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In vitro antimicrobial activity of stem bark extracts of frangipani (*Plumeria rubra*) obtained from Plateau State, Nigeria.

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ABSTRACT: The emergence of drug resistant strains has called the need for newer and novel antibiotics. More so, plants have shown promise as a source of diverse antibiotics. This research therefore reports the *in vitro* antibacterial activity of *Plumeria rubra* stem bark against *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Staphylococcus aureus*, *Aspergillus flavus*, and *Candida albicans*. The stem bark of *Plumeria rubra* L. (*Apocynaceae)* from Plateau State extracted successively with dichloromethane (DCM), ethyl acetate (EA), and methanol (MET) gave yields of 8.34%, 9.57% and 17.65% respectively. The phytochemical screening carried out on the crude extracts showed the presence of balsams, terpenoids, steroids, saponins, flavonoids, cardiac glycosides and resins. The three crude extracts demonstrated activity against the test microorganisms with zones of inhibition ranging from 10 to 28mm, minimum inhibitory concentration (MIC) and minimum bacteriocidal concentration (MBC) ranging from 25-100mg/ml. The potency of these extracts is in the order methanol (MET), ethyl acetate (EA) and dichloromethane (DCM). This confirms the traditional use of this plant for the treatment of bacterial infection; however, the researchers did not examine the cytotoxicity of this plant extract

Keywords: Stem bark, Plateau State, *In vitro*, Antimicrobial, Phytochemical.

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INTRODUCTION

Medicines from plants were in use since the beginning of the ages, with numerous successes. Such that today more than sixty percent of modern drugs are from natural sources (Newman and Cragg, 2007). More so, Farnsworth and Morison (1976) observed that plants are the goldmine of pharmaceutical companies.

Herbal medicine provides for the primary healthcare need of two-third of the population of the world. This is due to the high cost and inaccessibility of modern drugs. In addition to the emergence of drug resistant strains, this has led to the search for newer and more efficacious remedy to new and old diseases.

Plumeria rubra also called 'frangipani' in English and 'Rumand' in Hausa is a deciduous tree with milky sap that is a skin irritant. Its leaves are leathery and oval, alternatively arranged of about 30-50cm in length with white or pink flowers with yellow centers having five petals. Not much work is available on the plant that is readily available in our environment.

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However, Tohar *et al*., (2006) reported the hydro distillation of the oil of the flower of *Plumeria rubra*, *Plumeria obtuse*, and *Plumeria acuminate*. They reported the presence of a non-terpenoidal ester and/or acid as the major component of the oil. Whereas Egwaikhide *et al*., (2007) extracted the leaves and flowers with aqueous methanol using cold extraction, reported the presence of tannins, flavonoids, and reducing sugars, and carried out in vitro antimicrobial activity on fourteen pathogens. This research however examines the antimicrobial activity of the stem bark of *Plumeria rubra* extracted successively with dichloromethane, ethyl acetate, and methanol and its phytochemical contents.

METHODOLOGY

Collection of Sample/Sample Preparation

The stem bark of Plant was collected in the month of May, 2011 at the University of Jos Senior Staff Quarters along Bauchi Road Jos, Plateau State Nigeria. Mr. Arzila identified and authenticated the sample at the herbarium of the Federal College of Forestry, Jos, and Plateau State. The bark was washed, air-dried, pulverized and extracted successively with dichloromethane, ethyl acetate, and methanol using cold extraction method. Further filteration and the filtrate concentrated in vacuum.

Preliminary Phytochemical Screening

Crude extract of the three solvents was screened phytochemically using standard procedures (Harbone, 1984; Sofowora, 1984).

Antimicrobial Assay

The antimicrobial screening was carried using the agar disc diffusion method. Method was achieved by preparing nutrient agar plates then seeded for 24hours. Five well were bored on cork borer. Each of the wells were filled with 0.1ml of the different concentration of the crude extract of the three solvents extracted successively. While the fifth- well was filled with gentamicin 280mg/ml solution. Then plates were inoculated and incubated at 37^{o}C for 24hours without turning the plate. The measurements of the zones of inhibition were in millimeters.

