

## Antimicrobial Guided Phytochemical Evaluation of the Pods of *Caesalpinia pulcherrima*

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The alcoholic extract of the pods of the plant *C. pulcherrima* afforded five antimicrobial compounds: four homoisoflavonoids (4-O-methylsappanol, protosappanin A, brazilin and caesalpin J) and ethyl 2,4,6-trihydroxy benzoate. The structures of the compounds were established by means of NMR spectroscopic analysis. Antimicrobial activity of the compounds was evaluated against pathogenic gram -ve and gram +ve bacteria and fungi.

**Key Words:** *Caesalpinia pulcherrima*, Antimicrobial activity.

### INTRODUCTION

*Caesalpinia pulcherrima* Swartz. (Family: Caesalpinaceae) is an ornamental plant in India due to its variety of flowers, which appear yellow, pink, off-white and red with yellow margins. The red with yellow margins variety is the national flower of Barbados, known as pride of Barbados, and it is the pods of this variety that were used for this investigation. *C. pulcherrima* also has uses in traditional medicine: the stem is used as an abortifacient and emmenagogue, while fruits are employed to cure diarrhoea and dysentery<sup>1</sup>, while decoctions of the leaves, roots and bark are used as febrifuge and to treat liver disorders as well as ulcers of the mouth and throat<sup>2</sup>. Earlier works reported the isolation of diterpenoids and isovoucapenols A–D<sup>3–5</sup>, pulcherrimins A–D, peltogynoids and homoisoflavonoids<sup>6, 7</sup>. Some of the constituents were found to possess antitumour properties<sup>3, 5</sup>. As there is no reference in literature regarding the antimicrobial components of the pods of *C. pulcherrima*, it was, therefore, considered worthwhile to study the isolation and characterization of antimicrobial components of *C. pulcherrima*.

### EXPERIMENTAL

Pods of the plant *Caesalpinia pulcherrima* (Caesalpinaceae) were collected from the medicinal plant garden of Dr. B.V. Raju Foundation Campus, Bhimavaram, Andhra Pradesh, during January 2000. A voucher specimen was deposited in the herbarium of Shri Vishnu College of Pharmacy, Bhimavaram.

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The shade dried pods of *C. pulcherrima* (1 kg) were separated from seeds (seeds discarded), pulverized by a mechanical grinder, passed through a 40 mesh sieve and stored in a closed vessel. Powdered material (500 g) was extracted with alcohol<sup>8</sup> using Soxhlet apparatus for 8–10 h. The alcoholic extract was concentrated at 40°C under vacuum using a rotary evaporator to obtain crude dark brownish gum (yield: 12% w/w).

**Column chromatography of the alcoholic extract of the pods of *C. pulcherrima*:** This crude slurry was subjected to silica gel (100–200 mesh) column chromatography by gradient elution with solvents of increasing polarity using hexane, ethyl acetate and methanol. Fractions (250 mL) were collected and monitored on silica gel-TLC. The visualization of spots on TLC plates was carried out either in UV light or by exposing TLC plates to iodine vapours or by spraying 10% sulfuric acid in methanol and heating at 110°C. Similar fractions were combined together and tested for antimicrobial activity. Five compounds were isolated and subjected to spectral studies for the determination of their chemical structures.

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**Antimicrobial activity:** The crude alcoholic extracts of *C. pulcherrima* and isolated compounds were tested for activity against various pathogenic gram –ve and gram +ve bacteria and mold type fungi.

**Bacterial strains:** Gram –ve bacteria: *Escherichia coli* (enteropathogen), *Pseudomonas aeruginosa* (NCIM 8162); Gram +ve bacteria: *Bacillus subtilis* (NCIM 2063), *Staphylococcus aureus* (NCIM 2127); Mold type fungi: *Rhizopus oligosporus* (NCIM 1215). All microorganisms, except *E. coli*, used were obtained from the National Collection of Industrial Microorganisms (NCIM), Pune, India. *E. coli* was obtained from Department of Pharmaceutical Sciences, Andhra University, Visakhapatnam.

