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PRILIMINARY PHYTOCHEMICAL, ANTIOXIDANT AND ANTIMICROBIAL ANALYSIS OF CAESALPINIA PULCHERRIMA

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ABSTRACT

Plants are known to produce antimicrobial agents as their defense mechanism; they can be considered as potential sources of new antibacterial agents. In the present study, phytochemical analysis, antimicrobial and the antioxidant activities of leaves of *Caesalpinia pulcherrima* were investigated. Phytochemical analysis revealed the presence of alkaloids, flavonoids, saponins, tannins, and terpenoids in ethanol extract. The antimicrobial activity of ethanol, methanol, acetone and petroleum ether extracts of *Caesalpinia pulcherrima* was evaluated using disc diffusion method. Among the different extracts, the ethanol extract showed significant antibacterial and antioxidant activities. The obtained results suggested that the ethanol extract of the leaves of *C. pulcherrima* possess antimicrobial and antioxidant properties which can be used for the discovery of new bioactive natural products.

KEYWORDS: Phytochemical screening, antioxidant, antimicrobial agents.

INTRODUCTION

Caesalpinia pulcherrima is a species of flowering plant in the pea family, in traditional Indian medicine. The tree was formerly cultivated in South-East Asia for the red dye, obtained from its heartwood.[1] *C. pulcherrima* is distributed in Tamilnadu, Kerala, Karnataka, Andra Pradesh and West Bangal.[2] Bark of this plant shows strong antimicrobial and cytotoxic activities.[3] *C. pulcherrima* is used in the treatment of tridosha, fever, ulcer, abortifacient, emmenagogue, asthma, tumors, vata and skin diseases. Antiviral, antiulcer, anti-inflammatory activity of different parts of *C. pulcherrima* has been reported.[4]

Medicinal plants have been the mainstay of traditional herbal medicine amongst rural dwellers worldwide since antiquity to date. According to the World Health Organization, a medicinal plant is any plant which, in one or more of its organs, contains substances that can be used for therapeutic purposes, or which are precursors for chemo-pharmaceutical semi synthesis. Such a plant will have its parts including leaves, roots, rhizomes, stems, barks, flowers, fruits, grains or seeds, employed in the control or treatment of a disease condition and therefore contains chemical components that are medically active. The study of natural products on the other hand is called photochemistry.[5]

The number of multi-drug resistant microbial strains and the appearance of strains with reduced susceptibility to antibiotics are continuously increasing. This increase has been attributed to indiscriminate use of broad-spectrum antibiotics, immunosuppressive agent, intravenous catheters, organ transplantation and ongoing epidemics of HIV infection. In addition, in developing countries, synthetic drugs are not only expensive and inadequate for the treatment of diseases but also often with adulterations and side effects. Therefore, there is need to search new infection-fighting strategies to control microbial infections.[6]

In view of the above considerations, it was thought of interest to investigate the phytochemical, antioxidant potential and the antimicrobial activity of *Caesalpinia pulcherrima*. The main goal of the present work was to assess the phytochemical compound, total phenolic content, antioxidant properties and antimicrobial activity of various solvent extracts of the leaves of *C. pulcherrima.*

MATERIALS AND METHODS

Collection of plant materials

The plant specimens, *Caselpinia pulcherrima* for the proposed study was collected from poottety, Kanyakumari District and these specimens were identified on the basis of morphological characterizes and comparison with voucher specimens recorded in the Central Herbarium of Botanical Survey of India.

Preparation of samples

The healthy leaves of *Caselpinia pulcherrima* were air dried under shade. The leaves were then powered, sieved and stored in an air tight container for examination.

Preparation of solvent extracts

About 50g of powdered material was successively extracted with 250 ml of solvents such as ethanol, acetone, petroleum ether and methanol. They were placed in shaker for 3 days and the extract was collected from the conical flask by filtration. Then the plant extract kept in a water bath at 60°C to evaporate the solvent from the solution.

Phytochemical screening

The preliminary qualitative analysis of the plant extracts were performed to screen for the presence of bio active compounds in the *Caselpinia pulcherrima* leaves.[7]

1. Test for alkaloids

To 1 ml of the filtrate, a drop of Mayer's reagent was added along the side of the test tube. The test solution was observed for the presence of yellowish or white precipitate.

2. Test for tannin

To 1 ml of sample, 1 ml of ferric chloride solution was added. The test solution was then observed for the presence of black or green precipitate.

3. Test for saponin

To 2 ml of sample, 5 ml of distilled water was added shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously and observed for the formation of emulsion.

4. Test for carbohydrate

To 1 ml of the extract, was added 2-3 drops of 1% alcoholic alpha-naphthol and 2 ml concentrated sulphuric acid. This was added along the sides of the test tube (violet ring at the junction of two layers).

5. Test for terpenoids

To 5 ml of the sample, 2 ml of chloroform and concentrated sulphuric acid was carefully added to form layer. It was observed for the formation of reddish brown coloration at the interphase.

