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INVESTIGATING THE IMPLICATIONS OF ANTI-MICROBIAL AND ANTI-INFLAMMATORY PROPERTIES OF *PLUMERIA RUBRA* (FRANGIPANI) IN PREVENTING AND TREATING OF DISEASES IN LIVESTOCK FARMS.

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ABSTRACT

Plumeria rubra is small tree commonly known as White Champa Leaf. The leaves and flowers were evaluated for phyto-constituents, used in several traditional medicines to cure various diseases. The plant is mainly grown for its ornamental and fragrant flowers. Leaves arrangement is lanceolate to oblanceolate with flowers, fragrant in corymbose fascicles while the fruit is edible. Their medicinal properties are often due to their latex which is frequently drastic and corrosive. Latex is applied to ulcers, herpes and scabies. Seeds possess hemostatic properties. *Plumeria rubra* is also used as purgative, cardiotonic, diuretic and hypotensive. The medicinal value of *Plumeria* rubra is used in the treatment of a large number of human and livestock ailments. The zones of inhibition ranges from 10-28 mm 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*. Also, the ethyl acetate crude extract was effective against *Pseudomonas aeruginosa* which is resistant to most antimicrobial agents. The extracts were also effective against the fungi *Candida albicans*. The results of the study can provide suitable standards for the prevention and treatment of diseases in livestock farms.

KEYWORDS: *Plumeria rubra,* Antimicrobial, Anti-inflammatory, Leaves, Flowers, Inhibitory concentration; Bactericidal concentration; Lethal concentration

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INTRODUCTION

Plumeria rubra (Apocynaceae) is a small laticiferous tree, native to tropical America (Dubey *et al.,* 2014 and Radha, 2008). It is 4.5m high, occasionally grown for its ornamental and fragrant flowers. Leaves are lanceolate to oblanceolate while flowers have white fragrant which are in corymbose fascicles (Kumar *et al.,* 2012). The fruit is edible, latex is applied to ulcers, herpes and scabies while seeds possess haemostatic properties (Bawa *et al.,* 2019). Moreover its bark is bruised and applied as plaster over hard tumours (Kumar *et al.,* 2009). Uduji *et al.,* (2020) reported that the plant contains amyrinacetate mixtures of amyrin, ß- sitosterol, scopotetin, the iriddoids isoplumericin, plumieride, plumieride coumerate and plumieride coumerate glucoside.

The plant material is widely used as a purgative, febrifuge and remedy for diarrhoea and cure for itch. The leaves were reported to have analgesic-antipyretic, anti-inflammatory, and antioxidant properties. Odoemelam *et al.,* (2020) indicated that 10kg of Plumeria rubra leaf meal added to the diet of Hy-line brown birds favours hen day production, egg weight, shell weight and feed efficiency of the tested animals. Uduji *et al*., (2020) further reported that the appreciable level of fat in the *Plumeria rubra* flower meal based diets as additives might have accounted for the egg weight of birds fed these diets. The flowers were also reported to be useful as antioxidant and hypolipidemic (Sura *et al*., 2018).

Leaves are simple, arranged in a whorl, with prominent veins, crowded at the end of branches. In traditional medicinal system different parts of the plant have been mentioned to be useful in a variety of diseases (Dubey *et al.,* 2014 and Dhanapal *et al.,* 2018).

