VOLUME 9 ISSUE 1 2023

ISSN 2454 – 3055

INTERNATIONAL JOURNAL OF ZOOLOGICAL INVESTIGATIONS

Forum for Biological and Environmental Sciences

Published by Saran Publications, India

International Journal of Zoological Investigations Vol. 9, No. 1, 954-960 (2023)

International Journal of Zoological Investigations
Contents available at Journals Home Page: www.ijzi.net
Editor-in-Chief: Prof. Ajai Kumar Srivastav
Published by: Saran Publications, Gorakhpur, India

ISSN: 2454-3055

In Vitro Antimicrobial Activity of Flower Extracts of *Plumbago indica* L. against Pathogenic Bacteria and Fungi

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Received: 4th April, 2023; **Accepted:** 14th May, 2023; **Published online:** 3rd June, 2023

https://doi.org/10.33745/ijzi.2023.v09i01.105

Abstract: Medicinal plants are traditionally used for the treatment of human diseases. *Plumbago indica* is popularly known as "chittiramulam" in Tamil and belongs to the family Plumbaginaceae. Its root contains plumbagin, sucrose, fructose and protease which possess antiseptic and antipyretic properties. The present study was undertaken to investigate *Plumbago indica* flower for potential activity against pathogenic bacteria and fungi. *Plumbago indica* flower extracts were prepared in ten different solvents like ethanol, methanol, butanol, chloroform, ethyl acetate, acetone, hexane, petroleum ether, aqueous and saline. The activity of flower extracts was evaluated against six bacterial pathogens, *Bacillus subtilis*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Proteus vulgaris* and three fungi, *Aspergillus niger*, *A. flavus* and *Rhizopus stolonifer* using standard disc diffusion method. The results of the flower extracts displayed possible activity against bacterial and fungal pathogens. The highest mean diameter of zone of inhibition was observed against bacterial pathogen *Pseudomonas aeruginosa* and highest mean diameter of zone of inhibition was observed against the fungal pathogen *A. flavus* in the ethanol extract. Together, the results of this study demonstrate, for the first time, the antimicrobiological effects of the flower of *Plumbago indica* which seems to possess therapeutic molecules capable of inhibiting, the growth of all the pathogenic bacteria and fungi.

Keywords: Antimicrobial activity, Chittiramulam, *Plumbago indica*, Microbes, *Bacillus subtilis*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Proteus vulgaris*, *Aspergillus niger*

Citation: Anitha C., Basil Rose M.R., Arokya Glory P.T., Prakash Shoba S. and Josephine Priyatharshini C.: *In vitro* antimicrobial activity of flower extracts of *Plumbago indica* L. against pathogenic bacteria and fungi. Intern. J. Zool. Invest. 9(1): 954-960, 2023.

https://doi.org/10.33745/ijzi.2023.v09i01.105

Introduction

Disease is an integral part of human from the beginning of their existence. The subject of drugs is also as old as disease and the search for remedies to combat it is perhaps equally old and

for more than a millennium. Herbal medicines have been extensively used, apparently safely and effectively to alleviate various symptoms of disease (Zhang *et al*., 2011). Most of the people are dependent on medicinal plants due to no/least side effects as compared to synthetic chemicals. Ecofriendly and bio-friendly plant-based products are very helpful for the prevention and cure of different human diseases. People searching natural remedies because those are safe and effective but the synthetic drugs contain lots of adverse effects. The use of plants for healing purposes predates recorded history and forms the origin of much of modern medicine. Over 50% of all modern conventional drugs originate from plant sources: a century ago, most of the few effective drugs were plant-based (Suffness and Douros, 1982), play an important role in drug-development programs in the pharmaceutical industry. Plants are an amusing source of bioactive compounds; they have played a dominant role in the maintenance of human health since ancient times. Examples include aspirin (from willow bark), digoxin (from foxglove), quinine (from cinchona bark), and morphine (from the opium poppy). The development of drugs from plants continues, with drug companies engaged in large-scale pharmacologic screening of herbs (Vickers *et al*., 2001).

