

Potential Antibacterial Activity of *Indigofera Arrecta* Against Some Drug Resistant Strains of *Salmonella typhi* and Methicillin Resistant *Staphylococcus aureus*

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Abstract: The present study aimed to investigate the potential antibacterial activity of the aqueous, hexane, methanol and ethanol extracts of the plant *Indigofera arrecta* using the agar well diffusion method. The extracts were tested against methicillin-resistant *Staphylococcus aureus* (MRSA) strains and *Salmonella Typhi* strains. The plant showed the presence of phytochemical constituents such as tannins, saponins phenolic groups, flavonoids, triterpenes among others. The hexane extract showed the maximum inhibition activity against the tested bacterial strains when compared with the other extracts. No activity was found with aqueous extract for MRSA strains tested while low activity was observed for strains of *Salmonella Typhi*. The methanol and ethanol extracts showed moderate activity against the tested pathogens. The study suggests that leaves of *I. arrecta* possess components with antimicrobial properties which might be useful for production of natural antimicrobial drugs.

Key words: Antibiotics • Medicinal Plants • Treatment • Infections

INTRODUCTION

Indigofera species, belonging to the family *Papilionaceae*, have been used in traditional medicine for the treatment of a wide range of infections [1-3]. Several species in the family are promising anticancer therapy [4]. Leite *et al.* [5], reported antimicrobial activity against *Staphylococcus aureus*, *Trichophyton rubrum* and *Microsporum canis* of *Indigofera suffruticosa* when tested against five bacterial pathogens and seventeen fungal strains. The authors suggested that the leaves could be used in the treatment of skin infections caused by dermatophytes [5]. Musa *et al.* [6] also reported the antimicrobial activity of *I. conferta* against *S. aureus* and some other organisms. In a recent study, Natarajan *et al.* [7] reported that both aqueous and hexane extracts of *I. caerulea* exhibited wide spectrum of activity against all the tested bacterial strains including *E. coli*, *Klebsiella pneumoniae*, *Salmonella Typhi*, *Vibrio parahaemolyticus*, *Vibrio cholera* and *Bacillus subtilis*.

However, unlike the above mentioned species and other species like *I. aspalathoides*, *I. dendroides*, *I. oblongifolia*, *I. grandulosa*, *I. tinctoria*, *I. heterantha*, *I. uniflora*, *I. colutea*, *I. macrocalyx*, *I. nigritana* and *I. pulchra* which have been investigated to a large extent, there is paucity of report on the antibacterial activity of *Indigofera arrecta*.

I. arrecta, called “elujaja” in Yoruba and commonly known as Natal indigo is an erect, woody, large shrub that can grow up to 2 metres tall. The decoction of the leaves is reported to be used in herbal medicine to treat colic, diarrhea and dysentery [8]. The plant has been granted a patent use to relieve ulcer pain [9].

Salmonella species and methicillin resistant *Staphylococcus aureus* (MRSA) are a major concern worldwide and a public health problem in developing countries as agents of food-borne and nosocomial infections. *Salmonella Typhi* is of particular interest because most infections associated with it have been attributed to the consumption of poultry products. Moreover, MRSA is a major cause of nosocomial

infections and are difficult to treat because most strains are resistant to most of clinically available antibiotics. Cases of resistance to antibiotics by *Salmonella* Typhi also abound in literatures; hence natural products could provide alternative therapeutic agents. The main objective of this study was to investigate the antimicrobial activity of the aqueous, hexane, methanol and ethanol extracts of *I. arrecta* against some clinical strains of *Salmonella* Typhi and MRSA to ascertain the scientific basis for its use in treating infections associated with these organisms.

MATERIALS AND METHODS

Plant Material: The fresh and healthy leaves of *I. arrecta* were collected from Akinsola village, Eruwa, a part of southwestern Nigeria. The taxonomic identification of the plant was confirmed by both Mr. Ibrahim Lawal of the Department of Sustainable Forest, Forestry Research Institute of Nigeria, Ibadan and Dr. J.S. Ashidi of the Department of Plant Science and Applied Zoology, OlabisiOnabanjo University, Ago-Iwoye, Nigeria.

Extraction of Plant Material: The leaves were air-dried for two weeks; ground into fine powder using a mortar and pestle and then preserved in air-tight bottles for further studies. Twenty-five grams of the powdered leaves were then soaked separately in 250 mL of distilled water, hexane, methanol and ethanol contained in 500 mL sterile conical flasks. All the extracts were kept overnight in rotary shaker, filtered using Whatman No.1 filter paper and centrifuged at 5000rpm for 5 minutes. The filtrate was evaporated to dryness using Soxhlet apparatus. Each extract was preserved in a vial and kept at 4°C till use. The yield of each extract was calculated based on the initial plant material weight.

Bacteria Tested: The test organisms included three strains of *Salmonella* Typhi and four strains of methicillin resistant *Staphylococcus aureus* (MRSA). All the bacterial strains were obtained from Microbiology Laboratory, Department of Medical Microbiology, University College Hospital, Ibadan. The bacterial strains were grown in nutrient broth and maintained on tryptone soy agar slants at 4°C. The *Salmonella* Typhi strains were sensitive to ciprofloxacin and the MRSA strains were sensitive to gentamicin.

Preliminary Phytochemical analysis: Qualitative phytochemical studies of *I. arrecta* leaf powder were

carried out by the methods of Parekh *et al.* [10] and Tamilselvi *et al.* [11]. The plant extracts was assayed for the presence of tannins, saponins, alkaloids, glycosides, flavonoids, steroids, phenolic compounds and triterpenes.

