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## **PHYTOCHEMICAL SCREENING AND ANTICANDID ACTIVITY OF SELECTED PLANTS OF GENUS CAESALPINIA**

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### **ABSTRACT**

Phytochemical screening and the determination of antifungal activity of floral extracts of six plants used in treating various ailments were undertaken. The presence of secondary metabolites and their antifungal efficacy attests to their use as potent drugs for different ailments. Medicinal plants such as *Caesalpinia bonduc*, *Peltophorum pterocarpum*, *Parkinsonia aculeata*, *Delonix regia*, *Bauhinia purpurea*, *Cassia fistula* were screened for the metabolites and antifungal activity with *Candida albicans*, which were studied in different solvents and at different fractions. Results revealed that presence of transparent zone around the paper discs showed that the selected medicinal plants of *caesalpinian* genus have the greatest effect against *Candida*. Cardiac glycosides, Tannins, Saponins are present abundantly than other metabolites.

**Key Words:** *Caesalpinia, Phytochemistry, Anti Candid Activity*

### **INTRODUCTION**

*Caesalpinia* is a genus of flowering plants in the legume family, Fabaceae. Phytochemical constituents exhibited significant antifungal activity and showed properties that support folkloric use in the treatment of some diseases as broad-spectrum of potent metabolites. *Caesalpinia* genus is well anchored in medicines of allelopathy, ayurveda. The aim of the present study was to assess the anti-Candida activities against some clinical isolates of fungal species by using paper disc diffusion to find alternative materials to synthetic antifungal drugs. In the current investigation carried out, a screening of crude extracts of flowers against pathogenic fungi is done.

### **MATERIALS AND METHODS**

#### **Collection of Plant Materials**

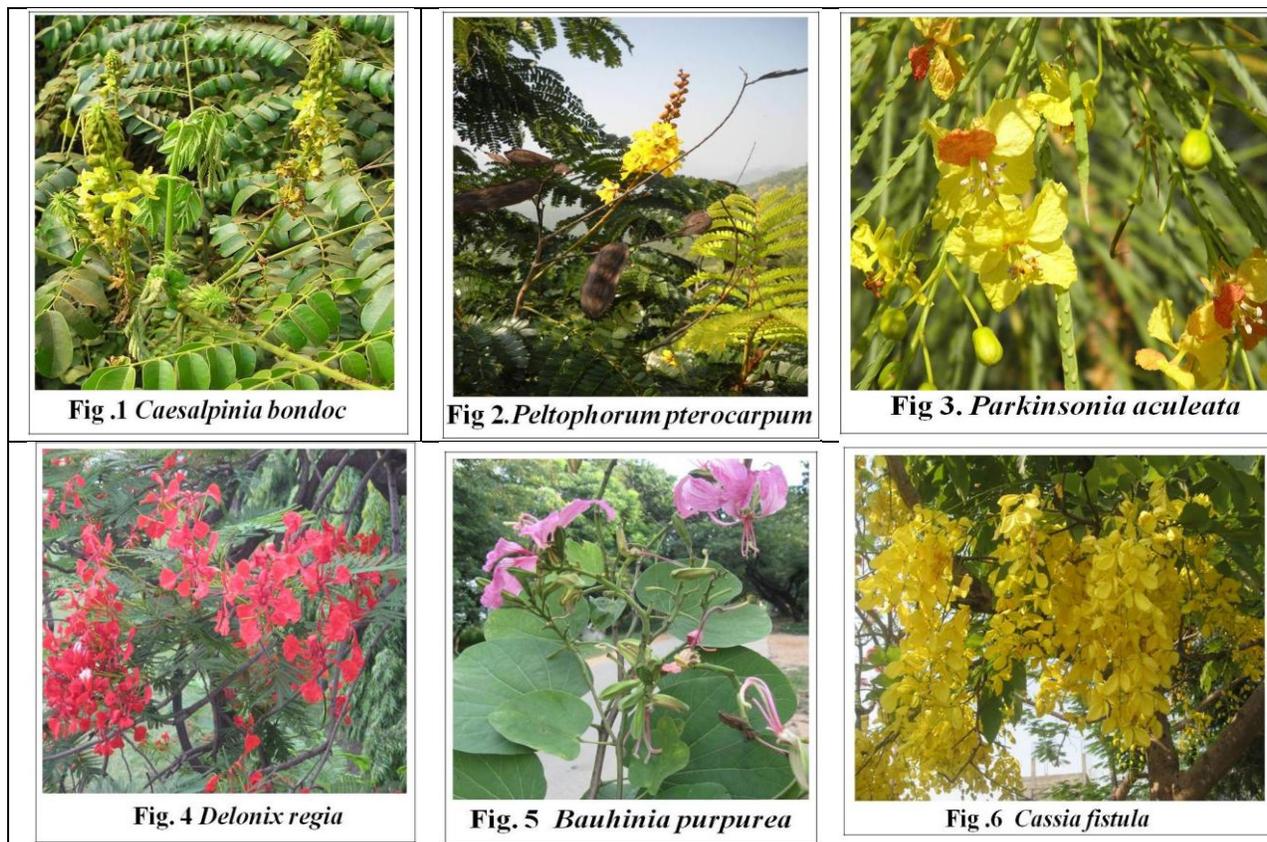
The fresh and healthy flowers of the plants *Caesalpinia bonduc* (L.) Roxb, *Peltophorum pterocarpum* (DC.) K. Heyne, *Parkinsonia aculeata* L., *Delonix regia* (Hook.) RAF, *Bauhinia purpurea* L. and *Cassia fistula* L. was collected from various areas of chittoor district, Andhra pradesh, India. The plant specimens were identified authenticated by K.Madhava chetty, department of botany, S.V. University, Tirupati. Plant parts were collected on the basis of the information provided in the ethnobotanical survey. Each specimen/plant material was labeled, numbered, a noted with the date of collection, locality, and their medicinal uses were recorded.