The genus *Plumbago* belonging to the family Plumbaginaceae, comprises 10 genera and 280 species. Among these species, *Plumbago indica* L. is more popular due to its therapeutic properties. *Plumbago indica*, commonly known as red chitrak, it also named as chitramula and chitrak is a highly reputed Indian medicinal plant mentioned in Ayurvedic literature. It grows to 2 m (7 ft.) tall by 1 m (3 ft.) wide. Chitrak is a perennial herb cultivated in shady places in the garden for its oval leaves and racemes inflorescence of deep pink or scarlet flowers in winter. It is widely distributed in the tropics, more specifically in Southern India. The tuberous roots of the plant have been used in traditional medicine for the treatment of rheumatism, edema, piles, common wart, secondary syphilis, and leprosy. It has also been used to treat skin diseases and intestinal worms, these properties are believed to be attributed due to the presence of plumbagin, a natural quinoid constituent. Plumbagin from other *Plumbago* species possess various pharmacological activities such as antibacterial, antifungal, anticancer and anti-inflammatory activity. Hence, the aim of the present study was to investigate the antibacterial and antifungal activity of *Plumbago indica* flower extracts.

Materials and Methods

Collection of plant material:

The plants selected for this present research *Plumbago indica* L. (Family: Plumbaginaceae) was collected from Elavuvilai, Killiyoor taluk of Kanniyakumari District, India. After collection, suitable herbarium sheets were prepared for plant which contain all basic information of the plant and were sent to Department of Botany, Holy Cross College (Autonomous), Nagercoil for authentication.

Extraction:

At first the flowers were separated from the plant, they were cleaned with tap water followed by distilled water. About 10 g of flower of *Plumbago indica* L. (Family: Plumbaginaceae) was taken in a clean, dry and flat-bottomed glass beaker (Borosil) and ground it with 100 ml of each solvent (acetone/ methanol/ butanol/ chloroform/ ethyl acetate/ethanol/hexane/petroleum ether/water/ saline). Then these ground mixtures were further centrifuged and filtered through Whatman filter paper and the solvents were evaporated at the room temperature. The remaining extract was collected and the residues were preserved in a refrigerator for further antibacterial and antifungal studies.

Microbial strains used:

Antimicrobial activity of flower extract was determined against six human pathogenic bacteria *Bacillus subtilis*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Proteus vulgaris* and three

fungi, *Aspergillus niger*, *A. flavus* and *Rhizopus stolonifer*.

Assay of antibacterial activity:

Antibacterial activity of the flower extracts in the different solvents of the plant *Plumbago indica* were tested using standard disc diffusion method of Bauer *et al*. (1966). Sterilized Muller Hinton Agar (20 ml) was poured into sterile glass petriplates and allowed to solidify. After solidification, 100 µl of fresh culture of human pathogenic bacteria were spread on the surface of Muller Hinton Agar plates. Sterile disc of 6 mm, loaded with 25 µl of flower extract as well as positive and negative control discs for comparison were placed in the plates. For positive control streptomycin disc (25 µg disc) and negative control sterile disc were used. The plates were incubated for 24 h at 37°C. After incubation the diameter of inhibitory zones formed around each disc were measured (mm) and recorded.

Assay of antifungal activity:

Sterilized potato dextrose agar (20 µl) was poured into sterile petriplates and allowed to solidify. After solidification, 100 µl of fresh culture of human pathogenic fungal strain was distributed uniformly on the surface of potato dextrose agar plate with the help of sterile cotton swab. The sterile disc of 6 mm, loaded with 25 µl of flower extract and Fluconazole (100 µg /disc) used as positive control and sterile disc used as negative control were placed in the fungal plates and were incubated at 37°C for 48 h and the antifungal activity was measured based on the diameter of zone of inhibition formed around each disc.

Results and Discussion

In India, plants are usually used to treat certain diseases. Pharmacological studies have indicated that *P. indica* extract has antiplasmodial (Simonsen *et al*., 2001), antimicrobial (Ahmad *et al*., 2000), antifungal (Mehmood *et al*., 1999), anti-inflammatory (Oyedapo, 2010), antihyperglycemic (Olagunju *et al*., 2018), hypolipidaemic and antiatherosclerotic activities (Sharma *et al*., 1996). The methanolic extracts of *Plumbago indica* have been screened against different microorganisms responsible for various infections. *Plumbago indica* has been used especially as a laxative, an expectorant, an astringent and an abortifacient. The roots of *Plumbago indica* are used in traditional systems of medicine to cure various ailments such as body pain, headache, fever and inflammation (Singh *et al*., 2017).

