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PHYTOCHEMICAL ANALYSIS AND ANTIMICROBIAL ACTIVITY OF PLUMBAGO ZEYLANICA LEAF EXTRACT

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ABSTRACT In India, *Plumbago zeylanica* is a beneficial medicinal herb. The current study examines the antibacterial, and antifungal properties and phytochemical analysis of *Plumbago zeylanica* leaf extract. Using the well diffusion technique, the antibacterial activity was evaluated against gram-positive and gram-negative microorganisms. the antifungal activity was carried on *Aspergillus niger*. The study's findings imply that the plant leaf extract possesses an inhibitory effect on both gram-positive and gram-negative bacteria and fungi and it could be used as phytomedicine for bacterial and fungal diseases

KEYWORDS :

INTRODUCTION:

Plumbago zeylanica is an herbaceous plant with ascending, prostrate, or erect glabrous stems. Additionally, the ovate petiolate or sessile leaves have leaves. It belongs to the *Plumbago* genus and the family *Plumbaginaceae.* Tropical and subtropical areas across the world support the growth of the native *Plumbago zeylanica* plant. Deciduous woodland in (Australia, and India). The herb has a variety of therapeutic benefits [1-4]. Chitrak, chitramoolam, plumbago, Ceylon leadwort, doctor bush, or wild leadwort are some of the native names for the plant. Chitra is the name in Hindi, while Chitramoolam is the name in Tamil [5,6]. Molubda scandens (L.) Raf., Plumbagidium scandens (L.) Spach, Plumbago scandens L., and Plumbago zeylanica var. glaucescens Boiss are some synonyms for the plant [7,8].

The *P. zeylanica* plant grows to a height of 0.5-2 meters. The leaves are simple, alternating, elliptic, or oblong, 0.5–12 cm long, with a tapering base, and sometimes have a hairy edge. They can also be ovate or ovate-lanceolate. Young leaves lack stipules and have a thin petiole with tiny auricles. The inflorescence has numerous flowers and is of the terminal raceme type, measuring 6 to 30 cm in length [4-7]. White flowers are produced in terminal and axillary elongated spikes. They are pentamerous, pedicellate, bisexual, regular, and sweet-scented. Free and included are the stamens. The ovary is superior and single-celled, and the style is filiform with five elongated stigma lobes. The flowers also have a tubular calyx with glandular trichomes (hairs) that secrete gooey mucilage, which is another distinguishing feature [3]. The plant blooms all year round, and insects are the main pollinators. The mucilaginous glands facilitate animal fruit distribution and insect capture. The plant's fruit is a single-seeded, oblong capsule with five ridges. Each seed has an oblong shape, is 5–6 mm long, and ranges in color from reddish-brown to dark brown. Roots are at least 30 cm long, smooth, branching, or unbranched, with or without secondary roots, and 6 cm in diameter. When they are young, they are light yellow, and as they dry, they turn reddish-brown. The roots taste caustic and bitter and have a potent, distinctive smell [7].

Description:

The generic name comes from the Latin words plumbum, which means lead since this plant was thought to be able to treat lead poisoning and eye illness. The particular plant comes from Sri Lanka and is known as Ceylon. In India, the plant *Plumbago zeylanica* is also known as Chitrak, and it has several conventional and effective medical applications. The plant's blossoms may be eaten and are used to adorn salads, desserts, and cold beverages. The plant thrives in West Bengal, Madhya Pradesh, and Chhattisgarh. It also grows in a variety of soil types, including deep soil, deep black soil, and red literate soil. It may be quickly propagated via stem cuttings [8].

Medicinal uses:

The plant is an Indian medicinal plant with therapeutic qualities including cardiotoxicity, atherogenicity prevention, and cardiotonic neuroprotection. It may also be used to lower blood cholesterol levels and improve the poor density of lipoproteins. Diabetes, cancer, skin infections, rheumatoid arthritis, constipation, asthma, and muscle discomfort are all treated with this herb [9].

Growth and Development:

The plant *Plumbago zeylanica* is a wild species that grows in many locations and has a variety of medicinal uses. The plants are cultivated in tropical and subtropical areas where it rains throughout the rainy season [10]. To identify the numerous bioactive substances that are significant from a pharmacological standpoint, including alkaloids, steroids, flavonoids, phenolic compounds, and many more, a phytochemical examination was carried out. The plant's bioactive components are a source for making medicines [11]. The bioactive substances are known to behave as microbial inhibitors [12]. The hunt for novel structural medications to replace synthetic antioxidants that are reportedly harmful has led researchers to explore plants in the present [13].

MATERIALS AND METHODS:

Collection of plant material:

The plant *Plumbago zeylanica* was harvested from the Nallamala forest in the Kurnool district, Andhra Pradesh, India. The gathered plant leaves are divided into bits, let to air dry for 10 days, and then ground into a fine powder for use in further research.

Preparation of plant leaf extract:

Up to 10 gms of the dried leaf powder were used, and 100 ml of distilled water was used to dissolve it. The leaf extract that had been dissolved was utilized to analyze phytochemicals and test the antibacterial and antifungal activities.

Phytochemical analysis of leaf extract of *Plumbago zeylanica*:

The phytochemical examination of the *Plumbago zeylanica* leaf extract was used to identify the bioactive components, such as alkaloids, flavonoids, tannins, phenolic compounds, and saponins [14].

Test for carbohydrates:

Molisch's test:

Plant leaf extract (2ml) was mixed with 1ml of Molisch's reagent and a few drops of concentrated H_2SO_4; the production of a violet color at the intersection of the two layers shows the presence of carbohydrates.