RESULTS AND DISCUSSION

The preliminary phytochemical screening of the stem bark of *Plumeria rubra* showed the presence alkaloids balsam, cardiac glycosides, flavonoids, terpenes, steroids, and absence of resins and saponins for the dichloromethane crude extract. Whereas the ethyl acetate crude extract showed the presence of alkaloids, cardiac glycosides, terpenes and resins and the absence of balsam, flavonoids, saponins and tannins, Table 1.

In addition, the methanol crude extract showed the presence of alkaloids, cardiac glycosides, balsam, Flavonoids, saponins, and tannins.

More over, the bark of *Plumeria rubra* showed the presence of such primary metabolites as protein/amino acids and the absence of fats and oils for the dichloromethane and methanol crude extract. It was not determined for the ethyl acetate extract, Table 1. Proteins/amino acids, fats and oils are primary plant metabolites, since they form part of the plant cell membrane and are important for organ communication and division [Sofowora, 2008]

Nevertheless, the secondary metabolites are important for cell survival and defense. Egwaikhide *et al*., (2007) reported the presence of flavonoids, steroids, diterpenoids, cardiac glycoside, tannins, phlobatannins, saponins and reducing sugar in the crude methanol extract of *Plumeria rubra* leaves and flowers.

Table 1: Preliminary Phytochemical Screening of the Crude Extract of Stem Bark of *Plumeria rubra*

Analyte	DCM	EA	MET
Alkaloids	+	+	+
Balsam	+	-	+
Flavonoids	+	-	+
Cardiac glycosides	+	+	+
Proteins/amino acids	+	ND	-
Tannins	+	-	+
Saponins	-	-	+
Terpenes	+	+	+
Fats & oil	-	ND	-
Steroids	+	ND	+
Resins	-	+	ND
Phenols	-		ND

\+ = present - = absent ND = not determined.

Tables 2, 3 and 4 show the various zones of inhibition for the stem bark extract of *Plumeria rubra*. The zones of inhibition obtained for most of the concentrations are worthy of further research to isolate/identify the active principle(s), because some gave values greater than gentamicin at 280mg/ml.

The zones of inhibition gave a strong antibacterial effect for the methanol, dichloromethane and ethyl-acetate crude extracts, Table 2-4. However, the ethyl acetate crude extract gave an intermediary effect at 50mg/ml for *S. aureus* and *P. aeruginosa* Table 4. Further, the methanol extract also gave a middle of road effect on *S. aureus* at 25mg/ml. More so, Ibeh and Uraih (2003) indicated that zone of inhibition of <10mm as weak, <15mm as moderate and >16mm as strong. The values obtained are in close agreement with those of the methanol extract of the root bark of *Psorospermum corymbiferum* when tested against the same organs (Zubair *et al*., 2009). The Minimum Inhibitory Concentration (MIC) for the dichloromethane was found to be 25mg/ml for *P.*

Table 2: Zones of Inhibition for the Bark of *Plumeria rubra* for DCM in mm

Pathogens	100mg/ml	50mg/ml	25mg/ml	12.5mg/ml	6.25mg/ml	Gentamycin 280mg/ml
Staphylococcus aureus	28	25	21	-	-	38
Pseudomonas aeruginosa	28	24	20	-	-	38
Proteus mirabilis	24	21	-	-	-	22
Candida albicans	-	-	-	-	-	20
Aspergillus flavus	-	-	-	-	-	-

\- = no inhibition.

Table 3: Result Zones of Inhibition for the Methanol Extract Stem Bark of *P. rubra*

Pathogens	100mg/ml	50mg/ml	25mg/ml	12.5mg/ml	6.25mg/ml	Gentamycin 280mg/ml
S. aureus	20	19	16	14	-	24
P. aeruginosa	26	24	21	-	-	28
P. mirabilis	28	27	25	-	-	30
C. albicans	15	-	-	-	-	19
A. flavus	-	-	-	-	-	-

- = no inhibition.

Table 4: Zones of Inhibition for the Ethyl acetate Extract of Stem Bark of *P. rubra*

Pathogens	100mg/ml	50mg/ml	25mg/ml	12.5mg/ml	6.25mg/ml	Gentamycin 280mg/ml
S. aureus	20	15	-	-	-	27
P. aeruginosa	18	15	-	-	-	24
P. mirabilis	28	26	24	-	-	24
Candida albicans	14	10	15	-	-	15
A. flavus	-	-	-	-	-	-

- = no inhibition.