#### **Preparation of Plant Extract**

The extraction of the specimens was carried out using known standard procedures of Harborne JB. and Chapman and Hall (1973). The plant materials were collected on the day when extraction was performed and were dried in shade and powdered in a mechanical grinder. The powder (25.0 g) of the plant materials were initially defatted with petroleum ether (60-80°C), followed by 900 ml of hydroalcohol by using a Soxhlet extractor for 72 hours at a temperature not exceeding the boiling point of the solvent. The extracts were filtered using Whatman filter paper (No.1) while hot, concentrated in vacuum under reduced pressure using rotary flask evaporator, and dried in a desiccator. The crude extract yields a dark greenish solid. More yields of alcoholic extracts were collected with by determine concentration in mg/ml. The

**Research Article**

extract was preserved at 2- to 4°C. This crude extracts of hydroalcohol was used for further investigation for potential of antifungal properties.



**Preliminary Phytochemical Screening**

The extracts were subjected to preliminary phytochemical testing to detect for the presence of different chemical groups of compounds. Air-dried and powdered plant materials were screened for the presence of saponins, tannins, alkaloids, flavonoids, triterpenoids, steroids, glycosides, anthraquinones, coumarin, saponins, gum, mucilage, carbohydrates, reducing sugars, starch, protein, and amino acids, as described in literatures of Khandelwal KR (2009) and Kumar A *et al.*, (2009)

**Test Microorganisms and Growth Media**

Fungal strain *Candida albicans* (MTCC 227) were chosen based on their clinical and pharmacological importance. The bacterial strains obtained from Institute of Microbial Technology, Chandigarh, were used for evaluating antimicrobial activity. The bacterial and fungal stock cultures were incubated for 24 hours at 37°C on nutrient agar and potato dextrose agar (PDA) medium (Microcare laboratory, Surat, India), respectively, following refrigeration storage at 4°C. The bacterial strains were grown in Mueller-Hinton agar (MHA) plates at 37°C (the bacteria were grown in the nutrient broth at 37°C and maintained on nutrient agar slants at 4°C), whereas the yeasts and molds were grown in Sabouraud dextrose agar and PDA media, respectively, at 28°C. The stock cultures were maintained at 4°C.

**Anticandid Activity and Determination of zone of inhibition method**

In vitro antifungal activity were examined for hydroalcohol extracts and determination of zone of inhibition against fungi were investigated by the agar disk diffusion method, and fungal strains were taken as a standard antibiotic for comparison of the results. All the extracts were screened for their antifungal activities against the fungi *Candida albicans*. The sets of five dilutions (5, 25, 50, 100, and 250 µg/ml) of floral extracts of *Caesalpinia bonduc*, *Peltophorum pterocarpum*, *Parkinsonia aculeata*, *Delonix regia*,

### **Research Article**

*Bauhinia purpurea* and *cassia fistula* and standard drugs were prepared in double-distilled water using nutrient agar tubes. Mueller-Hinton sterile agar plates were seeded with indicator bacterial strains ( $10^8$  cfu) and allowed to stay at 37°C for 3 hours. Control experiments were carried out under similar condition by using nystatin and griseofulvin for antifungal activity as standard drugs. The zones of growth inhibition around the disks were measured after 48 to 96 hours for fungi at 28°C. The sensitivities of the fungal species to the plant extracts were determined by measuring the sizes of inhibitory zones (including the diameter of disk) on the agar surface around the disks, and values <8 mm were considered as not active against microorganisms.