Flowers are of different kinds like white, reddish pink and bluish with fragrance (Rupali and Alka, 2014). The Pink flowers of *Plumeria* is due to phenolic compound and is found to be a good source of natural dye for cloth (Kalam *et al.,* 2014 and Reddy *et al.,* 1999). Root is bitter, carminative, and thermogenic (Ilyas *et al.,* 2016 and Sura *et al.,* 2018). Leaves are useful in inflammation, rheumatism, antibacterial, antifungal, bronchitis and antipyretic (Gunja *et al.,* 2017). Extract of leaves of *Plumeria rubra* (L.) showed significant antibacterial activity against *Streptoccocus. Epidermidis* and *Escherischia strains* (Singh, 2010). Methanolic extract showed antimicrobial activity against *Bacillus anthracis, Pseudomonas aeruginosa*. The plant is reported to contain amyrinacetate, mixture of *amyrins, ß-sitosterol, scopotetin, the iriddoids isoplumericin, plumieride, plumieride coumerate and plumieride coumerate glucoside* (Egwaikhide *et al.,* 2009 and Kumar *et al.,* 2011). Pod has abortifacient and hepatoprotective effects (Dawada, 2015 and Santhi, 2010). Bark is antinociceptive and anti-inflammatory. Leaves are found to have antiulcer activity, whereas flowers have

profound antioxidant effects (Ogunwande *et al.,* 2015). Flower of *Plumeria* was found to be a good source of natural dye for producing various green, ivory and brown shades on silk cloth.

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 veterinary 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 (Oladipupo *et al.,* 2015).

This study is therefore designed to investigate the implications of the anti-microbial and anti-inflammatory properties of true frangipani (*Plumeria rubra*) for the prevention and treatment of diseases in livestock farms, test for the activities of the hexane, ethyl acetate and methanol leaf and flower extracts of *Plumeria rubra* against four species of Gram negative and ten species of Gram positive bacteria strains. The results of the preliminary phytochemical analysis will provide suggestions as to the secondary metabolites responsible for the activities of the extracts.

MATERIALS AND METHODS

Collection and Authentication

Plumeria rubra leaves were collected, from in and around the botany garden of the Forestry Department, Imo State Polytechnic Umuagwo, Nigeria and authenticated by taxonomist and the plant authenticated specimen is deposited in the Department of Science Laboratory Technology of the institution. Authentication specimens of the fresh leaves were kept for shade drying. Dried specimen was powdered using mechanical grinder and passed through 60 mesh sieve to get the powder of desired coarseness. Powdered material was preserved in an air tight container.

Extraction of Plant material

For preliminary phytochemical analysis, extract was prepared by weighing 1kg of the dried powdered leaf was subjected to hot successive continuous extraction with different solvents as per the polarity, petroleum ether, benzene, chloroform, ethanol and aqueous. The extracts were filtered in each step using Whatman filters paper (Aggarwal and Paridhavi, 2007). The filtrate was concentrated using a rotary evaporator at low temperature (40-45^{0}C) and pressure. The presence or absence of the primary and secondary Phyto-constituents was detected by usual prescribed methods (Dhanapal *et al.,* 2018).

Preparation of Crude Extract

The flowers collected was dried under shade and then powdered with a mechanical grinder and stored in airtight container. The dried powder material of the flowers was defatted with n-hexane and allowed to dry. The product thus obtained was then extracted with methanol in a Soxhlet apparatus. The solvent was completely removed under reduced pressure and a semisolid mass was obtained.

Microorganisms

The two positive bacterial strains *Bacillus subtilis, Staphylococcus aureus* and two negative bacterial strains *Pseudomonas aeruginosa, Escherichia coli* including one fungal strain *Candida albicans* are collected for their antimicrobial testing from Department of Science Laboratory Technology, Imo State Polytechnic Umuagwo, Nigeria.

Antimicrobial assay

Disc diffusion method (Garba and Okeniyi, 2010) was used to test the antimicrobial activity of the extracts against four bacterial strains and one fungal strain (Table-5). Dried and sterilized filter paper discs (6 mm diameter) were then impregnated with known amount of the test substances dissolved in methanol (30 µg/ml) and water separately using micropipette and the residual solvents were completely evaporated (Doughari, 2006). Discs containing the test material with different concentrations each were placed on nutrient agar medium for bacterial strains and Sabouraud Dextrose Agar (SDA) for fungal strain uniformly seeded with the test microorganisms.