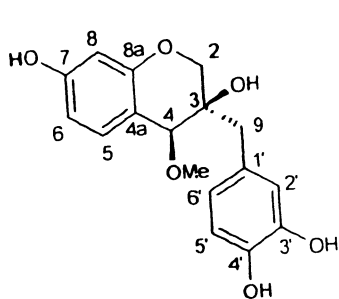
Bacterial cultures were sub-cultured on nutrient agar medium, incubated at 37°C for 24 h and stored at 4°C in a refrigerator to maintain stock culture. Fungal cultures were sub-cultured on Sabouraud dextrose agar medium, incubated at 28°C for 120 h and stored at 4°C in a refrigerator to maintain stock culture. Antimicrobial activity was assayed by the following standard methods.

**Disc diffusion method:** Antimicrobial activity of the alcoholic extracts was tested using the disc diffusion method<sup>9</sup>. Sterile nutrient agar medium (pH 7.2) was inoculated and aseptically transferred into a sterile petri dish. Sterile filter paper discs of 6 mm diameter, impregnated with alcoholic extract of plants (1 mg/mL) and isolated compounds (1 mg/mL), were introduced on the surface of the medium. Each disc absorbed 50 µg of extract/compound. Petridishes were incubated at 37°C for 24 h to obtain inhibition zones. The tests were conducted in triplicate. Antifungal activities of plant extracts were tested using Sabouraud dextrose agar, incubated at 28°C for 120–144 h. The standard antibiotics used were ampicillin (40 µg/mL) and nystatin (50 IU/mL).

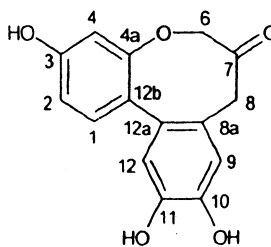
## RESULTS AND DISCUSSION

**Structure elucidation of Compound 1:** 4-O-Methylsappanol was obtained as a colourless solid, m.p. 156–158°C,  $[\alpha]_D^{25} + 58.2^\circ$ . UV absorptions at  $\lambda_{\max}(\text{MeOH})$ : 220, 279 and 284 nm underwent a bathochromic shift (221, 280, 286) on addition of base, indicating the presence of phenol and also indicating that it is having conjugation.

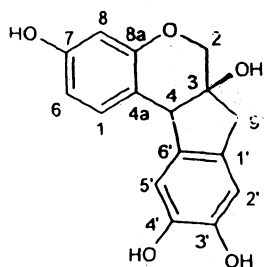
$^1\text{H NMR}$  spectrum showed signals for 1,3,4-trisubstituted benzene at  $\delta$  6.70 (1H, d,  $J = 2.0$  Hz, H-2'), 6.69 (1H, d,  $J = 8.2$  Hz, H-5') and 6.45 (1H, dd,  $J = 2.0, 8.2$  Hz, H-6') and signals at  $\delta$  7.05 (1H, d,  $J = 8.2$  Hz, H-5), 6.45 (1H, dd,  $J = 2.0, 8.2$  Hz, H-6), 6.35 (1H, d,  $J = 2.0$  Hz, H-8) for the 1,2,4-trisubstituted benzene, a methylene connected to oxygen atom at  $\delta$  3.62 (1H, d,  $J = 10.2$  Hz, H<sub>a</sub>-2) and 3.89 (1H, d,  $J = 10.2$  Hz, H<sub>b</sub>-2), a methine bearing oxygen atom at  $\delta$  3.69 (1H, br s, H-4), a methoxyl group at  $\delta$  3.32 (3H, s, 4-OMe), and a benzylic methylene at  $\delta$  2.55 (2H, br s, H-9).



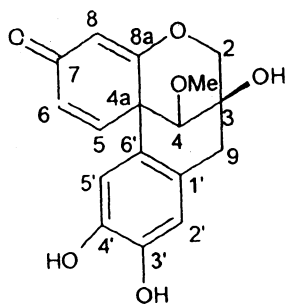
4-O-Methylsappanol



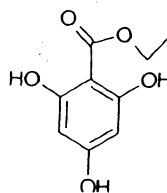
Protosappanin



Brazilin



Caesalpin J.



Ethyl-2,4,6-trihydroxybenzoate

From the spectral data and literature survey<sup>7,10</sup>, compound 1 was confirmed as 4-O-methylsappanol.