6. Test for flavonoids

To 5 ml of dilute ammonia solution a portion of the aqueous filtrate of each sample followed by addition of concentrated sulphuric acid. It was observed for the formation of yellow coloration.

7. Test for steroids

To 1 ml of aqueous extract, 10 ml of chloroform and equal volume of concentrated sulphuric acid was added by sides of the test tube. The upper layer turns red and sulphuric acid layer showed yellow with green fluorescence. This indicates the presence of steroids.

8. Test for amino acids

To 1 ml of extract, few drops of Ninhydrin reagent was added. Appearance of purple color shows the presence of amino acids.

9. Test for phenols

To 1 ml of each extract dissolved in alcohol or water, separately few ml of neutral ferric chloride solution was added. Any change in color indicated the presence of phenolic compounds.

10. Test for protein

To 1 ml of diluted extract, 1 ml of 5% $CuSO_4$ and 1% of NaOH solution was added. Deep blue color confirmed the presence of proteins.

Determination of the total phenolic content

Crude methanolic extract of 100µl and 100-500µl of Gallic acid as working standard were made up to 500µl and then mixed with 1.5ml of Folin-Ciocalteu reagent (10%) and allowed to stand at room temperature for 5 minutes and then added 1.5ml of Sodium carbonate(6%) solution to the above mixture. After 90 min incubation at room temperature, absorbance was measured at 725nm. The content of total phenolic in each extract was determined from the standard curve and expressed as mg/100g fresh weight of Gallic acid equivalents (GAE).

DPPH free radical scavenging assay

The free radical scavenging activity of ethanol extract of *C. pulcherrima* was measured by using 2, 2-diphenyl-1-picryl-hydrazyl (DPPH) by the modified method.[8] The reaction mixture consisting of DPPH in methanol (0.3 mM) and different concentrations of the ethanol extract (1 ml) were incubated for 10 min. in dark, after which the absorbance was measured

at 517 nm. Ascorbic acid was used as positive control. The radical scavenging activity was expressed in terms of the amount of antioxidants necessary to decrease the initial DPPH absorbance by 50% (IC50).

% DPPH radical scavenging = [(B – A) / B] × 100

Where B is the absorbance of the blank (DPPH in methanol) and A is the absorbance of the sample (DPPH in methanol with sample).

Antibacterial assay

Antibacterial assay of plant leaf extracts was determined using Kirby Bauer disc diffusion method. Sterile disc (6 mm) impregnated with plant leaf extracts and then placed on the culture plates inoculated with the test microorganisms. The test microorganisms were spreaded on the petri plates and incubated at 37°C for 24 hours. Check for antimicrobial activity, by looking for the clear area called zone of inhibition was measured in millimeters and compared with antibiotic disc Kanamycin.

RESULTS AND DISCUSSION

PHYTOCHEMICAL EVALUTION

Table 1: Phytochemical analysis of *Caselpinia pulcherrima* leaf extract.

PHYTOCHEMICALS	ETHANOL	ACETONE	METHANOL	PETROLEUM ETHER
Alkaloid	-	+	+	+
Tannin	+	+	+	-
Saponin	+	+	+	-
Carbohydrate	+	-	+	-
Terpenoids	+	-	+	+
Flavanoids	-	-	+	+
Phenol	-	+	-	+
Aminoacids	-	+	+	-
Protein	-	-	-	-
Steroids	-	-	-	+

Plants produce a number of substances and most are secondary metabolites which act as defense against predators, responsible for typical odors and characteristic pigmented nature of plants. Most of the phytochemicals are extensively used as medicinal compounds for treatment of various ailments all over the world.[9]

The quantitative screening of phytochemical components in leaves ethanol extract of *Caselpinia pulcherrima* revealed the presence of, tannin, saponin, carbohydrates, terpenoids, whereas amino acids, phenol, alkaloids flavonoids, steroids, and protein are absent. Analysis

in methanol revealed the presence of alkaloids, tannin, saponin, carbohydrates, terpenoids, flavonoids and amino acids whereas steroids, protein and phenol were absent. The phytochemical components indicates the presence of alkaloids, tannin, saponin, amino acid and phenol in acetone whereas terpenoids, flavonoids and steroids carbohydrates, and protein are absent. Analysis in petroleum ether revealed the presence of alkaloids, terpenoids, flavonoids, steroids and phenol, whereas tannin, saponin, carbohydrates, amino acid and proteins are absent which are shown in (Table.1).

Total Phenolic Content Assay

The content of total phenolic in the leaf extract of Caesalpinia *pulcherrima* was estimated by FCR method. Total phenolic content, as estimated in terms of mg/10g fresh weight and shown in Table 2. A number of studies have focused on the biological activities of the phenolic compounds, which are the potential antioxidants and free radical- scavengers. In the present study ethanolic extract of *Caesalpinia pulcherrima* contained the maximum amount of phenolics and followed by petroleum ether. Hence this may be a good source for phenolic compounds. The phenolic compounds are also thought to contribute directly to the antioxidant activity.[10-11] suggested, there is a correlation between phenolic content and antioxidant activity.