Negative controls were prepared using the same solvents as employed to obtain the extracts. As positive controls, Ciprofloxacin (10 µg/ml) was used for Gram-positive and Gram-negative bacteria and Fluconazole (10 µg/ml) for *Candida* spp. The inoculated plates were incubated at 37°C for 24 h for clinical bacterial strains and at 35°C for 48 h for fungal strain (yeast). The test materials having antimicrobial activity inhibited the growth of the microorganisms and a clear, distinct zone of inhibition was visualized surrounding the disc. The antimicrobial activity of the test agents was determined by measuring the diameter of zone of inhibition in millimeter.

Physico chemical features

The powdered drug was evaluated for its physico-chemical parameters like total ash values, acid insoluble ash, water soluble ash and loss on drying, and the results were tabulated (Table 1).

Table 1. Physico - Chemical Evaluation of the Crude Drug of Leaf of *Plumeria rubra.*

S/No	Physical Evaluation	%w/w
1.	Total Ash	6.03
2.	Acid Insoluble Ash	3.94
3.	Water Soluble Ash	2.42
4.	Loss on Drying	0.5

Table 2. Preliminary Phytochemical Tests for Drug Powder and Various Extracts of Leaf of *Plumeria rubra.*

S. No	Test	Drug Powder	Petroleum Ether Extract	Benzene Extract	Chloroform Extract	Ethanol Extract	Aqueous Extract
1.	Sterols	+	+	+	+	+	-
2.	Terpenoids	-	-	-	-	+	-
3.	Carbohydrates	+	-	-	-	+	+
4.	Flavonoids	+	-	-	-	+	+
5.	Proteins	+	-	-	-	+	+
6.	Alkaloids	+	-	-	-	+	+
7.	Glycosides	-	-	-	-	-	-
8.	Saponins	+	-	-	-	+	+
9.	Tannins	+	-	-	-	+	+
10.	Mucilages	+	-	-	-	+	+
11.	Volatile Oil	+	-	-	-	-	-

+ indicates positive reaction, -indicates negative reaction.

+ indicates positive reaction, -indicates negative reaction.

Table 3: Fluorescence analysis of leaf of *plumeria rubra.*

S. No	Sample	Colour in Day Light	Colour in UV Light
1.	Petroleum ether extracts	Pale green	Dark green
2.	Benzene Extract	Green	Light green
3.	Chloroform Extract	Brownish green	Green
4.	Ethanol Extract	Green	Dark Green
5.	Aqueous Extract	Brownish green	Yellowish green

Fluorescence analysis of the extracts

The extracts were prepared as per their polarity in hot successive extraction technique and they were treated with reagents and the colour changes were observed under Ultra Violet light and the results were tabulated (Table 3).

Table 4: Extractive values of leaf of *plumeria rubra* with different solvents.

S. No	Sample	Extractability (%)
1.	Petroleum ether extracts	9.5
2.	Benzene Extract	7.2
3.	Chloroform Extract	5.8
4.	Ethanol Extract	6.7
5.	Aqueous Extract	9.2

Extractive values

The extracts were prepared according to the polarity and they were concentrated and their values were calculated with reference to air dried drug and the results were tabulated (Table 4).

All the extracts of the drug was subjected to different tests for detecting the presence of various phyto-constituents present in the drug, which revealed the presence of sterols, flavonoids, alkaloids, saponins, proteins, carbohydrate, volatile oil and tannins. Preliminary phytochemical analysis indicated a high percentage of quercetin and flavonoids and this may be one of the reasons behind the sedative activity of the plant. These parameters, which are being reported for the first time in this plant, are significant towards establishing the Pharmacognostic standards for future identification and authentication of genuine plant material.

Table 5. Antimicrobial activity of *Plumeria rubra* bark Linn.

