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Research Article

Determination of Total phenolic acids, condensed tannins and flavonoids in the leaves of *Caesalpinia pulcherrima* (Linn.)

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Abstract:

Phenolic compounds which include tannins phenolic acids and flavonoids are responsible for various activities and are considered to be an important class of phytoconstituents. In the present study the total flavonoids, phenolic acids and condensed tannins were quantified by Folin ciocalteu method, Aluminum chloride colorimetric method and vanillin hydrochloride assay respectively. The phenolic acid content of the leaves extract was found to be 32 µg/g , amount of flavonoids was found to be 11.05 µg/g, while the amount of condensed tannins which was evaluated by vanillin hydrochloride assay was found to be 1.6 mg. This study gives an insight to the phytoconstituents present in the plant.

Key words: Total phenolic acids, condensed tannins, flavonoids, Caesalpinia *pulcherrima*

INTRODUCTION

Caesalpinia pulcherrima is an important plant that belongs to the family Caesalpiniaceae distributed through India. It is a small shrub growing to a height up to 5m in height[1]. The branches contain a few spines, leaves are bipinnate, usually 4-8 pairs, 6 to 12 cm long,leaflets are without a stalk. Flowers range from yellow to red and are present on terminal racemes [2]. Review of literature has revealed the presence of pulcherrimin A, peltogynoids, methoxypulcherrimin, bonducellin, flavonoids, isobonducellin,Bsitosterol, hydrocyanic acid, 5,7-dimethoxyflavanone diterpenoids, glycosides, rotenoids, flavanones, chalcones, flavones and sterols in the different part of the plant[3-8].The plant is reported to be used in various disorders which include antimicrobial ,cytotoxic activities, as emmenagogue, purgative, stimulant, abortificient, in bronchitis and as an malarial . Leaves used as mouth fever, wash, antitumor and gargle for mouth ulcers. Flowers used for erysipelas and inflammation of the eyes. Fruit is

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astringent and used for diarrhea and dysentery. The other activities reported are analgesic, anti inflammatory anticonvulsant, anti oxidant, anti ulcer, anti bacterial etc [9-13].The present study was carried out with an aim to estimate the amount of phenolic acids, flavonoids and condensed tannins which are the constituents that may be responsible for the various pharmacological activities exhibited by the plan.

MATERIALS AND METHOD:

Plant material:

The leaves of *Caesalpinia pulcherrima* were collected from the botanical garden of Krupanidhi College, Bangalore , Karnataka and were identified and authenticated by Prof Manjula Srinivisan, HOD, Department of Botany, Krupanidhi College, Bangalore. The collected material was then dried, pulverized and preserved in air tight containers.

Chemicals:

All chemicals for extraction and phytochemical analysis were obtained from Merck and SD fine chemicals.

Preparation of the extracts:

The methanolic extracts of both the dried powder (1 kg) of the leaves was prepared by using Soxhlet apparatus. The extracts were filtered using Whatman filter paper and then concentrated using a Rotary evaporator

Phytochemical screening;

The extracts were then subjected to preliminary phytochemical analysis i.e., alkaloids, phenolic compounds, tannins, carbohydrates, proteins, amino acids and saponins using standard reagents and procedures [14].

Determination of total Phenolic content in the leaves: [15, 16]:

The percentage of total phenol content of the methanolic extract was estimated by the Folin ciocalteu method. The extract and different dilutions of standard gallic acid were mixed separately with 1ml of Folin ciocalteu reagent and a solution of 7% sodium carbonate was also added. The mixtures were incubated at room temperature for one and half hours. The total phenolic content for the extract was determined by colorimetry at 750 nm. A standard curve for gallic acid in methanol was prepared using different concentrations (100 -700µg/ml). The total phenolic content was expressed in terms of gallic acid equivalents.

Determination of flavonoids [15]:

The amount of total flavonoids in the extract was determined using Aluminum chloride colorimetric method. The extract was dissolved in methanol (1mg/ml) and mixed with 0.1ml of 1M potassium acetate, 1.5ml of methanol, 0.1ml of 10% Aluminum chloride and 2.8ml of distilled water. This was maintained at room temperature for about 30mts. The absorbance was measured at 415 nm. Standard quercitin was prepared and a calibration curve for the standard quercitin was obtained by taking 12.5ml, 25ml, 50ml, 75ml and 100ml in methanol. A plot of absorbance versus concentration was plotted and the total flavonoid contents were calculated as quercitin equivalent.

Colorimetric estimation of condensed tannins by vanillin hydrochloride assay [16]:

The amount of condensed tannins was estimated by vanillin hydrochloride assay. Catechin was taken as the standard for estimating the total

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amount of condensed tannins in the methanolic extract. The amount of condensed tannins contents were calculated as catechin equivalent from the calibration curve of standard catechin by plotting the absorbance versus concentration Different dilutions of standard catechin were prepared ranging between 50-350 mcg/ml. This was then transferred to two sets of tubes and the volume in each of the tubes was made up to 1ml with methanol. These tubes were then incubated at 30^0 C in a water bath and to this 5ml of working reagent which was prepared by mixing one part 1% vanillin with one part 8% concentrated HCl was added at an interval of 1mt to one set of the tubes and 5ml of 4% HCl was added to the other set at intervals of 1.0 mt. The absorbance was recorded at 500nm after keeping the samples in the water bath for 20 mts. A difference of one minute was maintained as the color continues to develop when left for long time. The absorbance of the blank was subtracted from that of the sample containing vanillin reagent. An amount equivalent to 200mg of the extract was dissolved in 10ml of methanol and 1.0 ml of this was transferred to a tube and the same procedure was followed..

RESULTS AND DISCUSSION:

The preliminary phytochemical analysis revealed the presence of tannins, steroids, alkaloids, carbohydrates,flavonoids and proteins. The total phenolic acid content was estimated using the Folin ciocalteu method. This method measurers the amount of the substance (sample) that is needed to inhibit the oxidation of the Folin ciocaltteu reagent. It is one of the important methods for the quantification of plant phenolics.Aluminum chloride method was used for total flavonoid content. This is based on the principle that aluminum chloride forms acid stable complexes with the C-4 keto group and either the C-3 or C-5 hydroxyl group [17]. Vanillin HCl method was used for the estimation of condensed tannins.The amount of Phenolic acids, Flavonoid content and Condensed tannins is shown in table :1

Table 1: The amount of Phenolic acids, Flavonoid content and Condensed tannins

Constituents	Quantity Present
Phenolic acids	32 µg/g
Flavonoid content	11.05 µg/g
Condensed tannins	1.6 mg

The phenolic acid content of the leaves extract was found to be 32 µg/g.The amount of flavonoids was evaluated using aluminum chloride and was found to be 11.05 µg/g. The amount of condensed tannins which was evaluated by vanillin hydrochloride assay was found to be 1.6 mg. It has been reported that phenolic compounds, tannins and flavonoids show various activities like analgesic,anti inflammatory ,antimicrobial etc[18]. Hence the activities reported for this plant can be attributed to the presence of these constituents.

CONCLUSION:

Form the results of our study it can be concluded that the pharmacological activity of this plant reported by various researchers can be attributed to the presence of the phenolic phytoconstituents .The presence of these constituents is a significant finding of this study and further studies can be conducted to identify and estimate the important phytoconstituents of the methanolic extract.

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