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Preliminary Phytochemical and Anti-Bacterial Studies on the Leaf Extracts of *Plumeria Rubra Linn*

Lawal U., Egwaikhide P. A., and Longbap D.B.
Department of Chemical Sciences, Federal University Wukari. Nigeria,P.M.B 1020 Wukari
Taraba State. Nigeria
lawal@fuwukari.edu.ng

Abstract
Preliminary phytochemical screening and anti-bacterial activity of dried leaf extracts of *Plumeria rubra* using three solvent in the order of polarity (hexane, ethyl acetate and methanol) was investigated. The phytochemical screening performed on the crude extracts revealed that the three extracts contained saponins and steroids. Tannins in ethylacetate and methanol extracts. Cardiac glycosides in ethylacetate extract, phlobatannins, flavonoids, terpenes and reducing sugar in methanol extract. The crude extracts were tested for their anti-bacterial activity on some pathogenic bacteria. Almost all the crude extracts displayed higher inhibitory effects at the tested concentration (20mg/ml), against four species of Gram negative (*klesbsieva pneumonia*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Pseudomonas fluorescence*) and ten Gram positive (*Bacillus subtils, Staphlococcus aureus*, *Clostridium sporogenes*, *Staphlococcus epiderm*, *Bacillus stearothermophilus, Bacillus cereus*, *Bacillus anthracis*, *Streptococcus faecalis*, *Corynebacterium phyogenes*, and *Bacillus polymyxa*) bacterial strains; hexane and methanol extracts were the most active of the three extracts of *Plumeria rubra* leaf.
Keywords: Phytochemical screening, anti-bacterial activity, *Plumeria rubra*.

1.0 INTRODUCTION

Plumeria rubra is a deciduous plant species belonging to the family apocynaceae (Botanica, 2004). Originally native to Mexico, Central America, Colombia and Venezuela, it has been widely cultivated in subtropical and tropical climates worldwide. *Plumeria rubra* commonly known as Frangipani (temple tree) is well known for its intense fragrance and medicinal values (Raven *et al*, 1992). The flowers, root bark and leaves of *Plumeria rubra* are known for their therapeutic uses. Decoction of bark is used as purgative and antiherpetic. Root-bark is used as abortifacient, remedy for gonorrhoea and venereal sores. In Yucatan, latex is used for toothache. Decoction of flowers in Mexico is used for diabetes (Bailey, 1976). *Plumeria rubra* is used in ulcer, inflammation, bronchitis, fever (Baghel *at el*, 2010), diarrhoea (Basavaraju *et al*, 2009), rheumatism, cancer (Kuete and Efferth, 2011), snake bite (ethnoveterinary) (Deshmukh *et al*, 2011), infusion or extract from leaves is used for respiratory disorder (asthma) (Patil *et al*, 2008), A poultice of heated leaves is beneficial for swellings.
The emergence of antimicrobial resistant bacteria pathogens has become a major public health concern. The use of antimicrobials in any area including disease treatment can potentially lead to widespread dissemination of antimicrobial resistant bacteria. The increasing prevalence of antimicrobial drug-resistant bacteria is a major concern to human and vet nary medicine. Resistant bacteria include both pathogens and commensal organism, with the later serving as a potential reservoir for mobile resistant elements. Since the plant kingdom still holds many species of plants containing substances of medicinal values, which are yet to be discovered. *Plumeria rubra* is one of the plants which have been used in traditional medicine for many years. Therefore, this study is designed to test for the activities of the hexane, ethylacetate and methanol leaf extracts of *Plumeria rubra* against four species of Gram –ve and ten species of Gram +ve bacteria strains. The results of the preliminary phytochemical analysis will provide suggestions as to the probable secondary metabolites responsible for the activities of the extracts.

2.0 MATERIALS AND METHOD

2.1 PLANT SAMPLE

Mature leaves of *Plumeria rubra* were collected from cultivated trees growing in the premises of Kaduna state university, Kaduna. The leaf of *Plumeria rubra* was confirmed and authenticated at the herbarium of the Department of Biological Sciences, Kaduna state university.