S.No	Sample Name	Organism	Concentration of Sample	Zone Diameter	Bore Size
1.	Methanol Extract	*Bacillus Subtilis*	10mcg\ml (Ciprofloxacin) 8000mcg\ml 4000mcg\ml 2000mcg\ml 1000mcg\ml	25.87mm 19.91mm 18.64mm 17.24mm 14.12mm	6mm 6mm 6mm 6mm 6mm
		Staphylococcus aureus	10mcg\ml (Ciprofloxacin) 8000mcg\ml 4000mcg\ml 2000mcg\ml 1000mcg\ml	28.33mm 23.11mm 19.58mm 16.56mm 12.71mm	6mm 6mm 6mm 6mm 6mm
		Pseudomonas aeruginosa	10mcg\ml (Ciprofloxacin) 8000mcg\ml 4000mcg\ml 2000mcg\ml 1000mcg\ml	28.75mm 24.14mm 22.08mm 18.36mm 15.47mm	6mm 6mm 6mm 6mm 6mm

		Escherichia Coli	10mcg\ml (Ciprofloxacin) 8000mcg\ml 4000mcg\ml 2000mcg\ml 1000mcg\ml	30.00mm 26.35mm 23.17mm 16.68mm 11.88mm	6mm 6mm 6mm 6mm 6mm
		Candida albicans	10mcg\ml (Ciprofloxacin) 8000mcg\ml 4000mcg\ml 2000mcg\ml 1000mcg\ml	17.36mm 11.23mm 10.41mm 9.15mm 8.58mm	6mm 6mm 6mm 6mm 6mm
2.	Water Extract	*Bacillus Subtilis*	10mcg\ml (Ciprofloxacin) 8000mcg\ml 4000mcg\ml 2000mcg\ml 1000mcg\ml	23.19mm 13.38mm 9.21mm Nil Nil	6mm 6mm 6mm 6mm 6mm
		Staphylococcus aureus	10mcg\ml (Ciprofloxacin) 8000mcg\ml 4000mcg\ml 2000mcg\ml 1000mcg\ml	25.91mm 9.22mm 8.61mm 8.01mm 7.38mm	6mm 6mm 6mm 6mm 6mm
		Pseudomonas aeruginosa	10mcg\ml (Ciprofloxacin) 8000mcg\ml 4000mcg\ml 2000mcg\ml 1000mcg\ml	24.5mm 7.88mm 7.01mm Nil Nil	6mm 6mm 6mm 6mm 6mm
		Escherichia Coli	10mcg\ml (Ciprofloxacin) 8000mcg\ml 4000mcg\ml 2000mcg\ml 1000mcg\ml	31.75mm 8.58mm 7.51mm 7.04mm Nil	6mm 6mm 6mm 6mm 6mm
		Candida albicans	10mcg\ml (Ciprofloxacin) 8000mcg\ml 4000mcg\ml 2000mcg\ml 1000mcg\ml	15.36mm 8.22mm 8.07mm 7.62mm 7.11mm	6mm 6mm 6mm 6mm 6mm

Kumar *et al.*, (2012).

Antimicrobial activities

These were done according to the disc diffusion method (Bawa *et al*., 2019). For the test, 100 mg of the crude extract of *Plumeria rubra* was accurately measured by the electronic balance and taken into vial. Then one ml of ethanol was added and triturated in uni-directional manner. Both gram positive and gram negative bacteria were used. The bacteria used for the anti-microbial activity of Ethanolic crude extract of the *Plumeria rubra were Escherichia coli, Salmonella typhi, Salmonella paratyphi, Shigella dysenteriae,*

Staphylococcus aureus, Streptococcus pyogenes. In this method, measured amount of the test samples were dissolved in definite volumes of solvent to give solutions of known concentration (µg/ml). Then sterile Matricel (BBL, Cookeville, USA) filter paper discs are impregnated with known amount of test substances using micropipette and dried. Standard antibiotic discs and discs on which the solvent used to dissolve the samples is adsorbed and dried were used as positive and negative controls respectively. These discs are then placed in petri-dishes (120 mm in diameter) containing a suitable agar medium seeded with the test organisms using sterile transfer loop for anti-microbial evaluation. The plates are then kept at 40◦C for facilitating maximum diffusion. The test material diffuses from the discs to the surrounding medium. The plates are then kept in an incubator for 18-24 hours to allow the growth of the microorganisms. The antibacterial activity of the test agent is determined by measuring the diameter of the zone of inhibition in term of millimeter. It is concluded that the plant possesses potent antimicrobial activity (Gupta *et al.,* 2008).

