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Research Article

IN VITRO ANTIMICROBIAL AND ANTIOXIDANT ACTIVITIES EVALUATION OF METHANOLIC EXTRACT OF *CAESALPINIA PULCHERRIMA* FLOWERS

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ABSTRACT

The current study was conducted to prove ethno medicinal value of the plant, investigating anti-microbial, antioxidant, and total phenolic content of crude methanolic extract of flowers of *Caesalpinia pulcherrima*. The methanolic extract revealed narrow spectrum antimicrobial activity at the concentration of 400 µg/ disc. The results obtained were compared with standard ciprofloxacin of 5µg/ disc. The extract exhibited moderate amount of total phenolic compound (44.0 ± 0.08 mg/g of gallic acid equivalent). In DPPH free radical scavenging test, IC_{50} value of the crude extract was found fairly significant (41.59 ± 0.05 µg/ml) while compared to that of the reference standards butylated hydroxyl toluene (21.90 ± 0.05 µg/ml). Since, the plant, *Caesalpinia pulcherrima* got cabalistic antimicrobial, and antioxidant activities, other phytochemical and pharmacological studies can be carried out to justify its traditional uses, as the plant is available and being used traditionally in the rural areas of Bangladesh.

Keywords: *Caesalpinia pulcherrima*, Antioxidant, DPPH, Antimicrobial, TPC

INTRODUCTION

Molecular oxygen is essential to aerobic organisms to fuel biological process for the production of energy. The derivatives of oxygen generated as by-products during cellular metabolism and other exogenous environmental factors such as UV light, ozone, tobacco smoke, different xenobiotics, ionizing radiation herbicides, and pesticides[1]. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) play an important roles in many biological processes and are involved in host defense, overproduction of these species contributes to the immunopathology of a vast variety of conditions including development of degenerative diseases[2]. They also have been implicated in the pathogenesis of diabetes[3], liver damage, nephrotoxicity[4], inflammation, cancer, cardiovascular disorders and neurological disorders and in the process of ageing[5]. Therefore much attention has been focused on the use of antioxidants, especially natural antioxidants to inhibit per oxidation and to protect from damage developed due to free radicals. When infectious diseases lead death worldwide then antibiotic resistance has risen up as global concern[6]. Emergence of multidrug-resistant pathogens is threatening the clinical efficacy of many existing antibiotics whereas many infectious diseases have been known to be treated with herbal remedies throughout the history of mankind[7]. Natural products, either as pure compounds or as standardized plant extracts, provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity. Therefore, researchers are increasingly turning their attention to folk medicine, looking for new leads to develop better drugs against microbial infections[8]. *Caesalpinia pulcherrima* (family – Fabaceae), a shrub or small tree up to 6 m in height, locally known as Krisnochura and available all over Bangladesh. The bark of this plant shows strong antimicrobial and cytotoxic activities[9]. Plant is used as emmenagogue, purgative, stimulant and abortificient and also used in bronchitis, asthma, malarial fever[10]. In this present work antioxidant and antimicrobial activities of the crude methanolic extract of the flowers has been investigated. The total phenolic content of the plant have also been estimated.

MATERIALS AND METHODS

Plant Materials Collection

For this research, *Caesalpinia pulcherrima* was collected from, Noakhali, Bangladesh in July 2012 and was identified and authenticated by Bangladesh National Herbarium (DACB), Mirpur, Dhaka (Accession number: 38323).

Chemicals

Analytical grade chemical reagents used in the research were purchased from Merck, KGaA (Germany) and remaining are from BDH Laboratories (England).

Preparation of Plant Materials

The flowers of the plant were dried in the sun (under a shadow) for five days. They were further dried in the oven at a temperature below 40°C for 24 h. 300 g of the dried flowers was weighed by an electronic balance and grinded with a grinding machine.

Extraction of Plant Materials

Three hundred gram of the flower powder was macerated with 800 ml of 80 % methanol with sporadic shaking. After 15 days, the solvent was decanted and filtered using sterile cotton and Whatman® filter paper No. 1 (Sargent-Welch, USA), and then evaporated at room temperature, and freeze-dried (17 g deep greenish-black gummy extract was found).

