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Study on the Phytochemical and Antimicrobial Screening of Ethylacetate Extract of Plumeria Rubra Leaves and Stems Bark

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Abstract

Plumeria rubra leaves and stems bark was extracted using ethyl acetate by maceration process of solvent extraction. The leaves and bark were screened for phytochemicals, antifungal and antibacterial activity. The leaves and bark extract showed the presence of tannins, alkaloids, balsam, cardiac glycosides, phenols, terpenes and steroids. The extract indicates the absence of flavonoids, saponins and resins. The bark extract showed the presence of alkaloids, cardiac glycosides, resins, terpenes and steroids; absence of flavonoids, tannins, saponins and balsam. The zones of inhibition ranges from 10-28mm and the plant extracts showed a broad spectrum of antimicrobial activity against gram positive and gram-negative bacteria. It was more pronounced on gram negative bacteria especially Proteus mirabilis. Furthermore, the ethyl acetate crude extract was effective against *Pseudomonas aeruginosa* which is usually resistant to most antimicrobial agents. The extracts were also effective against the fungi Candida albicans.

Keywords: Phytochemical; Plumera rubira; Antimicrobial; Antifungal; Stems bark; Minimum inhibitory concentration; Minimum bactericidal concentration; Minimum lethal concentration

Introduction

Medicinal plants have been used for centuries as remedies for human diseases; they contain components of therapeutic value [1]. Researches into biologically active compounds from natural sources have always been of great interest for scientist looking for new sources of drugs against infectious diseases. It is an undisputable fact that the western pharmaceuticals have their origin in plants [2].

The use of herbs in the treatment of man and animal diseases has been practiced before the advent of antibiotics. In England, Digatalis purpurea L. has been used for centuries as an effective treatment for dropsy a condition in which the in efficient working of the heart leads to retention of fluids and general swelling of the body.

In South America, the decoction of the leaves of Mango tree is used as oral contraceptive abortifacient [3]. In Taiwan Waltheria indica has recently been reported to be effective in the treatment of inflammatory diseases. Recently, a list of plants that people of Kenya and Tanzania use as remedies to treat infections and wounds that have not been studied extensively were compiled for an ethno botanical research project [4]. The root and bark of Zambia's Africana hiern is popular remedy for various skin diseases in Tanzania [5]. The few examples described above point towards the need for continual research into plants used in traditional medicine worldwide especially in countries where folk knowledge is still available.

Antimicrobial

An antimicrobial is a substance that kills or inhibits the growth of microbes such as bacteria, fungi, protozoa, virus etc. Antimicrobial drugs have different mode of actions. Antimicrobial drugs which kill the microbes is said to be microbiocidal and that which prevents the growth of microbes is said to be microbiostatic. The main classes of antimicrobial include antibiotic drugs, antiviral drug, antifungal drugs, antiparasitics and non-pharmaceutical antimicrobials.

Phytochemicals

Phytochemicals are chemical compounds that occur naturally in plants. The term is generally used to refer to those chemicals that may affect health but are not established as essential nutrients. Phytochemicals have been used for drug for millennia e.g. Hippocrates may have prescribed willow tree leaves to abate fever. They are bioactive agent found in plants. Phytochemicals include flavonoids, resins, phenols, steroids, alkaloids, cardiac glycosides, tannins etc.

Aim: The aim of this research is to determine the phytochemical and antimicrobial activity of Plumeria rubra leaves and stem bark.

Objectives of the study: The objectives of the research include:

a. Extracting the leaves and stem bark of Plumeria rubra leaves and stem bark using ethyl acetate as the solvent.

b. Determining the phytochemicals present in the leaves and stem bark.

c. Determining the antimicrobial activity of the plant against gram positive and gram-negative microorganism.

Materials and Methods

Sample collection

The plant samples were collected at the University of Jos, staff quarters Bauchi road. The plant is called 'Rumand' in Hausa, 'True frangiapani' in English and 'Lal champa' in Hindu.

Sample preparation

The leaves and stems bark of Plumeria rubra were collected and dried at room temperature (25˚C). The dried leaves and stems bark were pulverized using a mortar and pestle and sieved using a mesh. The leaves (80.5g) and stems bark (96.30g) were respectively extracted in 250mL of ethyl acetate in a conical flask for 50 hours and filtered using a whatsman filter paper. The crude extract of leaves and stems bark were concentrated in a rotary evaporator after which they are evaporated to dryness on a hot plate.

Sterilization of apparatus

All glass wares used were sterilized using a hot air oven at 106˚C for one and half hours. The water used in making the solution was sterilized in an autoclave at 121˚C for 15 minutes.

Antimicrobial screening

The general standard methods for detecting and determining an invitro antibiotic activity include:

a. Plate diffusion test.

b. Serial diffusion test.

c. Streak test.