**Structure elucidation of Compound 2:** Protosappanin A was obtained as optically inactive, crystalline compound, m.p. 254–255°C, and its molecular formula  $\text{C}_{15}\text{H}_{12}\text{O}_5$ . UV absorptions at  $\lambda_{\max}(\text{MeOH})$ : 211, 260, 284 and 287 nm underwent a bathochromic shift (212, 261, 285, 289) on addition of base, indicating the presence of phenol and also indicating that it is having conjugation.

$^1\text{H}$  NMR spectrum showed two overlapped singlets at  $\delta$  6.79 (2H, br s, H-9 and H-12) for the 1,2,4,5-tetrasubstituted benzene and signals at  $\delta$  6.74 (2H, dd,  $J = 2.1, 8.2$  Hz, H-2 and H-4),  $\delta$  7.13 (1H, d,  $J = 8.2$  Hz, H-1) for the 1,2,4-trisubstituted benzene. Further,  $^1\text{H}$  NMR spectrum showed the presence of two methylenes at  $\delta$  3.43 (2H, s, H-8) and 4.49 (2H, s, H-6) and  $\text{D}_2\text{O}$  exchangeable signals at  $\delta$  7.90 (2H, br s, 10-OH and 11-OH) and 8.69 (1H, br s, 3-OH).

The  $^{13}\text{C}$  NMR spectra of compound **2** showed fifteen signals comprising two benzene rings (seven singlets and five doublets), two methylenes at  $\delta$  49.86 (C-8) and 78.39 (C-6), and a ketonic carbon at  $\delta$  205.62 (C-7) ppm.

From the spectral data and literature survey<sup>7, 10</sup>, compound **2** was confirmed as protosappanin A.

**Structure elucidation of Compound 3:** Compound **3** was obtained as a crystalline compound, m.p. 156–157°C,  $[\alpha]_{\text{D}}^{25} + 125.6^\circ$  and its molecular formula  $\text{C}_{16}\text{H}_{14}\text{O}_5$ . UV absorptions at  $\lambda_{\text{max}}$ (MeOH): 209, 260 and 288 nm underwent a bathochromic shift (210, 261 289) on addition of base, indicating the presence of phenol and also indicating that it is having conjugation.

$^1\text{H}$  NMR spectrum of compound **3** indicates the presence 1,2,4,5-tetrasubstituted benzene at  $\delta$  6.71 (1H, s, H-8) and 6.60 (1H, s, H-11) and a 1,2,4-trisubstituted benzene at  $\delta$  6.31 (1H, d,  $J = 2.1$  Hz, H-4), 6.48 (1H, dd,  $J = 2.1, 8.2$  Hz, H-2) and 7.19 (1H, d,  $J = 8.2$  Hz, H-1) respectively. Furthermore, its  $^1\text{H}$  NMR displayed signals for a methylene bearing oxygen atom at  $\delta$  3.69 (1H, d,  $J = 11.8$  Hz,  $\text{H}_a$ -2) and 3.92 (1H, d,  $J = 11.8$  Hz,  $\text{H}_b$ -2), a methine proton at  $\delta$  3.98 (1H, br s, H-4), and a benzylic methylene at  $\delta$  2.78 (1H, d,  $J = 16.2$  Hz,  $\text{H}_a$ -9) and 3.03 (1H, d,  $J = 16.2$  Hz,  $\text{H}_b$ -9).

$^{13}\text{C}$  NMR spectrum showed sixteen signals comprising two benzene rings (seven singlets and five doublets), two methylenes at  $\delta$  42.87 (C-9) and 70.85 (C-2) ppm, a methine carbon at  $\delta$  51.06 (C-4) ppm and a quaternary carbon at  $\delta$  115.59 (C-4a) ppm.

From the spectral data and literature survey<sup>10</sup>, compound **3** was confirmed as brazilin.

**Structure elucidation of Compound 4:** Compound **4** was obtained as a crystalline compound, m.p. 242–243°C,  $[\alpha]_{\text{D}}^{25} + 447.78^\circ$  and its molecular formula  $\text{C}_{17}\text{H}_{16}\text{O}_6$ . UV absorptions at  $\lambda_{\text{max}}$ (MeOH): 212, 240 284 and 288 nm underwent a bathochromic shift (214, 241, 285, 290) on addition of base, indicating the presence of phenol and also indicating that it is having conjugation.