### **Phytochemical Analysis of Extract**

The methods described by Harborne, (1998) with slight modifications were used to screen the presence of the active ingredients in the floral extracts

#### **Test for Steroids**

10 ml of the bark extract was evaporated to dry mass and dissolved in 0.5 ml of solvent. Acetic anhydride (0.5 ml) and 2 ml of concentrated sulphuric acid were added. A blue or green colour or a mixture of these two shades was regarded as positive for the presence of steroidal compounds (Harborne, 1998).

#### **Test for Terpenoids**

The presence of terpenoids was determined as described for steroids except that red, pink or violet colour indicates the presence of terpenoids (Harborne, 1998).

#### **Test for Tannins**

- i) 1 cm<sup>3</sup> of freshly prepared 10% KOH was added to 1 cm<sup>3</sup> of the extract. A dirty white precipitate indicated the presence of tannins (Harborne, 1998).
- ii) Powdered stem bark of the test plant (1.0 g) was weighed into a beaker and 10 ml of distilled water added. The mixture was boiled for five minutes. Two drops of 5% FeCl<sub>3</sub> were then added. Production of greenish precipitate indicated the presence of tannins (Harborne, 1998).

#### **Test for Flavonoids**

A small piece of magnesium ribbon was added to extract of the plant material, this was followed by the drop wise addition of concentrated hydrochloric acid. Colours varying from orange to red indicated flavones, red to crimson indicated flavonols, crimson to magenta indicated flavonones (Harborne, 1998).

#### **Test for Alkaloids**

The extract of the plant stem bark sample (0.5 g) was stirred with 5 ml of 1% HCl on a steam bath. The solution obtained was filtered and 1 ml of the filtrate was treated with two drops of Mayer's reagent. The two solutions were mixed and made up to 100 ml with distilled water. Turbidity of the extract filtrate on addition of Mayer's reagent was regarded as evidence for the presence of alkaloids in the extract (Harborne, 1998).

#### **Test for Saponins**

Stem bark of the test plant was ground into powder form and 0.5 g of the powdered stem bark was introduced into a tube containing 5.0 ml of distilled water, the mixture was vigorously shaken for 2 min, formation of froth indicated the presence of Saponins (Harborne, 1998).

#### **Test for Glycosides**

Coarsely powdered stem bark (1g) was added into two separate beakers. To one of the beakers was added 5 ml of dilute sulphuric acid while 5 ml of water was added to the other beaker. The two beakers were heated for 3 – 5 min and the contents filtered into labeled test tubes. The filtrate was made alkaline with 5% sodium hydroxide and heated with Fehling's solution for 3 min. The presence of reddish precipitate in the acid filtrate and the absence of such precipitate in the aqueous filtrate were regarded as positive for glycosides (Harborne, 1998).

#### **Test for Gums and Mucilage**

About 10ml of various extracts were added separately to 25ml of absolute alcohol with constant stirring and then filtered. The precipitate was dried in air and examined for its swelling properties and for the presence of carbohydrates (Harborne, 1998).

**Research Article**

**RESULTS**

**Phytochemical Analysis**

Preliminary phytochemical screening of the bark extracts of *Caesalpinia bonduc*, *Pettophorum pterocarpum*, *Delonix regia*, *Bauhinia purpurea* and *Cassia fistula* were done. The medicinal plants showed positive results for the presence of phytochemicals such as steroids, saponins, tri terpinoidal saponins, alkaloids, carbohydrates, flavanoids, tannins, glycosides, polyphenols and gums and mucilage. Among these phytochemicals the gums and mucilage was commonly absent in all plant extracts except aqueous and ethanolic extract of *Delonix regia* and *Bauhinia purpurea* (Table 1).

