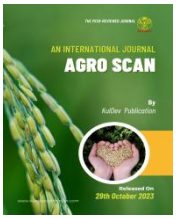
AgroScan: ISSN 0000-0000 (Online) 2023

Contents lists available at KulDevWeb

AgroScan

journal homepage: www.agroscan.kuldevpublication.com



Research Article

Studies on the ethyl acetate extract stem bark and leaves of *Plumeria rubra* for antibacterial and phytochemical screening

*[1]Subhash B. Pawar and [2]Pramod G. Jadhav

*[1]*Department of Botany, Sant Ramdas Arts, Commerce and Science College, Ghansawangi-431209, India*
[2]*Department of Agriculture, Kohinoor Arts, Commerce and Science College, Khultabad-431001, India*

ARTICLEINFO

Article history:
Received 11 September 2023
Accepted 15 October 2023
Available online xxxx xxxx

Keywords:
Phytochemical
Plumeria rubra
Extract

ABSTRACT

Plumeria rubra's stems and foliage Bark was extracted using a solvent extraction method called maceration and ethyl acetate. We looked for phytochemicals, antifungal, and antibacterial activities in the leaves and bark. Tannins, alkaloids, balsam, cardiac glycosides, phenols, terpenes, and steroids were detected in the leaves and bark extract. The extract shows that there are no flavonoids, saponins, or resins present. Alkaloids, cardiac glycosides, resins, terpenes, and steroids were detected in the bark extract; flavonoids, tannins, saponins and balsam were not present. The plant extracts demonstrated a wide range of antibacterial activity against both gram positive and gram negative bacteria with the zones of inhibition ranging from 10 to 28 mm. With gram-negative bacteria in particular, Proteus mirabilis, it was more noticeable. Additionally, *Pseudomonas aeruginosa* is a bacteria that is often resistant to the majority of antimicrobial treatments, was successfully combatted by the ethyl acetate crude extract. Additionally, the extracts showed efficacy against the fungus *Candida albicans.*

Selection and peer-review under responsibility of scientific committee of editorial board members of AgroScan and author(s) and suggested reviewer.

Introduction

Since ancient times, medicinal plants have been utilized to treat human illnesses because they contain useful ingredients [1]. Scientists searching for novel sources of medications against infectious illnesses have always been very interested in studies using biologically active chemicals derived from natural sources. There is no denying that plants are the source of modern medications in the west [2]. Prior to the development of antibiotics, herbal remedies were used to cure illnesses in both humans and animals. *Digatalis purpurea* L. has been used for centuries in England as a successful therapy for dropsy, a disorder where the heart's inefficiency causes fluid retention and overall body enlargement. The decoction of mango tree leaves is used as an oral contraceptive abortifacient in South America [3]. It has recently been discovered that Waltheria indica is useful in Taiwan in treating inflammatory illnesses. For an ethnobotanical study effort, a list of seldom researched plants used by the people of Kenya and Tanzania to cure wounds and diseases was recently collected [4].

* Corresponding author.
E-mail address: subhashpawar123@gmail.com (Subhash B. Pawar)
https://doi.org/10

Tanzanians often use the bark and root of Zambia's Africana hiern as a treatment for a variety of skin conditions [5]. The few instances mentioned above highlight the necessity for ongoing study into plants used in traditional medicine across the globe, particularly in nations where folk knowledge is still prevalent.

Antibacterial

A material that destroys or stops the development of microorganisms, including viruses, bacteria, fungus, and protozoa, is known as an antimicrobial. Antimicrobial medications function in various ways. Antimicrobial medications that eradicate bacteria are referred to as microbiocidal, while those that stop bacteria from growing are referred to as microbiostatic. Antibiotics, antivirals, antifungals, antiparasitics, and non-pharmaceutical antimicrobials are the primary classes of antimicrobials.

The phytochemicals

Chemical substances found in plants naturally are called phytochemicals. The phrase is often used to describe substances that have the potential to impact health but have not been shown to be necessary nutrients. Historically, phytochemicals have been used as medicines for thousands of years. It's possible that Hippocrates recommended willow tree leaves to treat fever. They are plant-based bioactive agents. Cardiac glycosides, steroids, alkaloids, resins, tannins, phenols and Flavonoids are examples of phytochemicals.

