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IN-VITRO 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 investigates the 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 some strains of methicillin- resistant *Staphylooccus aureus* (MRSA) and *Salmonella typhi*. 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 against all the strains of bacteria tested 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 *Indigofera arrecta* possess components with antimicrobial properties for production of natural antimicrobial drugs.

KEY WORDS: *Indigofera arrecta,* methicillin- resistant *Staphylooccus aureus* (MRSA) and *Salmonella typhi*

INTRODUCTION
Indigofera species, belonging to the family Papilionaceae, have been used in traditional medicine for the treatment of a wide range of infections (Mathabe *et al*., 2006; Singh *et al*., 2001; Chakrabarti *et al*., 2006). Several species in the family are promising anticancer therapy (Vieira *et al*., 2006). Leite *et al*. (2006), in a study on the antimicrobial activity of *Indigofera suffruticosa* against five bacterial pathogens and seventeen fungal strains reported some activity against *Staphylococcus aureus*, *Trichophyton rubrum* and *Microsporum canis*. The authors suggested that the leaves could be used in the treatment of skin infections caused by dermatophytes. Musa *et al*. (2008) also reported the antimicrobial activity of *I. conferta* against *S. aureus* and some other organisms. In a recent study, Natarajan *et al.* (2010) reported that both aqueous and hexane extracts of *I. caerulea* exhibited wide spectrum of activity against all the tested bacterial strains. However, unlike these species above 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*. The development of herbal products depend on local botanical flora. *I. arrecta*, called " elu aja" 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 (Orwa *et al*. 2009).The plant has been granted a patent for its use in the relieve of ulcer pain (Prabakaran *et al.*, 2011).

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. 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 literature, 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 the 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, Olabisi Onabanjo University, Ago-Iwoye, Nigeria.

Extraction of plant material

The leaves were air-dried for two weeks and ground into fine powder using a mortar and pestle and 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 of 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^{o}C before use. The yields 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 bacteria were grown in nutrient broth and maintained on tryptone soy agar slants at 4^{o}C.

Preliminary phytochemical analysis

Qualitative phytochemical studies of *I. arrecta* leaf powder were carried out by the methods of Parekh *et al*. (2006) and Tamilselvi *et al.* (2011). 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., 100 mg/ml, 75 mg/ml, 50 mg/ml and 25 mg/ml. Mueller-Hinton agar medium (Oxoid, UK) was used. A 24 h. old broth culture was swabbed using sterile cotton swab on the medium. Using sterile cork borer, wells (6mm wide) were made in each petri dish. The plant extracts and positive control drugs were loaded into the wells using micropipette. All the plates were prepared in duplicates and were incubated at 37oC 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 slvent 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 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 gpoups among others which are classes of secondary metabolites possessing antimicrobial activities (Cowan, 1999; Ogbolie *et al*., 2007; Owolabi *et al*., 2008), hence 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 (Prabakaran et al., 2011), I. aspalathoides (Tamilselvi et al., 2011; Mythili et al., 2011), I. caerulea (Natarajan et al., 2010), I. longeracemosa (Thangadurae et al., 2002), I. conferta (Musa et al., 2008) and I. uniflora (Sivagamy et al., 2012). Bakasso et al. (2008) also reported the antioxidant activities of five Indigofera species of Burkina Faso which was

attributed to the presence of high phenolic content. In the present study, I. arrecta in-vitro

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

Constituents	Extracts		
	Aqueous	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

Organism	Diameter of zone of inhibition (mm)															
	Extract concentrations (mg/ml)															
	Aqueous				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
strain 2	-	-	-	-	28	28	20	18	18	-	-	-	14	12	-	-
strain 3	-	-	-	-	24	20	16	16	16	16	-	-	15	12	-	-
strain 4	-	-	-	-	22	22	18	-	18	-	-	-	16	14	-	-
Salmonella typhi																
strain 1	10	9	-	-	24	19	15	10	-	-	-	-	20	14	-	-
strain 2	10	-	-	-	21	15	12	10	18	16	-	-	16	-	-	-
strain 3	16	15	15	-	30	24	20	18	14	14	14	-	12	10	-	-

assays showed some activity against MRSA and Salmonella typhi. Mythili et al. (2011) 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. The study of Natarajan et al (2010) 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, TajUrRehman et al. (2011) found no antibacterial activity but low antifungal activity.

A few workers have reported on the efficacy of Indigofera on Staphylococcus aureus. Selvakumar and Karunakaran (2010) 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 with 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. (2010) 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.

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