**Table 1: Phytochemical Screening of Ethanolic and Water Extract of *Caesalpinia bonduc*, *Pettophorum pterocarpum*, *Delonix regia*, *Bauhinia purpurea* and *Cassia fistula***

Phytochemical	<i>C. bonduc</i>		<i>P. pterocarpum</i>		<i>D. regia</i>		<i>B. purpurea</i>		<i>C. fistula</i>	
	E.E	A.E	E.E	A.E	E.E	A.E	E.E	A.E	E.E	A.E
Steroids	+	-	+	-	+	+	+	-	+	+
Triterpenes	+	+	-	+	+	-	+	-	+	+
Saponins	+	+	+	+	+	+	+	-	+	+
Triterpinoidal saponins	+	-	+	-	+	+	+	-	+	+
Alkaloids	+	+	+	+	-	+	+	-	+	+
Carbohydrates	+	+	+	-	+	-	+	+	+	+
Flavonoids	-	+	+	+	+	-	+	-	+	+
Tannins	+	+	+	+	-	+	+	+	-	+
Glycosides	+	+	+	+	-	+	+	+	+	-
Polyphenols	+	+	+	+	+	-	+	-	+	+
Gums and mucilage	-	-	+	-	-	+	+	-	-	-

E.E: Ethanolic extract; A.E: Aqueous extract; + = Present; - = Absent

**Table 2: Anti Candida activities of *Caesalpinia bonduc*, *Pettophorum pterocarpum*, *Delonix regia*, *Bauhinia purpurea* and *Cassia fistula***

Plant	Aqueous extract	Ethanol extract	Tetracycline Positive control	DMSO Negative control
<i>Caesalpinia bonduc</i>	8±0.57	18±0.28	21±0.38	NA
<i>Pettophorum pterocarpum</i>	11±0.98	15±0.51	23±0.94	NA
<i>Delonix regia</i>	ND	13±0.28	22±0.72	NA
<i>Bauhinia purpurea</i>	3±0.24	16±0.58	22±0.18	NA
<i>Cassia fistula</i>	15±0.58	19±0.67	21±0.63	NA

Zone of inhibition (in mm diameter) including the diameter of well (6mm) in agar well diffusion assay. Assay was performed in triplicate and results are the mean of three values. In each well, the sample size was 100µl. Tetracycline: one unit strength. ND: not detected, NA no activity

**Anticandida Activity**

The ethanolic and aqueous extracts of the flowers of *Caesalpinia bonduc*, *Pettophorum pterocarpum*, *Delonix regia*, *Bauhinia purpurea* and *Cassia fistula* were tested against *Candia albicans*. Among these plant extracts *Cassia fistula* and *Caesalpinia bonduc* ethanolic extract showed highest anticandida activity (19±0.67 and 18±0.28) against *Candia albicans*. Whereas aqueous of *Delonix regia* showed nil activity against *C. albicans* and *Pettophorum pterocarpum*, *Bauhinia purpurea* plant extract showed moderate anticandid activity (Table 1).

## Research Article

### DISCUSSION

The composition and concentration of phytochemical determines the antimicrobial efficacy of plants. The higher levels of steroids, polyphenols, tannins and alkaloids noted in bark extracts of *Caesalpinia bonduc*, *Peltophorum pterocarpum*, *Delonix regia*, *Bauhinia purpurea* and *Cassia fistula* could be responsible for the medicinal properties of this plant. Similar reports of antimicrobial activity was observed with extracts of *Azadirachta indica*, *Curcuma longa*, *Oscimum spp*, *Morinda citrifolia* L, *Zingiber officinale*, *Cassia auriculata* etc (Chopra et al., 1956; Chatterjee and Pakrashi, 1995; Nascimento et al., 2000; Ushimaru et al., 2007; Usha et al., 2010 and Maneemegalai and Naveen, 2010).

Among the two extracts of *Caesalpinia bonduc*, *Peltophorum pterocarpum*, *Delonix regia*, *Bauhinia purpurea* and *Cassia fistula* showed bioactive compounds like steroids, alkaloids, carbohydrates, Tannins, glycosides, polyphenols were present in ethanol and aqueous extract and these extracts found to be active against *Candida albicans* which is indicating broad spectrum of antimicrobial activity of ethanolic and aqueous extract. Our results authenticate the usage of *Caesalpinia bonduc*, *Peltophorum pterocarpum*, *Delonix regia*, *Bauhinia purpurea* and *Cassia fistula* by local people for treating fungal infections. Our study provide the basis for further isolation and evaluation of major active principles present in the plant material and test their efficacy against various infections.

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