Supplies and Procedures

Sample gathering

The staff quarters on Bauchi Road at the University of Jos is where the plant samples were gathered. The plant is referred to as "Lal champa" in Hinduism, "True frangiapani" in English, and "Rumand" in Hausa.

Sample setup

Plumeria rubra leaves and stem bark were gathered, then dried at room temperature (25˚C). Using a mortar and pestle, the dried leaves and stem bark were crushed, and then they were sieved through a mesh. After being extracted for 50 hours in a conical flask with 250 mL of ethyl acetate, the leaves (80.5 g) and stem bark (96.30 g) were filtered using Whatsman filter paper. A rotary evaporator was used to concentrate the crude extract of leaves and stem bark, which was then dried on a hot plate.

Equipment sterilization

A hot air oven set at 106˚C was used to sanitize all of the glassware for one and a half hours. An autoclave set at 121˚C for 15 minutes was used to sterilize the water used to make the solution.

Screening for antimicrobials

The plate diffusion test is one of the common, standard techniques for identifying and quantifying an in vitro antibiotic activity. c. Diffusion test in series. b. The streak test. Preliminary sensitivity testing, minimum bactericidal concentration (MBC), minimum lethal concentration (MLC) and minimum inhibitory concentration (MIC) along determination are among the analyses that were performed.

Phytochemical screening of *Plumeria rubra's* leaves, bark and stems in the ethyl acetate extract

Using an organic solvent and normal operating protocols adapted from Trease & Evans [6], the phytochemical screening was performed. The Wagner test for alkaloids, the Solkowski test for cardiac glycosides, the Liebermann-Burchard test for terpenes and steroids, the ferric chloride test for flavonoids, and general tests for saponins, phenols, resins and balsam are among the tests that are performed.

Findings and Conversation (Test)

In bulk: The yield of the ethyl acetate bulk extraction of the leaves and stem bark is 2.34g for the leaves and 2.08g for the stem bark. For the leaves and the stem bark the corresponding percentage yields were 2.90% and 2.10%. Antimicrobial efficaciousness Test for sensitivity: Tables 1 and 2.

Table 1: Result of the zone of inhibition for the leaves (Bacteria).

Test Organisms	Zones of Inhibition (mm) Ethyl Acetate Extract (mg/mL)					Gentamicin (Control) (mg/mL)
	100	50	25	12.5	6.25	280
Staphylococcus aureus	27	22	10	-	-	27
Pseudomonas aeruginosa	23	21	15	-	-	24
Proteus mirabilis	24	23	20	-	-	24

Key: - = No inhibition

Table 2: Result of the zone of inhibition for the stems bark (Bacteria).

Test Organisms	Zones of Inhibition (mm) Ethyl Acetate Extract (mg/mL)					Gentamicin (Control) (mg/mL)
	100	50	25	12.5	6.25	280
Staphylococcus aureus	20	15	-	-	-	27
Pseudomonas aeruginosa	18	15	-	-	-	24
Proteus mirabilis	28	26	24	-	-	24

Key: - = No inhibition.

Anti-fungal activity: (Table 3 & 4)

Table 3: Result of the zone of inhibition for the leaves for the dermatophytes.

Test Organisms	Zones of Inhibition (mm) Ethyl Acetate Extract (mg/mL)					Gentamicin (Control) (mg/mL)
	100	50	25	12.5	6.25	280
Candida albicans	25	22	15	-	-	26
Aspergillus falvus	-	-	-	-	-	-

Key: - = No inhibition.

Table 4: Result of the zone of inhibition for the stems bark for the dermatophytes.

Test Organisms	Zones of Inhibition (mm) Ethyl Acetate Extract (mg/mL)					Gentamicin (Control) (mg/mL)
	100	50	25	12.5	6.25	280
Candida albicans	14	10	-	-	-	15
Aspergillus falvus	-	-	-	-	-	-

Key: - = No inhibition.

Result of minimum inhibitory concentration (MIC): Table 5-8.

Table 5: Result of the MIC for the leaves (Bacteria).

