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Phytochemical and Antimicrobial Screening of *Indigofera conferta* GILLETT (Papilionaceae)

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Abstract: Antimicrobial activities of the crude methanol extract as well as the n-butanol and residual aqueous fractions from the aerial part of *Indigofera conferta* used in traditional medicine to treat infected wound were investigated using disc diffusion and broth dilution techniques. The extract and the fractions were tested against *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Candida albicans* using Ampiclox as standard antibiotic. The crude methanol extract and the aqueous fraction exhibited activity against all the organisms tested (zones of inhibition 16-34 and 14-31 mm, respectively). The n-butanol fraction showed activity on *Staphylococcus aureus*, *Bacillus subtilis* and *Pseudomonas aeruginosa* only (zones of inhibition 14-25 mm). Phytochemical screening on crude extract revealed the presence of tannins, flavonoids and steroids. This study showed that the leaves of *Indigofera conferta* contains active compounds and its antimicrobial activity justifies its use in traditional medicine.

Key words: Phytochemical, antimicrobial, *Indigofera conferta*

INTRODUCTION

Infectious disease is the number one cause of death world-wide, it accounts for approximately 50% of death in tropical countries (Iwu *et al*., 1999). This may be due to poverty and increasing incidence of multiple drug resistance. Bacterial resistance to almost all antibacterial agents has been reported (Truiti *et al*., 2003), this resistance is largely due to indiscriminate use of antimicrobial drugs commonly used in the treatment of infectious diseases (Afolayan and Aliero, 2006). Furthermore some antibiotics have serious undesirable side effects which limit their applications, so there is serious need to develop new antimicrobial agents that are very effective with minimal unwanted side effects and higher plants represent a potential source of novel antibiotic prototypes (Maureer-Grimes *et al*., 1996; Afolayan, 2003).

Indigofera conferta belongs to the family Papilionaceae, it is a grey branching herbs that can grow up-to 2 feet high and it is distributed throughout tropical region of West-Africa (Hutchinson and Dalziel, 1958). Ethnomedical information from the Hausa people of Northern-Nigeria revealed that the decoction of the leaves is used to treat sore feet (Personal communication). A survey of literature on *Indigofera conferta* revealed that there is no any report on phytochemical and antimicrobial studies of this plant, but broad spectrum antimicrobial activities of *Indigofera oblongifolia* leaves (Dahot, 1999)

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and that *Indigofera dendroides* (Esimone *et al*., 1999) have been reported. In this study the antimicrobial activities of the methanol extract as well as the n-butanol and residual aqueous fractions were investigated to ascertain the scientific basis for the use of this plant to treat sore feet.

MATERIALS AND METHODS

Plant Material

Indigofera conferta plant sample was collected from New Wuse village, Abuja-Nigeria in the month of October 2006 and was authenticated at the herbarium unit of Biological Sciences Department, Ahmadu Bello University Zaria-Nigeria, where a voucher specimen was preserved. The leaves were air-dried and made into powder using pestle and mortar.

Preparation of Extract and Fractions

The powdered air dried leaves (500 g) was extracted with 80% methanol using maceration. The extract was filtered and concentrated *in vacuo* to yield a residue referred to as the methanol extract (CME) with a yield of 82 g. The methanol extract (40 g) was suspended in water and filtered, the filtrate was partitioned exhaustively using n-butanol to give n-butanol fraction (NBF) 3.1 g and residual Aqueous Fraction (AF) 6 g.

Phytochemical Screening

The methanol extract was subjected to phytochemical screening for the presence of alkaloids, flavonoids, tannins, saponins and steroids using standard procedure (Silver *et al*., 1998).

Antimicrobial Assay

Microorganism Tested

The organisms used for this study include *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Candida albicans*. They were obtained from the Department of Medical Microbiology, Ahmadu Bello University Teaching Hospital, Zaria, Nigeria.

Antimicrobial Susceptibility Testing

Preliminary antimicrobial activities of the extract and the two solvent fractions were carried out using disc diffusion method as previously described by Nostro *et al*. (2000). The extract and fractions were tested at initial concentration of 150 μg $disc^{-1}$ with ampiclox a standard antibiotic at concentration of 75 μg $disc^{-1}$. The microorganisms were maintained on agar slant. The inocula were prepared by inoculating the test organisms in nutrient broth and incubating them for 24 h at 37°C for bacteria, while for incubated for 48 h. After incubation, the broth cultures were diluted to 1:1000 for the Gram-positive bacteria and 1:5000 for the Gram negative bacteria. One milliliter of the diluted cultures was inoculated into a sterile molten nutrient agar (45°C) and poured into sterile Petri dish. Similarly 1 mL of the diluted fungal suspension was poured into sterile saboraud dextrose agar plates and the excess sucked up with Pasteur pipette. These were swirled gently and allowed to solidify. The discs were gently placed on the solidified plates and the plates were incubated for 24 h at 37 and 25°C for bacteria and fungi, respectively. All tests were carried out in duplicate and the antimicrobial activity was expressed as the mean diameter of inhibition zones.