Test for reducing sugars:

Benedict's test:

Benedict's reagent (1 ml) and leaf extract (2 ml) were combined, and boiled in a water bath for 5 minutes, and the resulting brick-red color

indicates the presence of reducing sugars.

Test for anthraquinone glycosides:
Borntrager's test:
A few drops of diluted H_2SO_4 were added to leaf extract and heated for 5 minutes before filtering and cooling with an equivalent volume of dichloromethane. The production of rose pink to red color confirms the presence of anthraquinone glycosides.

Test for saponins:
Froth test:
The leaf extract was shaken for 15 minutes with 2ml of distilled water. The formation of foam up to 1cm indicates the presence of saponins.

Test for proteins:
Biuret's test:
The presence of proteins is indicated by the formation of a purplish violet color after the addition of a few drops of CuSO4 (0.7%) and 1 ml of NaOH (10%) to 2 ml of leaf extract.

Test for steroids:
Libermann-Burchard test:
To the plant leaf extract, a few drops of acetic anhydride were added boiled, and cooled later few drops of concentrated H_2SO_4 were added, Formation of a brown ring at the junctions of two layers, and the upper layer turned green indicating the presence of steroids.

Test for tannins and phenolic compounds:
Iodine test:
Plumbago zeylanica leaf extract was mixed with a few drops of diluted iodine solution; the appearance of a transient red color indicates the presence of tannins and phenolic compounds.

Test for alkaloids:
Wagner's test:
A few drops of Wagner's reagent were added after adding 5ml of diluted HCl to the plant leaf extract, and the formation of a reddish-brown precipitate indicates the presence of alkaloids.

Test for flavonoids:
Shinoda's test:
The addition of 95% ethanol, 0.5 grams of magnesium, and a few drops of concentrated HCl to the plant leaf extract results in the formation of pink color, which denotes the presence of flavonoids.

Antibacterial activity:
Using the Well diffusion method, the antibacterial activity of plant leaf extract was evaluated against Gram-positive *S. aureus* and Gram-negative *E. coli*. Using an L-rod, the bacterial cultures were evenly distributed on the nutrient agar plate before various concentrations of plant leaf extract were added to the wells of the agar medium. The plates were then incubated for 24 hours at 37°C. The control was streptomycin. The zone of inhibition was measured after incubation [15].

Antifungal activity:
Utilizing the Well diffusion method, the antifungal activity of plant leaf extract was examined against Aspergillus niger. Using an L-rod, the A. niger spore suspension was evenly distributed on the potato dextrose medium. Using a sterile borer, wells were later created in the PDA medium, and various concentrations of plant leaf extract were then added. The plates were incubated at room temperature for five to seven days. The zone of inhibition was assessed following incubation [16].

RESULTS AND DISCUSSIONS:

Phytochemical analysis of leaf extract of *Plumbago zeylanica*:
The phytochemical analysis of the plant leaf extract revealed the presence of carbohydrates, saponins, proteins, steroids, alkaloids, phenolic compounds, tannins, and the absence of anthraquinone glycosides, flavonoids, and reducing sugars. Table 1 displays the findings. Similar reports are examined in the phytochemical analysis of roots of *Plumbago zeylanica* [14].

Table – 1: Phytochemical analysis of leaf extract of *Plumbago zeylanica*

Phytochemicals	Test	Observation	Result
Carbohydrates	Molisch's test	Violet colour	+ve
Reducing sugars	Benedict's test	colour change negative	-ve
Anthraquinone glycosides	Borntrager's test	colour change negative	-ve
Saponins	Froth test	Formation of foam	+ve
Proteins	Biuret's test	Purplish violet colour	+ve
Steroids	Libermann-Burchard test	Reddish brown-green rings	+ve
Tannins & phenolic compounds	Iodine test	Transient red colour	+ve
Alkaloids	Wagner's test	Reddish brown precipitate	+ve
Flavonoids	Shinoda's test	colour change negative	-ve

Antibacterial activity:
The results of a study on the antibacterial activity of plant leaf extract against various bacterial strains are shown in Table 2. Both Gram-positive and Gram-negative bacteria were effectively eradicated by the plant leaf extract of *Plumbago zeylanica*. The zone of inhibition for gram-positive bacteria was 0.3cm at the lowest concentration of plant leaf extract, and it was 0.8cm at the highest concentration. The zone of inhibition for gram-negative bacteria was 0.4cm at the lowest concentration and it was 1.0cm at the highest concentration. The zone of inhibition also increased as plant leaf extract concentration did. Similar reports were seen in *Plumbago zeylanica* Methanolic Extract: A Stunning Antibacterial Agent Against a Wide Range of Human and Agricultural Pathogens [15].

Table – 2: Antibacterial activity

Conc of plant leaf extract (in µl)	Zone of inhibition (in cm)	
	S. aureus	E. coli
25	0.3	0.4
50	0.4	0.4
75	0.8	1.0
streptomycin	0.9	0.9

Antifungal activity:
Table 3 shows the plant leaf extract's antifungal effectiveness against *A. niger*. The zone of inhibition measured between 0.1 cm and 0.6 cm at the lowest and highest concentrations, respectively. As the concentration of plant leaf extract increased, the antifungal activity increased as well. Similar findings reported Cladosporium *oxysporum, Aspergillus flavus,* and *Lasiodiplodia theobromae* resistance in *Plumbago zeylanica* Extract [16].

Table – 3: Antifungal activity

Conc. Of plant leaf extract (in µl)	Zone Of Inhibition (in cm)
50	0.1
75	0.2
100	0.6

CONCLUSION:

According to the study's findings, plumbago zeylanica leaf extract contains a variety of phytochemicals, which are biologically active molecules that can serve and function as an inhibitor of microbial development.

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