Anti-inflammatory and anthelmintic activities

The methanolic extract of *Plumeria rubra* exhibited significant anti-inflammatory activity on the tested experimental animal models. The extract (500 mg) exhibited maximum anti-inflammatory effect. Carrageenan-induced oedema has been commonly used as an experimental animal model for acute inflammation and is believed to be biphasic. The cotton pellet method is widely used to evaluate the proliferative components of the chronic inflammation. The results obtained in this study indicated that the methanol extract of *P. rubra* possess potent anti-inflammatory activity in both acute and chronic models (Gupta *et al.,* 2006).

The saponins extract was used for testing anti-inflammatory and anthelmintic activity of *P. rubra* leaves. The anti-inflammatory activity was evaluated by determining the reduction in carrageenan induced hind paw edema in albino mice. The result of the maximum dose of 200mg/kg *P. rubra* extract exhibited a significant reduction in the volume of inflammation. The anthelmintic effect of *P. rubra* extract of 25mg/ml concentration is comparable with that of the effect produced by reference standards piperazine citrate on Indian adult earthworms (*Pheretima posthuma*) (Kumar *et al.,* 2012). The chloroform and ethanolic extract of *P .rubra* leaves shows antiulcer activity in albino rats.

The results of the antimicrobial screening have been represented in Table 5. The zone of inhibition of methanol extract ranged from 11.88 mm to 26.35 mm. The highest inhibition zone 26.35 mm was formed by the methanol extract of *Plumeria rubra* against *Escherichia coli* at the highest concentration followed by *Pseudomonas aeruginosa, Staphylococcus aureus and Bacillus subtilis.* The zone of inhibition of water extract of *Plumeria rubra* Linn. was less than that of the methanol

extract and ranged from 7.01 mm to 13.38 mm. The highest inhibition zone 13.38 mm was formed by water extract of *Plumeria rubra* against *Bacillus subtilis.* The methanol extract that showed antibacterial activity against the pathogens was active in all the given concentration i.e. 8000, 4000, 2000 and 1000 µg/ml, whereas water extract showed activity at the concentration of 8000 and 4000 mcg/ml but the lower concentrations 2000 and 1000 µg/ml showed no activity against *Bacillus subtilis* and *Pseudomonas aeruginosa.*

Methanol extract as well as water extract of *Plumeria rubra* also showed a significant zone of inhibition against a fungal species, *Candida albicans* at all the given concentrations (Table 5). In vitro antibacterial activity of *Plumeria rubra* bark against Gram positive *Staphylococcus aureus, Bacillus subtilis,* Gram negative *Pseudomonas aeruginosa, Escherichia coli,* and fungal species *Candida albicans* was carried out and methanol extract showed significant results against pathogens than that of water extract of *Plumeria rubra* Linn.

The results of this investigation should be helpful in the further experiments on antimicrobial activity of *Plumeria rubra* bark. These studies confirms the potential of this plant but further more mechanistic work is essential to prove it as one of the specific antimicrobial plant.

DISCUSSION

All the extracts of the drug was subjected to different tests for detecting the presence of various phyto-constituents present in the drug, which revealed the presence of sterols, flavonoids, alkaloids, saponins, proteins, carbohydrate, volatile oil and tannins (Tables 2, 3 and 4). Preliminary phyto-chemical analysis indicated a high percentage of quercetin and flavonoids and this may be one of the reasons behind the sedative activity of the plant. These parameters, which are being reported for the first time in this plant, are significant towards establishing the Pharmacognostic standards for future identification and authentication of genuine plant material. Though *Plumeria rubra* is a temple tree, it is a highly reputed drug used for the prevention and treatment of diseases in animal agriculture.

