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Research Article

ENZYMATIC AND NON-ENZYMATIC ANTI-OXIDANT ACTIVITIES IN LEAVES AND FLOWERS *CAESALPINIA PULCHERRIMA*

Jayalakshmi C[1] and Thangaraj N[2]

[1]Govt. Ats College for Women Salem-8
[2]Kandaswami Kander's College Namakkal

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ABSTRACT

Nature has been a source of medicinal agents since times immemorial. The herbal products today symbolize safety in contrast to the synthetics that are regarded as unsafe to human and environment. Traditional systems of medicine continue to be widely practiced or many accounts. Population rise, inadequate supply of drugs, prohibitive cost of treatments, side effects of several allopathic drugs and development of resistance to currently used drugs for infectious disease have led to increased emphasis on the use of plant materials as a source of medicines for a wide variety of human ailments. The blind dependence on synthetic is over and people are returning to the naturals with hope of safety and security.

INTRODUCTION

Plants have been model source of medicines as they are a reservoir of chemical agents with therapeutic properties. Since ancient times, plants are the richest source of drugs of traditional systems of medicine, modern medicines, naturaceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs.

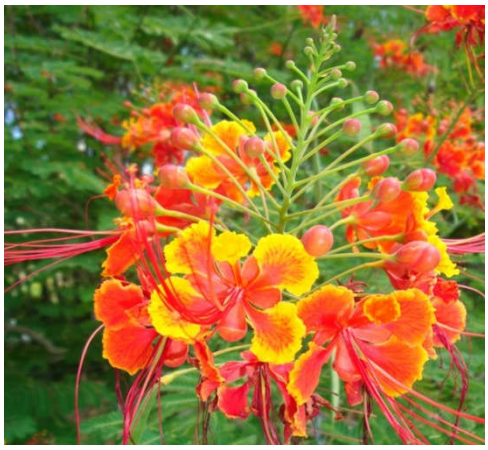
They synthesis and preserve a variety of biochemical products, many of which are extractable and used as chemical feed stocks or as raw material for various scientific investigations. Leaves, flowers, stem, roots, seeds, fruit and bark can all be constituents of herbal medicine. (Nonita p. peteros *et al*., 2010) Phytochemical components which produce definite physiological actions on the human body. These phytochemical, often secondary metabolites Present in smaller quantities in higher plants, include the alkaloids, steroids, flavonoids, terpenoids, tannis, and many others. Many secondary metabolites of plants are commercially important and find use in a number of pharmaceutical compounds.

Antioxidant may be defined as radical scavengers which protect the human body against free radicals that may cause pathological conditions such as ischemia, anaemia, arthritis, inflammation neurodegenertions, Parkinsons diseases, mongolism, ageing process and perhaps dementias. Flavonoids and flavones are widely distributed secondary metabolites with antioxidant and antiradical properties (polterait *et al* 1997).

Caesalpinia pulcherrima L is the most widely cultivated species in the genus *Caesalpinia*. *Caesalpinia pulcherrima*, commonly called peacock flower or Barbados Pride, is a fast-growing upright shrub or small tree. It is native to tropical America, but is now widely grown in tropical areas around the world for its long bloom of colorful flowers in upright racemes on prickly branches clad with twice pinnate green leaves. The leaves are anthelmintic, good for ulcers, infection.The main objective of the present study was designed to investigate the antimicrobial efficacy of *Caesalpinia pulcherrima* leaves and flower extracts.

Corresponding author:* **Jayalakshmi C
Govt. Ats College for Women Salem-8

Caesalpinia pulcherrima



Aim

To study the non-enzymatic and enzymatic antioxidant activities in flower of *Caesalpinia pulcherrima*

Objective

- To screen qualitatively the methanolic extract of the leaves and flowers of *Caesalpinia pulcherrima*.
- To analyze quantitatively the phytochemical constituents of the leaves and flowers of *Caesalpinia pulcherrima* evaluated by non enzymatic and enzymatic methods.
- To determine the phytochemical content of the leaves and flowers of *Caesalpinia pulcherrima* by standard method of quantitatively.

MATERIALS AND METHODS

Collection of Plant Material

The plant material namely leaves and flowers of *Caesalpinia pulcherrima* were collected from the field areas of P.Velur, Namakkal district, Tamilnadu, India.