Total Phenolic Content Determination

The amount of total phenolics in extracts was determined with the Folin-Ciocalteu reagent[11]. Gallic acid was used as a standard and the total phenolics were expressed as mg/g of gallic acid equivalents (GAE). Concentration of 6.25, 12.5, 25, 50, and 100 µg/ml of gallic acid and concentration of 250

µg/ml of plant extract were also prepared in methanol and 0.5 ml of each sample were introduced into test tubes and mixed with 2.5 ml of a 10-fold dilute Folin-Ciocalteu reagent and 2 ml of 7.5 % sodium carbonate. The tubes were covered with para-film and allowed to stand for 30 minutes at room temperature before the absorbance was read at 765 nm spectrophotometrically (UV-1800, Shimadzu, Japan). All determinations were performed in triplicate[12]. Total phenolic content was determined as mg of gallic acid equivalent per gram using the equation obtained from a standard gallic acid calibration curve.

DPPH Free Radical Scavenging Assay

The stable DPPH free-radical scavenging activity was measured using the modified method described by Chang *et al*.[13] Stock solution (1 mg/ml) of the methanol extract of the flowers of *Caesalpinia pulcherrima* was prepared in methanol solvent systems from which serial dilutions were carried out to obtain the concentrations of 500, 250, 125, 62.5, 31.25, 15.63, 7.81, 3.90, 1.95, and 0.98 µg/ml. In this assay, 2 ml of 0.1 mM methanolic DPPH solution was added to 2 ml of extract solution at different concentrations and the contents were stirred vigorously for 15 seconds. Then the solutions were allowed to stand at dark place at room temperature for 30 minutes occurring chemical reaction. After 30 minutes, absorbance was measured against a blank at 517 nm with the double beam UV-Visible spectrophotometer. The percentage of DPPH free radical-scavenging activity of plant extract was calculated as:

$$\text{DPPH free-radical scavenging activity (I \%)} = [(A_0 - A) / A_0] \times 100,$$

Where, A_0 is the absorbance of the control solution (containing all reagents except plant extract); A is the absorbance of the DPPH solution containing plant extract.

The DPPH radical-scavenging activity (%) was plotted against the plant extract concentration (µg/ml) to determine the concentration of extract necessary to decrease DPPH radical-scavenging by 50 % (called IC_{50}). Butylated hydroxyl toluene (BHT) was used as positive control standard.

Antibacterial and anti-fungal activity test

Test Organisms

Two strains of Gram-positive (*Staphylococcus aureus, Bacillus spizizenni*), three strains of Gram-negative bacteria (*Escherichia coli, Pseudomonas aeruginosa, Salmonella typhimurium*), and two strains of fungi (*Candida albicans, Aspergillus brasiliensis*) were used to evaluate the antimicrobial activity. The organisms were sub cultured in nutrient broth and nutrient agar media. The organisms were collected from the Department of Microbiology, Noakhali Science and Technology University, Bangladesh.

Table 1: Total phenolic content determination of methanolic extract of *Caesalpinia pulcherrima*

Methanol extract	Avg. absorbance at 765 nm ± SD	Total phenolic content of methanolic extract of *Caesalpinia pulcherrima*
Sample 01	0.129 ± 0.005	44.0 ± 0.08 mg gallic acid equivalent (GAE) per g of dry extract
Sample 02		
Sample 03		

SD = Standard deviation

Table 2: DPPH free radical scavenging activity of the methanolic extract of *Caesalpinia pulcherrima* flowers and standard

Concentration (µg/ml)	% Inhibition of different solvent extract and Standard	
	BHT (Standard)	*Caesalpinia pulcherrima*
500	92.90 ± 0.06	77.84 ± 0.04
250	92.26 ± 0.08	76.13 ± 0.23
125	88.39 ± 0.03	69.88 ± 0.03
62.5	76.77 ± 0.05	55.11 ± 0.56
31.25	51.94 ± 0.07	48.29 ± 0.91
15.63	38.71 ± 0.09	36.36 ± 0.53
7.81	24.19 ± 0.04	23.29 ± 0.71
3.90	15.81 ± 0.05	14.20 ± 0.36
1.95	11.61 ± 0.19	10.22 ± 0.09
0.98	9.03 ± 0.11	6.81 ± 0.15
IC_{50} (µg/ml)	**21.90 ± 0.05**	**41.59 ± 0.05**