The literature survey revealed that the various species of *Plumeria* is an important source of many pharmacologically and medicinally important chemicals such as *plumieride, isoplumeride, fluvoplumericin, irriod* glycoside and other various minor secondary metabolites. Study of pharmacological activities with different extracts obtained from different parts of the plant (Fig: 1, 2 and 3) with difference in vitro and in vivo model, which show that the compounds have beneficial effects against a number of diseases. The plant has been widely studied for its pharmacological activities and regarded as universal panacea in ethno - veterinary medicines and find its position as a versatile plant having a wide spectrum of medicinal activities. *Plumeria rubra appears* to have significant antimicrobial capacity resembling a broad spectrum

antibiotic against the common uro-gastro pathogenic *Escherichia coli*, one of the common bacteria with pathogenic strains and are relatively resistant towards synthetic drugs (Syakira and Brenda, 2010). As the global scenario is now changing towards the use of non-toxic plant products, development of modern drugs from *Plumeria* species should be emphasized.

Ursolic acid from the leaves, *plumeric* acid from the latex, leaves and *fulvoplumerin* from the bark of *P. rubra* possess local anesthetic, cardiotonic and bacteriostatic activities respectively. *P.rubra* containing *fulvoplumerin* acts as inhibitors of human immunodeficiency virus type 1 (HIV) *revereasetrancriptase*. Methanolic extract of *P.rubra* showed hepatoprotective action against paracetamol induced hepatic damage. Ethanolic extract of *Plumeria rubra*. (Apocynaceae) leaves and flowers were tested for antimicrobial activity against Gram-positive bacteria (*Bacillus subtilis, Enterococcus faecalis and Staphylococcus aureus*), Gram-negative bacteria (*Escherichia coli, Klebsiella Pneumonias, Pseudomonas aeruginosa, Salmonella typhimurium)* and fungi (*Aspergillus niger* and *candida albicans*) by disc diffusion method. The ethanol extract showed strong *in vitro* antimicrobial activity against *E. faecalis, B. subtilis, S. aureus, P. aeruginosa, S. typhimurium, A. niger* and *C. albicans* respectively (Rasool *et al.,* 2008).

Plumeria rubra extracts were evaluated for antimicrobial activity using cup plate method and minimum inhibitory concentration against *Escheria Coli*, *Bacillus subtilis, Staphylococcus aureus* and *Aspergillus niger*. It was observed that a methanol extract exhibited significant activity against bacterial strains. When compared with *Ciprofloxacin* as a standard, aqueous extract was active against fungal strains as compared with standard *Fluconazole* (Surendra *et al.,* 2012).

Methanolic extract of *P. rubra* leaves possesses significant antitumor activity against *Dalton lymphoma* ascites in mice result shows that methanolic extract of *P. alba* can significantly prolong the life span, reduce tumour volume and improve the hematological parameters of the host (mice) (Radha et al., 2008).

Fig 1 a: Plumeria rubra Flowers

Fig 1b: Plumeria rubra Plant Fig 1c: Plant displaying the Pods

Fig 2: Plumeria rubra flower meal.

Fig 3: Plumeria rubra leaf meal.

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.

In the present study various standardization parameters such as macroscopy, microscopy (histochemical and powder), physicochemical standards, preliminary phytochemical investigation, which are being reported for the first time in this plant could be helpful in authentication and preparation of a suitable monograph for the proper identification of *Plumeria rubra* for the future.

The prevention of oxidative damage to tissue could therefore be one of the mechanisms responsible for the anti-inflammatory effect shown by this plant. Confirmation of the anti-inflammatory activity in animal model further justifies the traditional use of this plant for inflammatory disorders. The ethno medical use of *P.rubra* as a useful remedy in inflammatory and arthritic disorders could possibly be because of its excellent anti-inflammatory and antioxidant potential.

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