Extraction

The leaves and flowers of *Caesalpinia pulcherrima* were shad dried in below 60°C for 2hrs. They were finely powered and extracted with 80% acetone chloroform methanol using soxhlet apparatus at 55°C. The soluble part was concentrated over water bath maintained below 60°C and dried in vacuum oven to obtain free flowing reddish brown powder. The extract obtained was termed as methanolic extract of *Caesalpinia pulcherrima.*

Phytochemical Analysis

The phytochemical in each sample was determined qualitatively and quantitatively.

Qualitative Analysis

The phytochemical analysis of the plant was carried out by the standard method.

Test for Tannins

0.1 ml extraction solution 0.5 ml distilled water 2 drops of Ferric chloride solution added observed for the blue or green black coloration.

Test for Saponins

0.2 ml of extract and 0.2ml of distilled water shaked well and small bubbles on surface of liquid.

Test for Flavonoids

0.5 ml of methanol added to 0.5 ml of extract and 1 ml of NaOH added shaken well yellow coloration.

Test for Glycosides

0.4 ml of extract solution added glacial acid 5 drops and few drops of Fecl3 and concentration sulphuric acid and observed reddish brown coloration 2 layers and bluish green colour in upper layer.

Test for Alkaloids

0.4 ml of extract 1drops of Mayer's reagents was added by the sides of the test tubes Creamy yellow or white precipitate indicates test for positive.

Test for Anthraquines

1.0 ml of extract and few drops CCl_4 (Carbon tetrachloride) from layer of solution remove the layer solution. Added 5 drops (Hcl+Fecl3) mixing solution cherry red colour indicate Anthraquines.

Test for Phenolic compounds

0.2 ml of extract was diluted to 0.5 ml with distilled water. To this few drops neutral 5% ferric chloride solution was added a dark color indicate phenolic compounds.

Quantitative Analysis

Tannins

500 mg of plant sample was weighed and transferred to 50 ml flask. Then added 50 ml of distilled water and stirred for 1 h. Sample was filtered into a 50 ml volumetric flask and the volume was made up to the mark. 5 ml of the filtered sample was pipette into test tube and then mixed with 2 ml of 0.1 M ferric chloride. The absorbance was measured using spectrophotometer at 395nm wavelength within 10 min (Tyler 1994; Harborne *et al.,* 1973).

Saponins

20g of each ground plant samples were put into a conical flask and 100 ml of 20% ethanol was added to the plant sample.60 ml of n- Butanol was added and combined n- Butanol extract were washed twice with 10 ml of 5% sodium chloride. The remaining solution was then heated on water bath and after evaporation; the samples were dried in oven to a constant weight.

Flavonoids

Extracted 10g of the plant sample with 100ml of 80% aqueous methanol at room temperature. The whole solution was filtered and the filtrate was then transferred into a water bath. The solution was evaporated to dryness and weighed to a constant weight (Mattila and Hellström, 2007; Williamson and Manach, 2005).

Alkaloids

5 g of the plant sample was prepared in a beaker and 200ml of10% CH_3CO_2H in C_2H_5OH is added to the plant sample. The mixture is covered and allowed to stand for 4 h. The mixture was then filtered and the extract is allowed to become concentrated in a water bath until it reaches ¼ of the original volume. Concentrated ammonium hydroxide was added until the precipitation is complete. The residue is alkaloid, which is then dried and weighed.

Phenols

Plants sample was boiled for 15 min with 50 ml of (CH3CH2)2O. 5 ml of the sample was pipette into 50 ml flask, and 10 ml of distilled water was added. Then 2 ml of NH4OH solution and 5 ml of concentrated CH3 (CH2)3CH2OH was added to the mixture. The sample was made up to the mark and left to react for 30 min for color development and measured for 505 nm wave length using a spectrophotometer (Tyler 1994; Harborne *et al*., 1973).