The analysis carried out include the preliminary sensitivity testing, determination of minimum inhibitory concentration (MIC), determination of the minimum bactericidal concentration (MBC) and the determination of the minimum lethal concentration (MLC).

Phytochemical screening of the leaves and stems bark of the ethyl acetate extract of plumeria rubra

The phytochemical screening was done using organic solvent and carried out using standard operating procedures adopted from Trease & Evans [6]. The tests carried out are the Wagner test for alkaloids, Ferric chloride test for flavonoids, Solkowski test for cardiac glycosides, Liebermann-Burchard test for terpenes and steroids, general tests for saponins, phenols, resins and balsam.

Results and Discussion (Experimental)

Results

Bulk extraction: The bulk extraction of the leaves and stems bark using ethyl acetate obtains a yield of 2.34g for the leaves and 2.08g for the stems bark. The percentage yield was 2.90% for the leaves and 2.10% for the stems bark respectively.

Antibacterial activity

Sensitivity test: Table 1 & 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.

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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.

Discussion

Solubility of each constituent in a plant is very specific to different solvents in the extraction process. Hence, chemical nature as well as the pharmacological activity of herbal extracts obtained using same herb with different solvents will differ [7]. The result of the phytochemical screening showed the presence of alkaloids, tannins, balsam, cardiac glycosides, terpenes and steroids, phenols and absence of flavonoids, saponins and phenols in the leaves extract. The bark extract showed the presence of

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alkaloids, cardiac glycosides, terpenes and steroids, resins and absence of flavonoids, tannins, saponins, balsam and phenols. This reveals the presence of tannins which could be responsible for the antibacterial activity of the plant [8]. The terpenoids reveals the reason the plant is widely used in herbal medicine [9]. Since the plant extract contains other constituents, such as alkaloids as revealed by the phytochemical screening in Table 13, the actual content of tannins which possess the antibacterial activity in the concentration used might not be much. If the tannins constituents of this plant are isolated and tested, there may be a resultant increase in antibacterial activity of the plant extract [8]. The plant extract containing chemical such as tannins with the antibacterial properties have been useful in treating bacterial and fungal infections [10].

The results of the antibacterial activity showed that the leaves and bark of the plant Plumeria rubra extracted with the solvent ethyl acetate posses' antibacterial activity as indicated in the zones of inhibition (Table 1-4). Staphylococcus aureus had the zone of inhibition of 27mm and ranged from 10.0mm - 27.0mm at concentrations of 25mg/mL - 100mg/mL for the leaves extract. From Table 1, the zones of inhibition of Staphylococcus aureus and Proteus mirabilis were equal to that of the positive control, gentamicin. From Table 2, the bark of the plant had more antibacterial activity on Proteus mirabilis with the highest zone of inhibition of 28.0mm and ranged from 24mm - 28mm with the concentration range of 25mg/mL - 100mg/mL. Interestingly, the zone of inhibition for Proteus mirabilis at 100mg/mL of the extract was greater than that of gentamicin. *Pseudomonas aeruginosa* had the least zone of inhibition at 100mg/mL for both leaves and bark which were 23.0mm and 18.0mm respectively.

Furthermore, from the dermatophytes, only Candida albicans showed appreciable activity against the plant extract, it had a significant zone of inhibition of 25.0mm and 14.0mm at 100mg/mL for the leaves and stems bark respectively. A point of note from Table 3 & 4 was that the plant extract was not effective against Aspergillus flavus. The growth of *Protus mirabilis* was inhibited at 25mg/mL, 50mg/mL and 100mg/mL. Staphylococcus aureus and *Pseudomonas aeruginosa* were inhibited in the broth at 50mg/mL and 100mg/mL for the leaves and stems bark extracts as seen in Table 5 & 6 respectively. In addition, from Table 7 & 8, growth was inhibited at 50mg/mL and 100mg/mL for the leaves extract while it was inhibited at 100mg/mL only for the stems bark.

The result of the minimum bactericidal concentration showed that the leaves and bark were bactericidal at concentration of 50mg/mL and 100mg/mL for Proteus mirabilis, Staphylococcus aureus and *Pseudomonas aeruginosa* at only 100mg/mL as shown in Table 9 & 10 respectively. The minimum lethal concentration for the leaves extract was 50mg/mL for Candida albicans, also the minimum lethal concentration for the bark extract was 100mg/mL which killed Candida albicans as shown in Table 11 & 12.

Conclusion

It is reasonable from the result obtained to suggest that the plant extracts possess broad spectrum antimicrobial activity. The antimicrobial activity was more pronounced in the gram-negative Staphylococcus aureus, a gram-negative bacterium. The plant extract was also effective against the fungi Candida albicans.

Recommendation

We would like to suggest that further work should be carried out on each of these plants extract since it was basically investigated for activity against bacteria and fungi. This research could be extended to other classes of organisms.

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