Values are expressed as mean ± SD (n = 3)

Table 3: Antimicrobial activity of the crude sample of *Caesalpinia pulcherrima*

Test Organisms		Crude sample (400 µg/disc)		Ciprofloxacin (5 µg/disc)
		Zone of inhibition	Relative % of inhibition	
Gram positive Bacteria	*Staphylococcus aureus*	9 mm	28.13 %	32 mm
	Bacillus spizizenni	8 mm	22.86 %	35 mm
Gram Negative Bacteria	*Escherichia coli*	8 mm	25 %	32 mm
	Salmonella typhi	6 mm	30 %	20 mm
	Pseudomonas aeruginosa	8 mm	22.86 %	35 mm
Fungi	*Candida albicans*	6 mm	19.35 %	31 mm
	Aspergillus brasiliensis	6 mm	20.69 %	29 mm

Disc Diffusion Assay (DDA)

Disc diffusion method is widely acceptable for the evaluation of antimicrobial activity[14]. Dried, sterilized filter paper discs (6 mm diameter, HI-Media, China) containing the known amounts of test samples (400 µg/disc) were placed on nutrient agar medium consistently seeded with the test bacteria. As positive and negative control, standard disk of ciprofloxacin (5 µg/disc) and blank discs, respectively were used. For the maximum diffusion of the test materials to the surrounding media, these plates were reserved at low temperature (4°C) for 24 h**.** The plates were then incubated at 37°C for 24 h to allow optimum growth of the organisms. The test materials with antimicrobial property inhibited microbial growth in plates and thereby yielded a clear, distinct zone defined as zone of inhibition. The activity of the test sample was then determined by measuring the zone of inhibition expressed in millimeter[12].

RESULTS AND DISCUSSION

Total Phenolic Content Determination

Based on the absorbance values of the extract solutions, the colorimetric analysis of the total phenolics of the extract was determined and compared with that of the standard solution of gallic acid equivalents. Result (Table 1) shows the total phenolic amount calculated for *Caesalpinia pulcherrima.* These results reported that total phenolic content of methanol extracts is correlated with the activity of gallic acid and showed the presence of moderate amount of phenolics which has been recognized that the effects of antioxidant are mainly due to the phenolic compounds of the plant[12].

DPPH Free Radical Scavenging Activity

DPPH free radical scavenging activity of the *Caesalpinia pulcherrima* was found to be increased with the increase of concentration of the extract (Table 2). The extract showed 77.84 ± 0.04 % radical inhibitions at 500 µg/ml whereas at the same concentration the standard BHT showed 92.90 ± 0.06 % inhibitions respectively. The IC_{50} value of extract of *Caesalpinia pulcherrima* was determined as 41.59 ± 0.05 µg/ml where BHT showed 21.90 ± 0.05 µg/ml. A method which is based on the scavenging of the stable radical 1, 1-diphenyl-2-picrylhydrazyl (DPPH) has been used extensively for the prediction of the antioxidant activities of extracts of plants[15].

Antimicrobial Activity Assay

Antimicrobial activity of methanolic extract was tested against seven bacteria and fungi at concentrations of 400 µg/ disc. Standard antibiotic disc of ciprofloxacin (5 µg/ disc) was used for the comparison. The result of antimicrobial activity is given in Table 3. The extract revealed highest activity against *Staphylococcus aureus* (28.13 %). The zone of inhibition of *Caesalpinia pulcherrima* was very low; therefore, the MIC (minimum inhibitory concentration) was not determined.

CONCLUSION

In light of the results of the present study, it can be summarized that the plant extract possesses moderate antioxidant, and narrow spectrum antimicrobial activities. Therefore additional studies may be suggested to better understand the mechanism of such actions scientifically.

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