Enzymatic Method

Catalase

Total catalase (CAT, E.C. 1.11.1.6) activity was measured as described (Beers and Sizer, 1952). The reaction mixture (1.5ml) consisted of 50 mM phosphate buffer (pH 7.0), 0.1 mM EDTA, 20 mM H_2O_2 and samples of CgLP or PrLP or EtLP. The reaction was started by adding the enzyme extract, and the decrease in H_2O_2 was monitored at 240 nm and quantified by its molar extinction coefficient (36 M-1cm-1). The results were expressed as μMol H_2O_2 min-1g-1DM.

$$2\ H2O2 \xrightarrow{\text{Catalase}} 2\ H2O + O2$$

Calculation

$$\text{Units/mg} = \frac{\Delta A240/\text{min} \times 1000}{4.36 \times \text{mg enzyme / ml reaction moisture}}$$

Activity of Peroxidase

Total peroxidase activity (APX, E.C. 1.11.1.1) was performed by two methods. Colorimetric assays were performed according to (Nakano and Asada, 1981). The reaction mixture (1.5 mL) composed of 50 mM phosphate buffer (pH 7.0), 0.1 mM EDTA, 0.5 mM ascorbate, 1.0 mM H_2O_2 and samples of CgLP or PrLP or EtLP. The reaction was started by adding H_2O_2 and ascorbate oxidation was measured at 290 nm for 1 min.The gels were then immersed in 100 mL phosphate buffer pH 7.0 containing 0.03% H_2O_2, 0.2% guaiacol and 0.03% 3-amino-9-etil-carbazole. Peroxidase isoforms were detected as brown bands (Johri *et al.,* 2005).

Activity of Superoxide Dismutase (SOD)

The assay of SOD was performed according to Madamanchi *et al.,* (1994). 1g of plant material was homogenized in 10mL ice-cold 50 mM potassium phosphate buffer (pH 7.8) and centrifuged at 10,000 rpm for 10 min at 4º C. The supernatant was used as the enzyme source.

Non Enzymatic Method

Determination of DPPH (2, 2-diphenyl-1-Picrylhydrazyl) Radical Scavenging Activity

Free radical scavenging activity against 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical was measured Oboh *et al.,*(2005) in about 50µL of the extract, where 1.5 mL of 0.1 mM DPPH was added and vortexed for 15 to 30 s and allowed to stand without any disturbance for 30 min at room temperature. Indication in the activity of DPPH was observed with a change in the colour from purple to yellow and was measured by reading the absorbance at 517 nm. Ascorbic acid (AA), was used as the standard, while the inhibition ratio for DPPH scavenging activity was calculated from the equation: Absorbance of control (Ac) Absorbance of test sample (As)

$$AA\ (\%) = \frac{A\,c - As}{Ac} \times 100$$

Determination of Total Phenolic Content

The total phenolic content (TPC) of the plant extracts was determined spectrophotometrically using Folin-Ciocalteau's reagent Kahkonen *et al.,* (2001). 50 µL of the samples in triplicate was added into the test tubes followed by 1.5 mL of 2N Folin-Ciocalteau reagent (diluted 10 times) and 1.2 mL of 20% sodium carbonate. The contents of the tubes were mixed thoroughly and stored at dark for 30 min.

Measurement of Tocopherol

Tocopherol Rosenberg *et al.,* (1992) was measured by pipetting 1.5mL of each plant extract, standard (α tocopherol) and water respectively in tubes separately. To the test and blank was added 1.5mL ethanol and to the standard was added 1.5mL water and vortexed, to which was added 1.5 mL of Xylene and centrifuged at 1000rpm for 10min. About 1.0 mL of Xylene layer was transferred into another stopper tube, taking care not to include any ethanol or protein and 0.1 mL of 2, 2`-dipyridyl reagent was added and read at 460 nm for both the extinction of test and standard against the blank. It was calculated using the formula

$$= \frac{(\text{Reading of test at 520nm}) - (\text{Reading of test at 460nm})}{\text{Reading of standard at 520nm}} \times 0.29 \times 15 \times \frac{\text{Total volume of homogenate}}{\text{Volume used} \times \text{weight of the tissue}}$$

Determination of Ferric Reducing Antioxidant Power (FRAP)

The FRAP assay was conducted using method of Wong *et al.,* (2006). 200mL of extract were added with 3 ml of FRAP reagent that was prepared with mixture of 300 mM sodium acetate buffer at pH 3.6, 10 mM 2, 4, 6-tri (2-pyridyl)-s-triazine (TPTZ) solution and 20mM $FeCl_6H_2O$ at the ratio of 10:1:1. The reaction mixture was incubated in a water bath at 37°C for 30 min. The increase in absorbance was measured using spectrophotometer at 593nm. The antioxidant capacity based on the ability to reduce ferric ions of the extracts was calculated as percent of antioxidant. The percent of antioxidant

was calculated using the formula, percent of antioxidant (%) = [(A593 of sample-A593 of control)/ A593 of sample] x 100.