The flower extract of the *Plumbago indica* showed different antibacterial activity on different bacteria tested. Among the six different bacterial strains tested, the ethyl acetate and ethanol extract of the *Plumbago indica* inhibited the growth of all the tested bacteria. The ethanol extract showed highest inhibition against *P. aeruginosa* (19±0.5 mm) and *S. aureus* (13±0.2 mm), and the growth of *B. subtilis* (16±0.5 mm), *E. faecalis* (13±0.5 mm) and *E. coli* (13±0.1 mm) were highly inhibited by ethyl acetate extract (Table 1). The flower extracts in methanol arrested the growth of all the tested bacteria except *P. aeruginosa*. The flower extracts in the acetone solvent inhibited the growth of *E. faecalis*, *S. aureus* and *B. subtilis*, but failed to inhibit the growth of *P. aeruginosa*, *P. mirabilis* and *E. coli*. The butanol extract of the flower affected the growth of *P. aeruginosa*, *B. subtilis* and *E. faecalis*, but inefficient against *S. aureus*, *P. mirabilis* and *E. coli*. Similarly, the petals with chloroform extracts stop the growth of only three bacteria and functionless in other three tested bacteria. Hexane, petroleum ether and saline extract were efficient for the bacterial growth inhibition on only two bacteria; but incapable for rest of the four bacteria. Among these ten extracts tested the aqueous extracts was unable to inhibit the growth of all the tested bacteria except *E. faecalis* (Table 1).

In the present investigation the extracts of the *P. indica* flower exhibited varying degrees of inhibition activity against the tested bacteria (Table 1); and the results were expressed in terms of the diameter of the growth-inhibition zone. The results clearly showed that tested bacteria were susceptible to all the extracts except flower in saline. The higher antibacterial activity of flower

Table 1: Antibacterial activity of *Plumbago indica* flower extracts

Extracts	Zone of inhibition (mm)					
	P. aeruginosa	***S. aureus***	***P. mirabilis***	***B. subtilis***	***E. faecalis***	***E. coli***
Acetone	-	8±0.02	-	7±0.01	11±0.01	-
Methanol	-	6±0.03	8±0.7	6±0.01	7±0.09	7±0.02
Butanol	11±0.01	-	-	8±0.02	11±0.5	-
Chloroform	-	-	-	7±0.5	12±0.3	9±0.5
Ethyl acetate	8±0.07	9±0.05	12±0.2	16±0.5	13±0.5	13±0.1
Ethanol	19±0.5	13±0.2	11±0.3	12±0.02	9±0.05	10±0.1
Hexane	-	-	09±0.5	-	8±0.03	-
Petroleum ether	10±0.01	-	-	-	-	07±0.02
Aqueous	-	-	-	-	6±0.02	-
Saline	-	-	-	8±0.1	-	12±0.05
Streptomycin	23±0.1	20±0.07	13±0.07	15±0.01	21±0.09	21±0.01
Sterile disc	-	-	-	-	-	-

extracts may be due to the extracts which are rich in secondary metabolites; however, activity does not depend only on secondary metabolites in the plant extracts, but also on their concentration and the possible interaction with other components (Dzotam *et al*., 2016). Bukar *et al.* (2015) reported that the extracts containing the secondary metabolites may be able to inter-chelate with DNA of both Gram positive and negative bacteria and interfere with cell division. Marjorie (1999) also reported that the antibacterial activity is due to their ability to bind with intracellular and soluble proteins and to bind with bacterial cell walls and due to complex with membrane lipids and exerts its action by causing leakage. The present investigation also agreed with Al-Mutairi *et al*. (2016) who have recorded that ethanol and methanol leaves extracts were also effective against most tested strains. The activity may be due to the plant which is rich in phyto-constituents like -- tannins, phytosterols, sitosterols, saponins, anthroquinones, glycosides and oleanolic compounds (Chen *et al*., 2005). Wanda (2018) also observed the bacterial growth inhibition which may be due to the synergic action of the antibacterial substances on peptidoglycan of bacterial cell wall to reduce the structural strength leading to degradation, inactivity and death of the bacteria. Lemma *et al.* (2002) reported the anti-bacterial action of polar (aqueous) and non-polar (pet. ether) extracts prepared from the roots of *P. zeylanica*. Minimum inhibitory concentration value of this particular compound showed comparative action resembling the commonly used broad spectrum antibiotic, tetracycline.