2.2 PREPARATION OF PLANT MATERIAL AND EXTRACTION

The plant materials (leaves of *plumeria rubra*) were air dried under the shade for two weeks in the laboratory and was subsequently pulverized using a grinding machine to increase its surface area. The method of cold maceration was used in the extraction by serial extraction method. 400g of Powdered was soaked in 1500ml of hexane for 72 hours (three days). The extract was filtered and concentrated using a rotary evaporator at 40^{o}C in vacuo. The process was repeated on the same plant material for ethylacetate and methanol respectively. The various extracts were store in the refrigerator until when needed.

2.3 Phytochemical screening assay

Qualitative assay, for the presence of plant secondary metabolites such as tannins, phloba-tannins, saponin, flavonoid, steroids, terpenes, cardiac glycoside and reducing sugar were carried out on the crude leaf extracts in accordance with the methods described by Trease and Evans (1989), Harborne (1998), Ushie and Adamu (2010).

2.4 Anti Bacterial Sensitivity test

2.4.1 Culture media used

Nutrient agar (Biotec,Ltd) was used for sub-culturing the bacterial isolate. Diagnostic Sensitivity test agar (Lab m,Ltd) was used for the sensitivity tests. The media were sterilized in the Autoclave at 121^{o}c and 1.05kgcm^{2} for 15minutes.

2.4.2 Preparation of the microorganism used for the experiment

The bacterial isolates used for the experiment were incubated overnight into nutrient broth (Biotec, Ltd) and incubated for 18 hours at 35^{o}c. Cultures stored on slants were sub-cultured onto fresh slants every three months.

2.4.3 Antimicrobial sensitivity testing of the extracts against selected bacterial isolates.

The dilute extracts were tested for their antibacterial properties using the agar-welltechnique. The medium used was diagnostic sensitivity agar (LabM, Ltd). The assay for antibacterial activity was carried out with *klesbsieva pneumonia*, *Bacillus subtils, Staphlococcus aureus*, *Clostridium sporogenes*, *Escherichia coli*, *Staphlococcus epiderm*, *Pseudomonas fluorescence*, *Bacillus stearothermophilus, Bacillus cereus*, *Bacillus anthracis*, *Streptococcus faecalis*, *Corynebacterium phyogenes*, *Pseudomonas aeruginosa*, and *Bacillus polymyxa.*

3.0 RESULTS AND DISCUSSION

The result of the preliminary phytochemical screening of the hexane, ethyl acetate and methanol extracts of *Plumeria rubra* dried leaves has been summarized in Table 1.

Table1: Summary of phytochemical results on *Plumeria rubra* leaf Extracts.

Phytochemicals	Tannins	Phloba-tannins	Saponin	Flavonoid	Steroids	Terpene	Cardiac glycoside	Reducing sugar
Hexane	-	-	+	-	+	-		-
Ethyl acetate	+	-	+	-	+	-	+	-
Methanol	+	+	+	+	+	+	-	+

Key: + = Present
- = Absent

The results of the preliminary phytochemical screening of the hexane, ethylacetate and methanol crude extracts of *Plumeria rubra* leaf revealed the presence of saponnins, steroids in the three extracts, tannin in ethylacetate and methanol extracts, cardiac glycosides in ethylacetate extract, terpenes, flavonoids, phlobatannins and reducing sugar in methanol extract. From the result, methanol extracted more of the bioactive constituents. Tannins are known to be useful in the treatment of inflamed or ulcerated tissues and they have remarkable activity in cancer prevention and anticancer (Ruch et al, 1989; Li et al, 2003). Thus, *Plumeria rubra* containing this compound may serve as a potential source of bioactive compounds in the treatment of cancer.

Flavonoids serve as health promoting compound as a results of its anion radicals (Hausteen, 1983). These observations support the usefulness of this plant in folklore remedies in the treatment of stress-related ailments and as dressings for wounds. (Fergusion, 2001; Grierson and Afolayan, 1999).

