were active in the lowest tested concentration of 62.5µg/ml, 5% active in a concentration of 250µg/ml, 75.7% active in a concentration of 500µg/ml, and 92.8% active in a concentration of 1000µg/ml. Ciprofloxacin (10µg) was used as standard drug. The results were shown in the table 3.

## DISCUSSION

The present study was conducted to study the *in vitro* antibacterial activity of *Thespesia populnea* used by Indian peoples to show that therapeutic properties. The antibacterial activity was expressed at varying degrees with the activity being both strain and dose dependent. Fourteen bacterias were used for antibacterial studies.

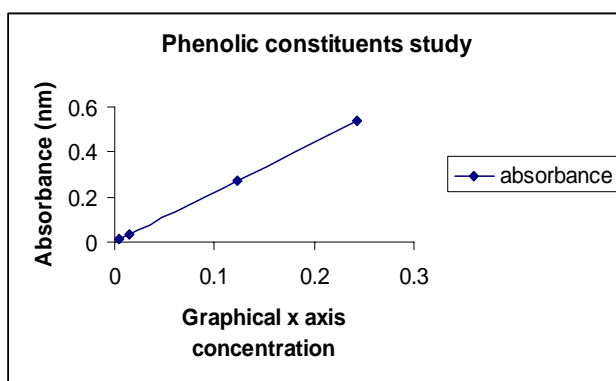
Polyphenols have been reported to exhibit antibacterial activities with distinguished characteristics in their reactivity with proteins related polyamides polymers (Haslam *et al.*, 1996). The inhibition of microorganisms by phenolic compounds may be due to iron deprivation or hydrogen bonding with vital proteins such as microbial enzymes (Scalbert *et al.*, 1991).

**Table 3:** Antibacterial study of methanolic extract of *Thespesia populnea*

Bacteria	Zone diameter (mm)*					
	1000µg /ml	500µg/ml	250µg /ml	125µg /ml	62.5µg /ml	Ciproflo-xacin 10µg /ml
<i>Rhodococcus terrae</i>	23	18	9	0	0	24
<i>Micrococcus flavum</i>	17	0	0	0	0	26
<i>Brevibacterium leuteum</i>	12	10	9	0	0	20
<i>Flavobacterium devorans</i>	15	10	9	0	0	23
<i>Shigella sonnei</i>	18	10	0	0	0	25
<i>Shigella flexneri</i>	21	18	17	16	14	25
<i>Shigella boydii</i>	16	13	12	0	0	22
<i>Streptococcus faecalis</i>	0	0	0	0	0	23
<i>Shigella dysenteriae</i>	15	12	20	0	0	22
<i>Escherechia coli</i>	18	16	10	0	0	20
<i>Bacillus lichenformis</i>	20	17	14	0	0	26
<i>Proteus mirabilis</i>	16	13	11	0	0	22
<i>Klebseilla pneumoniae</i>	21	18	17	16	14	25
<i>Micrococcus leuteum</i>	17	14	12	0	0	25

\*Each value in the table was obtained by calculating the average of three experiments

Medicinal plants are being used by large proportion of Indian population. The reasons for this include a) True improvement of diseases conditions after herbal treatment b) Harmful side effects and high cost of the other forms of treatment. In the present study, the results were encouraging, as the *Thespesia populnea* appeared to contain substances that had antimicrobial properties because of, the methanolic extract of *Thespesia populnea* flowers were active against 13 out of 14 bacteria's. Five concentrations of the extract were used (1000µg/ml, 500µg/ml, 250µg/ml, 125µg/ml and 62.5µg/ml). It is estimated that if an inhibition is obtained by 250µg/ml-1000µg/ml) of test solution, the extract can be considered worthy for further investigations.



**Fig. 1:** Calibration curve of Gallic acid.

The aqueous and organic extracts from the plants showed different activities. There are no common rules for this, but in most cases, organic extracts showed the same (or) greater activity than aqueous extracts. The plate hole diffusion method showed larger activity than disc diffusion method. Most likely this is because large

amount of solvents may influence bacterial growth, there by demonstrating that larger doses are required (Ray *et al.*, 1976).

Since the medicinal plants studied appear to have a broad antimicrobial activity spectrum, they could be useful in antiseptic and disinfectant formulations as well as in chemotherapy. The optimal effectiveness of a medicinal plant may not be due to one main active constituent, but to the combined action of different compounds originally in the plant (Gonzalez *et al.*, 1994).

In literature, it has been indicated that the antibacterial activity is due to different chemical agents in the extract, including essential oils (especially thymol), flavonoids and triterpenoids and other compounds of phenolic nature or free hydroxyl group, which are classified as active antimicrobial compounds. A complete study conducted with the purpose of finding these chemicals is worthwhile. These findings can form the basis of further studies to isolate active flavonoid compound, elucidate them against wider range of bacterial strains with the goal to find new therapeutic principles.

Under this experimental study the extract was active for bactericidal action. The findings revealed that the extract capability to penetrate the cell walls with hydrophobic environment (gram negative) and hydrophilic environment (gram positive) bacterias responsible for the bactericidal action which can be isolated and identified by some analytical techniques (Kudi *et al.*, 1999). The results of the study supports to a certain degree, traditional medicinal uses of the plants evaluated both for human and animal diseases therapy and reinforce the concept that ethno botanical approach to screening plants as potential sources of bioactive substances is successful (Valsaraj *et*

al., 1997). Plants showing significant activity may be due to the presence of alkaloids, flavonoids, tannins and polyphenols. Among the various microorganisms, the methanolic extract of *Thespesia populnea* was more active against *Rhodococcus terrae*. The aqueous extract generally exhibits a high degree of antibacterial activity this seems to confirm the traditional therapeutic claims of this plant (Perumal samy *et al.*, 1998) These results suggest the presence of either good antibacterial potency or high concentration of an active principle in the extract. This antibacterial activity would support the folk therapy of infections whose symptoms might involve bacteria (Verpoorte *et al.*, 1982).

Plant extracts and phytochemical are becoming popular as potential sources of antibacterial and several reviews have been written (Rojas *et al.*, 1992). Results from this investigation show the rationale behind the use of *Thespesia populnea* in traditional medicine. *Thespesia populnea* are not only interesting sources for antimicrobial activities but also potential source of phenolic antioxidants.

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