Minimum Inhibitory Concentration (MIC)

MIC was determined on the organisms that exhibited sensitivity against the tested organisms using broth dilution technique (Sidney *et al*., 1978; Vollokova *et al*., 2001).

Minimum Bactericidal Concentration

Minimum bactericidal concentrations were determined by assaying the test tube content resulting from MIC determinations. A loopful of the content of each tube was inoculated by streaking on a solidified nutrient agar plate and then incubated at 37°C for 24 h after which it was observed for microbial growth. The lowest concentration of the subculture with no growth was considered as minimum bactericidal concentration.

RESULTS AND DISCUSSION

The result of phytochemical screening of the methanol extract and the two solvent fractions revealed the presence of flavonoids and tannins while steroid was only detected in the methanol extract (Table 1). Flavonoids and tannins have been reported to posses antimicrobial activity (Cowan, 1999). The antimicrobial activity of flavonoids is due to their ability to complex with extracellular and soluble protein and to complex with bacterial cell wall while that of tannins may be related to their ability to inactivate microbial adhesions, enzymes and cell envelop proteins (Cowan, 1999). The methanol extract showed zone of inhibition of growth ranging from 16-34 mm against all the test organisms while the residual aqueous fraction produced zone of inhibition ranging from 14-31 mm against the same organisms and for n-butanol fraction, zones of inhibition ranging from 14-25 mm were recorded against *Ps. Aeruginosa*, *Bacillus subtilis* and *Staphylococcus aureus* (Table 2).

The crude methanol extract and residual aqueous fraction were found to have MIC values ranging from 4-6 mg mL^{-1} and MBC at 5-7 mg mL^{-1} while the n-butanol fraction had MIC values ranging from 5-6 mg mL^{-1} and MBC at 6-7 mg mL^{-1} (Table 3).

The extract and fractions showed strong activity against *S. aureus*, this organism is known to play significant role in skin diseases (Srinivasan *et al*., 2001), this indicate that the plant can be a source of compound that can be effective against skin infections. *Pseudomonas aeruginosa* and *Escherichia coli*, two organisms that cause infections that are very difficult to combat due to their multi-drug resistance (Salie *et al*., 1996; Afolayan and Aliero, 2006) were found to be susceptible to the methanol extract

Table 1: Phytochemical analysis of *Indigofera conferta* leaves

		Extract/ fractions		
Constituents	Test	CME	NBF	AQ
Alkaloids	Dragendoff	-	-	-
	Wagner's	-	-	-
	Meyer's	-	-	-
Flavonoids	Shinoda	+	+	+
	Sodium hydroxide	+	+	+
Saponins	Frothing	-	-	-
Tannins	Ferric chloride	+	+	+
	Lead acetate	+	+	+
Steroidal nucleus	Salkowski	+	-	-
	Liberman-Burchard	+	-	-

CME = Crude methanol extract, NBF = n-butanol fraction, AQ = Residual aqueous portion, + = Positive, - = Negative

Table 2: Results of sensitivity test of methanol extract and fractions against various organisms

	Zone of growth inhibition (mm)			
Test organisms	Methanol extract	Residual aqueous fraction	n-butanol fraction	Amplclox (5 mg mL^{-1})
S. aureus	30	31	22	20
B. subtilis	34	27	25	14
P. auregenosa	20	21	14	26
E. coli	18	20	0	16
C. albican	16	14	0	20

Table 3: MIC and MBC of crude extract and fractions (mg mL^{-1})

	Methanol extract		n-butanol fraction		Aqueous fraction	
Tested organisms	MIC	MBC	MIC	MBC	MIC	MBC
S. aureus	4	5	5	6	4	5
B. subtilis	4	5	6	7	5	6
P. aeruginosa	6	7	-	-	6	6
E. coli	5	6	-	-	5	6
C. albicans	-	-	-	-	6	7

- = Not determined

and residual aqueous fraction. This gives an indication that a compound can be obtained from the plant that can be used against infections caused by these organisms. It is important to note that the strong activity of methanol extract and the residual aqueous fraction against *Candida albicans* indicate that the plant can serve as a source of an antifungal agent and that antifungal compound can be isolated from the residual aqueous fraction.

The antimicrobial activities observed with the methanol extract, n- butanol and residual aqueous fractions suggest the presence of bioactive compound(s) which can serve as antimicrobial agent or lead compound for the synthesis of an effective and less toxic antimicrobial agents.

In conclusion, the study showed that the methanol extract, n-butanol and residual aqueous fractions from the leaves of *Indigofera conferta* have anti-microbial properties which explain the basis for its use in traditional medicine to treat sore feet.

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