Determination of Antibacterial Activity: Antibacterial activity of all the extracts was determined by the agar well diffusion method at four different concentrations *i.e.*, 100mg/mL, 75 mg/mL, 50 mg/mL and 25 mg/mL. Mueller-Hinton agar medium (Oxoid, UK) was used. Mueller-Hinton agar plates were swabbed with 24h. old broth using sterile cotton swabs. Using sterile cork-borer, wells (6mm wide) were made in each petri dish. The plant extracts and positive control drugs (gentamicin 10µg/mL and ciprofloxacin 5µg/mL for *S. aureus* and *Salmonella* Typhi respectively), were loaded into the wells using micropipette. 0.15mL of each concentration of extracts and drugs were loaded into the separate wells. All the plates were prepared in duplicates and were incubated at 37°C for 24 h. The diameter of the inhibition zones observed were measured and the mean values are presented.

RESULTS

The results of preliminary phytochemical analysis on the different solvent extracts of *I. arrecta* showed that the leaves contained tannins, flavonoids, alkaloids, saponins, phenolic groups, glycosides, steroids and triterpenes (Table 1).

The results of the antimicrobial activity of extracts revealed that hexane extract exhibited greater activity against the strains of MRSA and *Salmonella* Typhi. Activity was recorded at 100 mg/mL and 75 mg/mL for ethanol and methanol extracts and maximum activity was observed at highest concentration (100 mg/mL). No activity was found against MRSA strains by the aqueous extract of the plant (Table 2).

DISCUSSION

The phytochemical screening of the extracts of leaves of *I. arrecta* have shown the presence of many bioactive constituents including saponins alkaloids, phenolic groups among others which are classes of secondary metabolites possessing antimicrobial activities [12-14], hence these compounds may be responsible for the activity observed against the tested organisms. The antimicrobial activities of different species of *Indigofera* have already been reported, *I. grandulosa* [9], *I. aspalathoides* [11,15], *I. caerulea* [7], *I. longeracemosa* [16], *I. conferta* [6] and *I. uniflora* [17]. Bakasso *et al.* [18]

Table 1: Preliminary phytochemical screening of the extract of the leaves of *Indigofera arrecta*

Constituents	Extracts			
	Aqueous(cold water)	Hexane	Methanol	Ethanol
Tannins	-	+	+	+
Flavonoids	-	+	+	+
Alkaloids	-	+	+	+
Steroids	-	+	+	+
Glycosides	-	+	+	+
Triterpenes	-	+	+	+
Phenolic compounds	-	+	+	+
Saponins	-	+	+	+

+ = present - =absent

Table 2: Antibacterial activity of leaf extracts of *Indigofera arrecta* against MRSA and Salmonella Typhi strains

	Diameter of zone of inhibition (mm)																Contr.
	Organism Extract concentrations (mg/ml)																
	Aqueous (hot water)				Hexane				Methanol				Ethanol				
	100	75	50	25	100	75	50	25	100	75	50	25	100	75	50	25	
MRSA strain 1	-	-	-	-	24	22	18	16	22	18	14	10	14	14	12	10	18 g
strain 2	-	-	-	-	28	28	20	18	18	-	-	-	14	12	-	-	21 g
strain 3	-	-	-	-	24	20	16	16	16	16	-	-	15	12	-	-	21 g
strain 4	-	-	-	-	22	22	18	-	18	-	-	-	16	14	-	-	24 g
Salmonella Typhi																	
strain1	10	9	-	-	24	19	15	10	-	-	-	-	20	14	-	-	22 cf
strain2	10	-	-	-	21	15	12	10	18	16	-	-	16	-	-	-	21 cf
strain 3	16	15	15	-	30	24	20	18	14	14	14	-	12	10	-	-	21 cf

Contr. = positive control drugs used and diameter of inhibition

g=gentamicin

cf=ciprofloxacin

also reported the antioxidant activities of five *Indigofera* species from Burkina Faso which was attributed to the presence of high phenolic content.

In the present study, *I. arrecta in-vitro* assays showed some activity against MRSA and *Salmonella* Typhi. Mythili *et al.* [15] also reported some activity of methanol extract of *I. aspalathoides* against *Salmonella* Typhi in their study. However the zones of inhibition reported by them were lower than those recorded in the present study, possibly due to differences in the strain of organism and plant species used. The study of Natarajan *et al.* [7] also lend support to our findings since they reported the antibacterial activity of aqueous, hexane, chloroform and methanol extracts from leaves of *I. caerulea* against *Salmonella* Typhi and other bacterial pathogens. In a related study, but using a different species and part of *Indigofera*, *I.heterantha* roots, Taj UrRehman *et al.* [19] found no antibacterial activity but low antifungal activity.

A few workers have reported the efficacy of *Indigofera* on *S.aureus*. Selvakumar and Karunakaran [20] observed that *I. tinctoria* possess good antimicrobial activity against *S. aureus* at as low as 0.5 mg/mL concentration with sufficiently high zone of inhibition. This contrasts to our findings where activity was observed at a higher concentration. This may be due to the differences in the plant parts and strains of bacteria used in the separate studies. Rosy *et al.* [21] have also reported that the chloroform extract of *I. aspalathoides* Vahl. showed very promising antibacterial activity against *S. aureus* among other bacteria investigated in their study. The present findings support the need to identify the bioactive compounds of the *I. arrecta* for possible formulation of more potent natural antimicrobial drugs.

CONCLUSION

Since results indicate that the extracts possess bioactive substances even in their crude form, this provides the basis for the use of the plant in traditional medicine to treat various diseases.

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