Test Organisms	Ethyl Acetate Extract (mg/mL)				
	100	50	25	12.5	6.25
Staphylococcus aureus	-	-	+	+	+
Pseudomonas aeruginosa	-	-	+	+	+
Proteus mirabilis	-	-	-	+	+

Key: - = Complete inhibition; + = Growth.

Table 6: Result of the MIC for the stems bark (Bacteria).

Test Organisms	Ethyl Acetate Extract (mg/mL)				
	100	50	25	12.5	6.25
Staphylococcus aureus	-	-	+	+	+
Pseudomonas aeruginosa	-	-	+	+	+
Proteus mirabilis	-	-	-	+	+

Key: - = Complete inhibition; + = Growth.

Table 7: Result of the MIC for the leaves for the Dermatophytes.

Test Organisms	Ethyl Acetate Extract (mg/mL)				
	100	50	25	12.5	6.25
Candida albicans	-	-	+	+	+
Aspergillus flavus	+	+	+	+	+

Key: - = Complete inhibition; + = Growth.

Table 8: Result of the MIC for the stems bark for the Dermatophytes.

Test Organisms	Ethyl Acetate Extract (mg/mL)				
	100	50	25	12.5	6.25
Candida albicans	-	-	+	+	+
Aspergillus flavus	+	+	+	+	+

Key: - = Complete inhibition; + = Growth.

Results of minimum bactericidal concentration (MBC): Table 9 & 10.

Table 9: Result of the MBC for the stems bark (Bacteria).

Test Organisms	Ethyl Acetate Extract (mg/mL)				
	100	50	25	12.5	6.25
Staphylococcus aureus	-	+	+	+	+
Pseudomonas aeruginosa	-	+	+	+	+
Proteus mirabilis	-	-	+	+	+

Key: - = Complete inhibition; + = Growth.

Table 10: Result of the MBC for the stems bark (Bacteria).

Test Organisms	Ethyl Acetate Extract (mg/mL)				
	100	50	25	12.5	6.25
Staphylococcus aureus	-	+	+	+	+
Pseudomonas aeruginosa	-	+	+	+	+
Proteus mirabilis	-	-	+	+	+

Key: - = Complete inhibition; + = Growth.

Results of minimum lethal concentration (MLC):Table 11 & 12.

Table 11: Result of the MLC for the leaves.

Test Organisms	Ethyl Acetate Extract (mg/mL)				
	100	50	25	12.5	6.25
Candida albicans	-	-	+	+	+
Aspergillus flavus	+	+	+	+	+

Key: - = Complete inhibition; + = Growth.

Table 12: Result of the MLC for the stems bark.

Test Organisms	Ethyl Acetate Extract (mg/mL)				
	100	50	25	12.5	6.25
Candida albicans	-	+	+	+	+
Aspergillus flavus	+	+	+	+	+

Key: - = Complete inhibition; + = Growth.

Results of phytochemical screening: Table 13.

Table 13: Phytochemical screening of the ethyl acetate extract of *Plumeria rubra*.

Secondary Metabolites Test	Ethyl Acetate Extracts of Leaves	Ethyl Acetate Extracts of Stems Bark
Alkaloids	+	+
Flavonoids	-	-
Tannins	+	-
Saponins	-	-
Balsam	+	-
Cardiac Glycosides	+	+
Terpenes and Steroids	+	+
Resins	-	+
Phenols	+	-

Key: - = Absent; + = Present.

Discussions:

Every component of a plant has a certain solubility when it comes to the various solvents used in the extraction process. As a result, the pharmacological activity and chemical makeup of herbal extracts made from the same plant in various solvents will vary [7]. The results of the phytochemical screening indicated that the leaf extract included Resins, steroids, terpenes, cardiac glycosides, alkaloids but not flavonoids, saponins or phenols. Phenols, resins, steroids, terpenes, cardiac glycosides and alkaloids were detected in the bark extract, but flavonoids, tannins, saponins, balsam and phenols were absent. This indicates the presence of tannins, which may be the cause of the plant's antibacterial action [8]. The plant's widespread usage in herbal medicine may be explained by its terpenoids [9]. The actual level of tannins with antibacterial action at the concentration employed may not be very high since the plant extract includes other elements, such as alkaloids, as shown by

the phytochemical screening in Table 13. There could be a rise in the plant extract's antibacterial activity if the tannin components of this plant are identified and examined [8]. Fungal and bacterial infections may be effectively treated using plant extracts that include chemicals with antibacterial qualities such as tannins [10].