Also, the plant extract was revealed to contain saponins, known to produce inhibitory effect on inflammation (Just et al, 1998) and are major ingredients in traditional Chinese medicine and thus responsible for most of the observed biological effects (Liu and Henkel, 2002), and this tend to justify the use of *Plumeria rubra* in traditional medicine. The plant extract was also positive for steroids which are very important compounds especially due to their relationship with compounds such as sex hormone (Okwu, 2001)

3.1 ANTIBACTERIAL SENSITIVITY TESTING

Comparative antibacterial sensitivity testing results of the hexane, ethyl acetate and methanol extracts of *Plumeria rubra* leaf against bacteria isolates are shown in Table 2.

Table2: Comparative antibacterial sensitivity testing of the hexane, ethylacetate and methanol extracts of Plumeria rubra leaf and Streptomycin against bacteria isolates

Microorganism	Zones of inhibition (mm)				
	Gram	PHLE (20mg/ml)	PELE (20mg/ml)	PMLE (20mg/ml)	STR (500mg/ml)
Corynebacterium pyogenese(L10)	+	21	12	0	19
Staphylococcus aureus(NCIB8588)	+	14	21	15	21
Bacillus cereus (NCIB6349)	+	17	14	18	ND
Bacillus polymyxa(L10)	+	20	15	14	15
Bacillus anthracis (L10)	+	0	14	0	20
Bacillus subtilis(NCI B3610)	+	17	15	14	22
Clostiridrum sporogenes(NCIB 523)	+	14	15	13	28
Klebsiella pneumonia (NCIB418)	-	15	17	15	0
Pseudomonas aeruginosa (NCIB)	-	12	16	12	ND
Escherichia coli (NCIB 86)	-	12	14	12	0
Pseudomonas fluorescence (NCIB3756)	-	0	12	12	ND
Streptococcus faecalis (NCIB755)	+	0	16	12	24
Bacillus stecrothermophilus(822)	+	25	18	20	23
Staphylococcus epidermidis	+	0	17	14	ND

PHLE-*Plumeria rubra* leaf hexane extract
PELE- *Plumeria rubra* leaf ethylacetate extract
PMLE3- *Plumeria rubra* leaf methanol extract
NCIB-National collection of Industrial Bacteria
LIO-Locally isolated organism
ND-Not determined
STR-Streptomycin.

The results for the antibacterial sensitivity testing (inhibition zones (mm)) for the extracts (at 20mg/ml), using streptomycin (500mg/ml) as antibiotic standard possess a wide range of anti-bacterial activity against both Gram negative (*klesbsieva pneumonia*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Pseudomonas fluorescence*) and Gram positive (*Bacillus subtils, Staphlococcus aureus*, *Clostridium sporogenes*, *Staphlococcus epiderm*, *Bacillus stearothermophilus, Bacillus cereus*, *Bacillus anthracis*, *Streptococcus faecalis*, *Corynebacterium phyogenes*, and *Bacillus polymyxa*) bacterial strains (Table 2). The inhibition zones value for the bacteria strains which were sensitive to the hexane, ethylacetate and methanol extracts were in the range of 12-25mm, 12-21mm and 12-20mm respectively. *Plumeria rubra* leaf extracts revealed that the hexane and methanol extract were the most active of the three extracts showing activity against three Gram-ve and seven Gram +ve bacterial strains for the hexane extract, and four Gram-ve and eight Gram +ve for the methanol extract with the largest zone of inhibition (25 mm) for hexane extract on *Bacillus stecrothermophilus* and (20mm) for methanol extract. Also hexane extract showed high inhibition of 21mm on *Corynebacterium pyogenes*.

The ethyl acetate extract also showed the activity against four Gram –ve and ten Gram +ve bacterial strains and had the highest zone of inhibition (21mm) on *Staphylococcus aureus*. Thus, antibacterial activity was high on *Bacillus strecrothermophilus* for all extracts.

4.0 CONCLUSION

The findings provides evidence that the crude leaf extracts of *Plumeria rubra* is a potential source of secondary

metabolites and the active plant extracts in this study showed significant antibacterial activities against the bacterial strains which justifies its use in certain folk practices in the treatment of different ailments.

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