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Note

Antioxidant and cytotoxic activities of *Caesalpinia pulcherrima* wood

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Antioxidant and cytotoxic activities of the methanolic and aqueous extracts of *Caesalpinia pulcherrima* wood were studied in *in vitro* models. Both extracts exhibited strong antioxidant activity, as evidenced by the low IC_{50} values in both 1,1-diphenyl-2-picryl hydrazyl (DPPH), nitric oxide and superoxide scavenging methods; the values were found to be less or comparable to those of gallic acid, the standard used. To determine the cytotoxic activity, extracts were tested for toxic effects to brine shrimp larvae. In this assay, the methanolic extract had little effect, but aqueous extract was relatively toxic. The antioxidant and cytotoxic activities may be attributed to the total phenolic content in the wood.

Keywords: *Caesalpinia pulcherrima*, Antioxidant activity, Cytotoxic activity, Total phenolic content

Caesalpinia pulcherrima (Caesalpiniceae), a shrub or small tree up to 5 m in height, commonly known as Guleture is distributed throughout India[1]. The presence of diterpenoids isovouacapenol C and pulcherrimin A in root[2], peltogynoids bhonducellin, 6-methoxypulcherrimin and homoisoflavonoids in stem[3], lupeol, β-sitosterol, flavonoids, and myricetin in flowers[4], hydrocyanic acid, tannins, and benzoic acid in leaves[5] is reported. The bark shows strong antimicrobial and cytotoxic activities[6]. Plant is used as emmenagogue, purgative, stimulant and abortificient and also used in bronchitis, asthma, malarial fever[7]. In present work, the antioxidant and cytotoxic activities of the methanolic and aqueous extracts of *C. pulcherrima* wood have been investigated. The total phenolic content of the wood has also been estimated.

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Materials and Methods

The wood of *Caesalpinia pulcherrima* was collected from Nashik, Maharashtra, India. The plant was authenticated by Mr. S G Pradhan, Botanical Survey of India, Pune (Voucher no. BSI/WC/ Tech/2003/597) and preserved in the herbarium of the department. The dried wood (100 g) was extracted with 95% methanol for 48 h in soxhlet apparatus. For aqueous extract preparation, wood powder (100 g) was macerated with chloroform water mixture (4 ml chloroform in 1000 ml water) for 24 h. The extracts were filtered and concentrated to vacuum under reduced pressure in rotary evaporator and dried in desiccators.

Total phenolic content[8]

The total phenolic content of methanol and aqueous extracts of *C. pulcherrima* wood was determined by using the Folin-Ciocalteu assay. A stock solution (1 mg/ml) of the extracts was prepared in methanol. From the stock solution, 1 ml of the extracts of different concentrations ranging from 20 to 100 µg/ml was taken into a 25 ml volumetric flask and 10 ml of water and 1.5 ml of Folin-Ciocalteau reagent was added to it. The mixture was kept for 5 min, and then 4 ml of 20% sodium carbonate solution was added and made up to 25 ml with double-distilled water. The absorbance was recorded at 765 nm after 90 min. Percentage of total phenolics was calculated from calibration curve of gallic acid plotted by using the above procedure, and expressed µg of gallic acid equivalent.

Evaluation of free radical scavenging activity

The antioxidant activity of extracts of wood was studied with different concentrations ranging from 5 to 25 µg/ml. *In vitro* methods (DPPH, nitric oxide and superoxide scavenging) were used to screen the extracts for antioxidant activity. Gallic acid was used as positive control[9].

DPPH radical scavenging method[10]

The stock solutions of methanolic and aqueous extracts (50 µg/ml) were prepared by dissolving extracts in methanol and water. From stock solution, different concentrations range from 5 to 25 µg/ml was

prepared and then 0.075 ml of DPPH was added in each test tube of different extracts and volume was made up to 3 ml with methanol. Tubes were allowed to rest for 15 min and the absorbance was recorded at 516 nm. The decrease in absorbance with blank was also recorded. Experiment was repeated for three-times.

Scavenging of nitric oxide[11]

Different concentrations ranging from 5 to 25 µg/ml of methanolic and aqueous extracts of wood were prepared. To the extract, 1 ml of sodium nitroprusside (10 m*M*) was added and volumes were made up to 5 ml with methanol and incubated at room temperature for 150 min. The same reaction without the Greiss reagent, but equivalent amount of methanol served as control. After 150 min, 0.5 ml of Greiss reagent (1% sulphanilamide, 2% H_3PO_4 and 0.1% naphthylene dihydrochloride) was added. The absorbance was recorded at 546 nm. Experiment was repeated for three-times.

Scavenging of superoxide radical[12]

The assay was based on the capacity of the extract to inhibit formazon formation by scavenging superoxide radicals generated in riboflavin-light-NBT system. The reaction mixture contained 50 m*M* phosphate buffer, *p*H 7.6, 20 µg riboflavin, 12 m*M* EDTA and NBT 0.1 mg/3 ml, added in that sequence. Reaction was started by illuminating the reaction mixture containing different concentrations of sample extract for 90 s and the absorbance was measured immediately at 590 nm. Gallic acid was used as positive control.