Table 2: Total Phenolic Activity of *Caesalpinia pulcherrima*

S.NO	PLANT EXTRACTS	mg/10g of fresh wt.
1	Ethanol	63
2	Methanol	49
3	Acetone	23
4	Petroleum ether	55

DPPH free radical scavenging activity

DPPH radical is one of the most widely used strategies to evaluate the antioxidant activity of herbal extracts. This method is simple, rapid and measures the capacity of herbal extract to bleach the DPPH radical. In the present study, we monitored the decrease in DPPH absorption in the presence of varying concentrations of ethanol extracts at 517nm. Leaf extract of *Caesalpinia pulcherrima* showed highest percentage of scavenging potential at 20µg/ml (24%). It was evident that the extracts showed hydrogen donating ability and therefore the extracts could serve as free radical scavengers, acting possibly as primary antioxidants.[12]

Table 3: DPPH Radical Scavenging Activity of *Caesalpinia pulcherrima.*

CONCENTRATION (µg/ml)	ETHANOL (%)	STANDARD
20	24	14
40	10	28
60	12	42
80	12	57
100	15	71
120	14	85
140	12	99
160	14	114
180	15	128
200	15	142

ANTIBACTERIAL ACTIVITY

Antibacterial activity of *Caesalpinia pulcherrima* leaf extract was tested using four solvents against five gram positive and five gram negative bacteria. Among the four solvents the ethanol extracts showed the highest activity than other solvents. The gram negative bacteria was more susceptible than gram positive bacteria. The most susceptible bacteria was *Klebsillia pneumonia* followed by *E.coli.* In contrast[13] reported that gram positive bacteria were more susceptible than gram negative bacteria.

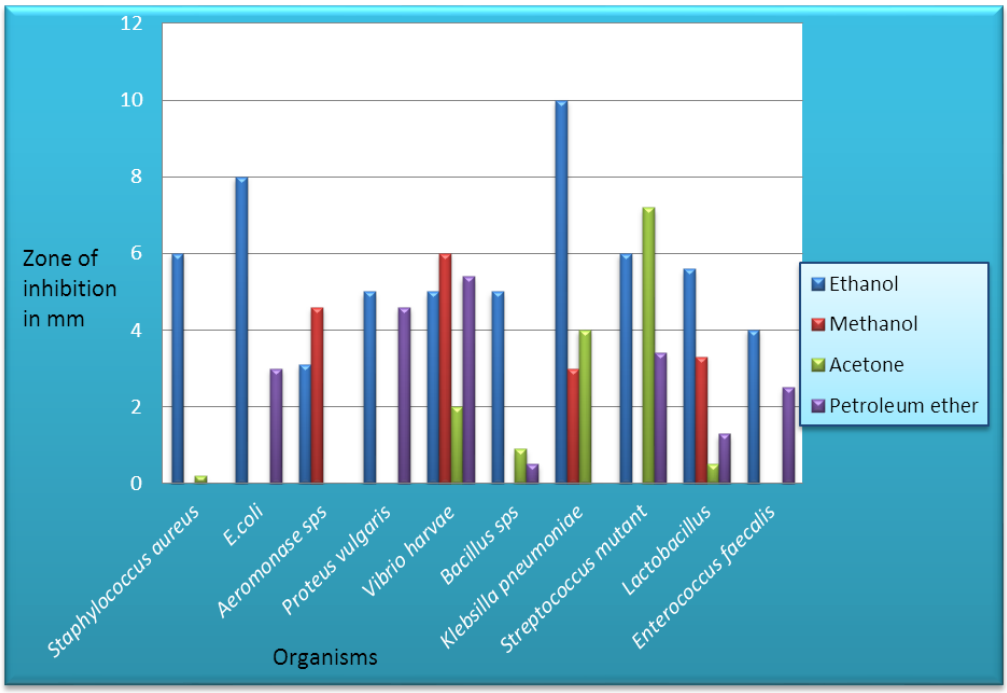

Fig 1: Antibacterial activity of different solvent extracts of *Caesalpinia pulcherrima* leaves.

CONCLUSION

Demand to herbal drugs is increasing day by day. Plants contain number of chemical moieties with varied pharmacological activities. Many potent and efficous medicinal principles used for treating dreadful diseases have been isolated from plant kingdom. So it is very clear that the study of the medicinal plant is important to meet the requirements in effective therapy. *Caesalpinia pulcherrima* has been examined to determine its phytochemical, antioxidant and antimicrobial activity. The plant extracts were found active against microbes and acts as a good antioxidant. Further work can be concentrated to separate bioactive compounds and to use in traditional medicine for diseases.

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