The plant *Plumeria rubra*, whose leaves and bark were extracted using ethyl acetate as a solvent, was shown to have antibacterial activity, as evidenced by the zones of inhibition (Table 1-4). When Staphylococcus aureus was exposed to leaf extract at doses ranging from 27 mg/mL to 110 mg/mL, its zone of inhibition measured 29 mm, with a range of 15.0 mm to 29.0 mm. According to Table 1, the zones of inhibition for Proteus mirabilis and Staphylococcus aureus matched those of the positive control, gentamicin. According to Table 2, the plant's bark exhibited greater antibacterial activity against Proteus mirabilis, with the greatest zone of inhibition measuring 29.5 mm and a concentration range of 26 mg/mL to 110 mg/mL. The bark ranged from 25 to 29 mm. It's interesting to note that at 110 mg/mL of the extract, Proteus mirabilis had a larger zone of inhibition than gentamicin. The zone of inhibition for Pseudomonas aeruginosa was the smallest at 100 mg/mL for both leaves and bark, measuring 25.0 mm and 17.0 mm, respectively.

Additionally, out of all the dermatophytes, only *Candida albicans* exhibited significant activity against the plant extract; for the leaves and stem bark, it had a notable zone of inhibition measuring 24.0 mm and 14.0 mm at 100 mg/mL, respectively. It's important to highlight that the plant extract did not work well against Aspergillus flavus, according to Tables 3 and 4. *Protus mirabilis* growth was suppressed at concentrations of 26 mg/mL, 70 mg/mL, and 120 mg/mL. As shown in Tables 5 and 6, respectively, *Pseudomonas aeruginosa* and *Staphylococcus aureus* were suppressed in the broth at 50 mg/mL and 100 mg/mL for the leaf and stem bark extracts. Furthermore, Tables 7 and 8 shows that growth was suppressed at 50 mg/mL and 100 mg/mL for the leaf extract and only at 110 mg/mL for the stem bark as shown in Tables 9 and 10, the minimum bactericidal concentration result revealed that the bark and leaves were bactericidal at concentrations of 50 mg/mL and 100 mg/mL for *Proteus mirabilis*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* respectively as shown in Tables 11 and 12, the minimum lethal dosage for the bark extract was 100 mg/mL, which also killed Candida albicans. The minimum lethal concentration for the leaf extract was 50 mg/mL.

In summary

The results obtained are consistent with the plant extracts having wide range antibacterial action. Gram-negative *Staphylococcus aureus* was the gram-negative bacteria with the strongest antibiotic activity. Additionally, the plant extract shown efficacy against *Candida albicans* fungus.

References

Bisgano (1999) Respiratory distress syndrome therapy with peptide analogs of human SP-B.
Shage RW, Pearcy RW (2000) The Physiological Ecology of C4 Photosynthesis. Photosynthesis. pp. 497-532.
Riddle JM, Estes JW (1992) Oral Contraceptives in Ancient and Medieval Times. American Scientist 80(3): 226-233.
Hugo FA, Egon Noe (2006) What makes organic agriculture move - protest, meaning or market? A polyocular approach to the dynamics and governance of organic agriculture. IJARGE. pp. 1-17.
Kokwara (1976) Synthesis and Characterization of Dichlorobis (Embelinate) Copper (II) Complex.
Trease GE, Evans WC (1989) Pharmacognosy. (11th edn), Brailah Tiridal and Macmillan publishers. pp. 45-50.
Kirtikar KR, Basu BD (1999) Indian Medicinal Plants. (2nd edn), Dehradun, India.
Soforowa A (1993) Historical review of traditional medicine in Africa, spectrum books limited, Ibadan, Nigeria, pp. 1-25.
Hayashi TK, Okanuka M, Kavaski M, Movita N (1993) Production of diterpenoids by cultured cells from two chemotypes Scoparia dulcis. Phytochemistry 35(2): 353-356.
Roger P, Barnett V, Steiner R (1992) Agricultural sustainability: economic, environmental and statistical considerations.