Brine shrimp lethality assay

The brine shrimp lethality assay is considered a useful tool for preliminary assessment of toxicity and is also suggested for screening pharmacological activities in plant extracts. However, it is necessary to evaluate the suitability of the brine shrimp method, before it is used as a general bioassay to test plant extracts for pharmacological activity[13].

Brine shrimp eggs were purchased from the fishery shop, Mastyakanya, Canada corner, Nashik. The bioassay was conducted, following the procedure described previously. The eggs were hatched in a conical flask containing 300 ml deionized water containing 3.8% sea salt (artificial sea water). The flask was well aerated with the aid of an air pump and kept in a water bath at 29-30°C. A bright light source was left on and the nauplii hatched within 48 h. The methanolic and aqueous extracts were dissolved in artificial sea water to obtain required concentration ranges from 40 to 200 µg/ml. A solution of each concentration (1 ml) was transferred into clean sterile universal vials with pipette, and aerated sea-water (9 ml) was added. About 10 nauplii were transferred into each vial with pipette. A check count was performed and the number alive after 24 h was noted. LC_{50} were determined using the probit method[14,15].

Results and Discussion

The preliminary phytochemical screening of the wood showed the presence of high amount of total phenolics and tannins along with flavonoids. The amount of total phenolics of methanolic and aqueous

Table 1— Free radical scavenging activity of the *Caesalpinia pulcherrima* wood

[Values represent mean ± SD, n = 3]

Concentration (µg/ml)	% Inhibition*								
	DPPH scavenging			Nitric oxide scavenging			Superoxide scavenging activity		
	Gallic acid	Methanolic extract	Aqueous extract	Gallic acid	Methanolic extract	Aqueous extract	Gallic acid	Methanolic extract	Aqueous extract
5	77.81 ± 2.49	48.58 ± 2.74	40.57 ± 1.38	73.89 ± 1.82	16.08 ± 0.74	14.39 ± 0.75	75.21 ± 1.36	36.47 ± 2.59	22.70 ± 2.68
10	81.21 ± 1.12	65.34 ± 0.83	61.29 ± 1.68	76.29 ± 0.61	41.25 ± 1.48	36.75 ± 2.59	79.19 ± 1.12	45.64 ± 1.38	37.70 ± 0.70
15	84.67 ± 2.34	78.25 ± 0.93	72.56 ± 1.59	82.34 ± 1.33	48.24 ± 2.66	44.12 ± 1.26	83.76 ± 1.78	58.44 ± 0.81	44.16 ± 5.69
20	88.59 ± 2.66	90.48 ± 3.07	85.51 ± 1.24	85.12 ± 0.74	64.32 ± 1.57	57.19 ± 1.48	87.42 ± 2.03	72.40 ± 4.47	51.52 ± 4.55
25	91.62 ± 0.25	94.33 ± 3.14	89.76 ± 2.05	91.62 ± 1.75	80.41 ± 1.83	74.98 ± 2.19	91.71 ± 1.56	84.09 ± 11.49	58.34 ± 1.20

extracts was found to be 28.2 and 20.5 µg of gallic acid equivalent, respectively. As phenolics including flavonoids, tannins and gallic acid were present in the wood, free radical scavenging property of the wood was tested in a few *in vitro* models and it was found to scavenge DPPH, nitric oxide and superoxide radicals (Table 1).

This finding was further supported by the brine shrimp lethality assay, which clearly indicated the toxic effects of extracts. The aqueous and methanolic extracts had LC_{50} at 99.572 and 110.886 µg/ml respectively, indicating the relatively lower toxicity of methanolic extract. Kaur *et al*[16] reported that phenolics such as gallic acid and flavonoids are the major components of extract and its capacity to inhibit cancer cell proliferation provide evidence that it may be the principle factor responsible for cytotoxic effect of extract. As the cytotoxicity is an indicator of a wide range of pharmacological activities, such as anticancer, antiviral, insecticidal, pesticidal, AIDS etc[17,18], further studies on the *C. pulcherrima* wood are required to investigate for such activities.

The free radical scavenging activity of natural compounds can be evaluated by their ability to quench the synthetic DPPH, nitric oxide and superoxide free radicals, in which absorbance of reaction mixture is taken in visible range to know whether the compound is having antioxidant property. The methanolic extract showed promising antioxidant activity against DPPH, nitric oxide and superoxide induced free radicals as compared to aqueous extract (Table 1).

Reactive oxygen species (ROS) are formed continuously in cells as compared of both oxidative biochemical reactions and external factors. However, they become harmful when they are produced in excess under certain abnormal conditions such as inflammation, ischemia and in the presence of iron ions. Under these conditions, the endogenous antioxidants may be unable to counter ROS formation. ROS formed may cause cellular damage which may involve in etiology of diverse human diseases. Exogenous antioxidant supplement is helpful in scavenging these free radicals[19].

In conclusion, the present study demonstrated that methanolic extract of wood of *C. pulcherrima* showed promising antioxidant activity. The antioxidant and cytotoxic activities of the wood might be attributed to total phenolic content.

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