$^1\text{H}$  NMR spectrum showed signals assignable to the aromatic ABX system at  $\delta$  6.98 (1H, d,  $J = 10$  Hz, H-5), 6.38 (1H, dd,  $J = 1.8, 10$  Hz, H-6) and 5.43 (1H, d,  $J = 1.8$  Hz, H-8) [shifted to the high field by 0.77 ppm] indicating that the carbonyl group is located at C-7, which is conjugated to two double bonds ( $\text{C}_5=\text{C}_6$  and  $\text{C}_8=\text{C}_{8a}$ ). Furthermore,  $^1\text{H}$  NMR spectrum displayed signals due to 1,2,4,5-tetrasubstituted aromatic proton at  $\delta$  6.41 (1H, s, H-2') and 6.63 (1H, s, H-5'), a methylene bearing oxygen atom at  $\delta$  4.05 (2H, dd,  $J = 5, 11$  Hz, H-2), a benzylic methylene at  $\delta$  3.20 (2H, br s, H-9), a methine proton at  $\delta$  3.85 (1H, s, H-4) and three  $\text{D}_2\text{O}$  exchangeable protons at  $\delta$  8.06 (2H, br s, 3'-OH and 4'-OH) and 4.52 (1H, br s, 3-OH).

$^{13}\text{C}$  NMR spectra showed signals due to a total of seventeen carbons composed

of the following functional groups: a methoxyl group at  $\delta$  62.02 ppm, an  $\alpha,\alpha',\beta,\beta'$ -unsaturated ketone system at  $\delta$  149.40 (C-5), 130.91 (C-6), 190.48 (C-7) and 109.90 (C-8) ppm, two quaternary carbons at  $\delta$  53.76 (C-4a) and 177.24 (C-8a) ppm, one benzoyl carbon at  $\delta$  43.19 (C-9) ppm, a methylene bearing oxygen atom at  $\delta$  85.42 (C-4) ppm, and six aromatic carbons at  $\delta$  125.20 (C-1'), 116.06 (C-2'), 146.60 (C-3'), 144.88 (C-4'), 113.60 (C-5') and 127.15 (C-6') ppm.

From the spectral data and literature survey<sup>11</sup>, compound 4 was confirmed as caesalpin J.

**Structure elucidation of Compound 5:** Compound 5 was obtained as a powder compound, m.p. 182–183°C, and its molecular formula  $C_9H_{10}O_5$ .

<sup>1</sup>H NMR spectrum in acetone  $d_6$ , 300 MHz showed signals assignable at  $\delta$  8.2 (3-OH, b, s), 7.1 (2H, d,  $J = 2$  Hz), 4.2 (2H, q) and 1.3 (3H, t).

From the spectral data, compound 5 was confirmed as ethyl-2,4,6-trihydroxy benzoate.

### Antimicrobial activity

Crude extract, compounds 1 and 2 showed a significant activity against all tested organisms. Compound 3 showed a moderate activity against pathogenic gram +ve bacteria, *S. aureus* and mold type fungus, *R. oligosporus*. compound 4 showed moderate activity against all tested organisms. Compound 5 showed good activity against *E. coli* and *S. aureus* (Table-1).

TABLE-1  
ANTIMICROBIAL ACTIVITY OF ISOLATED COMPOUNDS

Compound	Inhibition zone diameter (mm)				
	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>B. subtilis</i>	<i>S. aureus</i>	<i>R. oligosporus</i>
Crude extract	20	18	17	22	19
Compound 1	10	14	12	18	15
Compound 2	14	16	12	8	19
Compound 3	12	9	11	14	16
Compound 4	10	12	11	10	14
Compound 5	16	0	12	17	0
Ampicillin (40 $\mu$ g/mL)	17	16	21	23	—
Nystatin (50 IU/mL)	—	—	—	—	24
Control (60% v/v alcohol)	0	0	0	0	0

CP-crude: Alcoholic extract of the pods of *C. pulcherrima*  
Each disc contains 50  $\mu$ g of the compound.

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