RESULTS AND DISCUSSION

Table 1&2 Qualitative analysis of Phytochemicals on acetone, chloroform, methanol extract of *Caesalpinia pulcherrima* leaves & flowers

S.No	Phytochemicals	Acetone	Chloroform	Methanol
1.	Tannins	+++	+	+++
2.	Saponins	++	+++	+++
3.	Flavonoids	++	++	+++
4.	Steroids	+	+++	+
5.	Glycosides	++	++	++
6.	Alkaloids	+++	++	+
7.	Anthaquinones	++	+++	+
8.	Phenolic compounds	++	+++	+++

Phytochemicals	Acetone	Chloroform	Methanol
Tannins	+++	+++	+++
Saponins	+++	+++	++
Flavonoids	+	+++	++
Steroids	++	++	+++
Glycosides	+++	++	+
Alkaloids	+++	+++	++
Anthaquinones	+++	+	+
Phenolic compounds	+++	+++	+++

Table3 & 4 Qualitative analysis of Phytochemicals on acetone, chloroform, methanol extract of *Caesalpinia pulcherrima* leaves & flowers

S.No	Phytochemicals	Acetone	Chloroform	Methanol
1.	Tannins	0.092	0.041	0.092
2.	Saponins	0.082	0.078	0.094
3.	Flovonoids	0.082	0.074	0.086
4.	Steroids	0.036	0.086	0.042
5.	Alkaloids	0.093	0.082	0.032
6	Protein	0.966	0.966	0.968
7	Carbohydrates	1.232	1.074	1.254
8	Amino acid	0.190	0.212	0.140

Phytochemicals	Acetone	Chloroform	Methanol
Tannins	0.086	0.078	0.096
Saponins	0.093	0.082	0.074
Flovonoids	0.086	0.096	0.076
Steroids	0.064	0.072	0.086
Alkaloids	0.088	0.080	0.072
Protein	0.968	0.968	0.965
Carbohydrates	1.204	0.929	1.471
Amino acid	0.428	0.595	0.635

Enzymatic Methods

Table 4 Catalase scavenging activity of leaves leaves and flowers of *Caesalpinia pulcherrima.*

Solvents	Leaf Units /mg	Flower Units /mg
Acetone	71.10	43.96
Chloroform	63.45	42.04
Methanol	73.39	49.69

Table 5 Peroxidase scavenging activity of Flowers of *Caesalpinia pulcherrima.*

Solvents	Leaf Units /lit	Flower Units /lit
Acetone	35.20	18.99
Chloroform	33.68	15.95
Methanol	36.98	22.54

Table 6 & 7 Assay of polyphenoloxidase scavenging activity of leaves & flowers in *Caesalpinia pulcherrima*

Solvents	Leaf Units /mg	Flower Units /mg
Acetone	1.65	1.58
Chloroform	1.17	1.16
Methanol	1.76	1.63

Solvents	Acetone Units /min	Chloroform Units /min	Methanol Units /min
1	0.015	0.013	0.015
2	0.007	0.007	0.008
3	0.005	0.007	0.005
4	0.004	0.003	0.004
5	0.003	0.003	0.003

Non Enzymatic Methods

Table 8 & 9 Free radicals scavenging activity of leaves and flowers of Caesalpinia pulcherrima by DPPH & FRAP method

DPPH

Solvents	Leaf mg/g	Flower mg/g
Acetone	8.13	30..62
Chloroform	9.56	35.40
Methanol	7.17	280.22

FRAP

Solvents	Leaf µg	Flower µg
Acetone	39	30
Chloroform	38	33
Methanol	43	36

Table 10 Total phenolic contents scavenging activity of leaves and flowers of *Caesalpinia pulcherrima*

Solvents	Leaf µg /g	Flower µg /g
Acetone	0.165	0.125
Chloroform	0.175	0.135
Methanol	0.185	0.150

Table 11 Tocopherol scavenging activity of leaves and flowers of *Caesalpinia pulcherrima*

Solvents	Leaf µg /g	Flower µg /g
Acetone	9.43	6.63
Chloroform	11.36	7.23
Methanol	8.96	5.95

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