Mirabilis and *P. aeruginosa*, 50mg/ml for the *S. aureus* and 100mg/ml for *C. albicans* , Table 5. The MIC for the EA was obtained to be 50mg/ml for *S. aureus* and *P. aeruginosa* and 25mg/ml for the *P. Mirabilis* Table 5. This is good since *P. Mirabilis* is resistant to most antibiotics. However, the ethyl acetate crude extract inhibited the dermatophyte; *C. albicans* at 100mg/ml.

Moreover, the crude methanolic extract gave MIC of 25mg/ml for *P. Mirabilis* and *P. aeruginosa* and 50mg/ml for *S. aureus*. An MIC of 100mg/ml for *C. albicans* was obtained Table 5. Zubair *et al*.,(2009) obtained an MIC of 100mg/ml for *P. aeruginosa*, *S*. aureus and *P. Mirabilis* for the methanol and 100mg/ml for the ethyl acetate crude extract of the root bark of *Psorospermum corymbiferum*.

Furthermore, minimum bacteriocidal concentration (MBC) was also determined for these crude extracts. The MBC for the dichloromethane was found to be 50mg/ml for *S. aureus* and *P. aeruginosa* and 100 mg/ml for the *P. mirabilis*, Table 6. That for ethyl acetate was determined to be 50mg/ml for *P. mirabilis* and 100 mg/ml for the *P. aeruginosa*, *S. aureus* and *C. albicans* but ineffective for the *Aspergillus Flavus,* Table 6. Further, the methanol extract

indicated an MBC of 50 mg/ml for the *P. mirabilis* and *P. aeruginosa* and 100 mg/ml for the *S. aureus* and *C. albicans*, Table 6.

Meanwhile, Egwaikhide *et al.,* (2007) reported a zone of inhibition of between 12-28mm when they studied the crude methanol extract of the leaves and flowers of *Plumeria rubra*. They studied the anti-microbial effect of this plant against 14 microorganisms including *Corynebacterium pyogene*, *Bacillus anthracis*, *Streptococcus faecalis*, *S. aureus*, *P. aeruginosa*, *E coli* and reported the zone of inhibition of 23mm for the flower and 15mm for the leaves. They obtained a zone of inhibition of 21mm *for P. aeruginosa* for the flower and 14mm for the leaves and observed that the flowers had more inhibitory effect against the pathogens relative to the leaves. In addition, Dey *et al*., (2011) reported the antibacterial activity of the n-hexane fraction of the methanolic extract of the stem bark of *Plumeria rubra* L. They worked with *S. aureus*, *P. aeruginosa*, *E. Cloacae* and *Serratia marcescens* and reported a lower zone of inhibition from those of this research and also those of Egwaikhide *et al*., (2007). This might be because of the fact that they worked with a fraction of the methanol or the probability of the geographical location of the plant, which might affect the amount, and the type of constituents present in plants [Sofowora, 2008].

Overall, stem bark of *Plumeria rubra* has antimicrobial activity that promise to be of repute since this research showed a higher zone of inhibition then the well-known gentamicin. This research therefore confirms the use of this plant in traditional medicine for the treatment of bacterial infections.

Table 5: Minimum Inhibitory Concentration of Extracts of *P. rubra*

Pathogens	DCM (mg/ml)	EA (mg/ml)	MET (mg/ml)
S. aureus	25	50	50
P. aeruginosa	50	50	25
P. mirabilis	100	25	25
Candida albicans	0	100	100
Aspergillus flavus	0	0	0

0 = Resistance

Table 6: Minimum Bactericidal Concentration of the Stem Bark of *Plumeria rubra*

Pathogens	DCM (mg/ml)	EA (mg/ml)	MET (mg/ml)
S. aureus	**50**	**100**	**100**
P. aeruginosa	**50**	**100**	**50**
P. mirabilis	**100**	**50**	**50**
Candida albicans	**0**	**100**	**100**
Aspergillus flavus	**0**	**0**	**0**

0 = Resistance

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