In this study, different antifungal activity was observed on three different fungi tested by the flower extract of the *Plumbago indica* herb. Among the three different fungal strains tested, the ethanol and ethyl acetate extract of the *Plumbago indica* flower inhibited the growth of all the tested fungi. The ethanol extract showed highest inhibition against *A. flavus* (25±0.09 mm) followed by *A. niger* (23±0.07 mm) and *R. stolonifer* (15±0.1 mm), and the growth of *A. niger* (15±0.02 mm), *A. flavus* (14±0.01 mm) and *R. stolonifer* (12±0.07 mm) were also inhibited by ethyl acetate flower extract (Table 2). The flower extracts in acetone, methanol, butanol and chloroform arrested the growth of two fungi; but failed to arrest the growth of rest of the fungi. Flower extraction using saline affected the growth of *A. flavus* alone. The extracts of flower in solvents like hexane, aqueous

Table 2: Antifungal activity of *Plumbago indica* flower extracts

Extracts	**Zone of inhibition (mm)**		
	A. niger	***A. flavus***	***R. stolonifer***
Acetone	12±0.02	-	12±0.01
Methanol	15±0.07	-	19±0.05
Butanol	20±0.03	11±0.05	-
Chloroform	9±0.01	12±0.1	-
Ethyl acetate	15±0.02	14±0.01	12±0.07
Ethanol	23±0.07	25±0.09	15±0.1
Hexane	-	-	-
Petroleum ether	-	-	-
Aqueous	-	-	-
Saline	-	12±0.5	-
Streptomycin	20±0.05	18±0.1	22±0.01
Sterile disc	-	-	-

and saline failed to inhibit the growth of all the three tested fungi. Among the ten different solvent extracts tested, antimicrobial activity was prominent in the ethyl acetate extract followed by ethanol extract. The ethyl acetate and ethanol extracts of the flower inhibited the growth of all the tested pathogenic bacteria and fungi. The aqueous extract of the flower of *Plumbago indica* inhibited the growth of none of the tested microbes except *E. faecalis*, the acetone extract inhibited the growth of one pathogenic fungus and the petroleum ether extract inhibited the growth of a single pathogenic bacterium. Although, comparison of the antimicrobial activity was observed between tested bacteria and fungi, the maximum zone of growth arrest was evidently noted on pathogenic fungi than bacteria which suggests that the *Plumbago indica* flower extracts highly act on the fungi for therapeutic industry.

Antifungal effect of *Plumbago indica* on the three test fungal pathogens at the said zone of inhibition was very significant. The extracts showed antifungal properties against *R. stolonifer*, *Aspergillus niger* and *Aspergillus flavus*. The inhibition of the test fungal pathogens could be due to the presence of active secondary metabolites present in *Plumbago indica* which have been reported as antifungal agents. The results of present investigation showed that the different extract varied in their effectiveness in inhibiting fungal growth. The ethanol extracts, showed high diffusion in the growth media owing to their hydrophilic character (Talibi *et al*., 2012), making active biochemical groups available to the assayed fungi, which represents an important characteristic in the evaluation of new compounds. The diversity in the bio-composition of chemical components of plant extracts, i.e., the secondary metabolites of plants, even those obtained from the same species, may result in different responses, especially with regard to the potential for inhibition of microbial multiplication. Other associated factors include solubility, pH, volatility, diffusion characteristics in growth medium, and the type of microorganism under evaluation (Gillitzer *et al*., 2012; Talibi *et al*., 2012). Afeez *et al*. (2021) reported antifungal activities of *Plumbago zeylanica* extract against *Aspergillus flavus*, *Lasiodiplodia theobromae* and *Cladosporium oxysporum* for potential human cream development. This attest to the fact that flower of *Plumbago indica* ethanolic and ethyl acetate extracts contains highly active compounds of possibly therapeutic significance and thus could be a potential agent for drug development; and this justify pharmacological and traditional uses of the plant.

Conclusion

The present investigation revealed a real solution for antimicrobial resistance by using a natural herbal extract. The plant extracts of *Plumbago indica* have a powerful antimicrobial activity that inhibit or at least stop the growth of microbial populations of different bacterial and fungal categories; so these extracts may be used as food additives and food preservatives to control such microbial population and conserve human and animal health. The extracts were also found to possess significant antifungal activities, but the antifungal activities were tremendously higher than the antibacterial activities. Thus, this plant shows potential as a source for an antimicrobial agent. Further study is necessary to identify other bioactive compounds that can be used to fight microorganisms. The future scope of this research is to provide a complete description of phytochemical constituents as well as a participatory scientific assessment of the important phytocompounds and their pharmacological action for the future creation of novel ethnomedicine.

Acknowledgements

The authors acknowledge the authorities at Holy Cross College (Autonomous), Nagercoil for providing the resources needed to complete this work, for which the writers are grateful.

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