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Antimicrobial activity screening of crude extract from leaves of *Cassia nigricans* Vahl.

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ABSTRACT

The antimicrobial activity of the methanol, ethyl acetate, chloroform and petroleum ether extracts of the leaves of *Cassia nigricans* Vahl was investigated against *Staphylococcus aureus*, *Streptococcus pyogenes*, *Corynebacterium pyogenes*, *Salmonella typhi*, *Escherichia coli* and *Pseudomonas aeroginosa*, using agar diffusion technique. Results showed that the extract were effective against all the test microorganisms. The minimum inhibitory concentrations of the extracts of methanol, ethyl acetate and chloroform were found to be 2 x 10^4 µg/ml, while the petroleum ether showed no inhibition. The results of the study provide scientific basis for the use of the plant extract in the treatment of wounds and skin diseases. It is concluded that the active principles possessing antimicrobial activity may be extracted from the leaves of *Cassia nigricans* Vahl by methanol, ethyl acetate and chloroform.

INTRODUCTION

Cassia species have been of keen interest in phytochemical and pharmacological research due to their excellent medicinal values. Different classes of natural products, possessing potent physiological and pharmacological activities have been isolated from *Cassia* species, and they include anthracene derivatives, flavonoids and polysaccharides. Some of these compounds have been shown to possess considerable antimicrobial activity [1,2]. *Cassia* species are well known in folk medicine for their laxative and purgative uses. They are also widely used for treating skin diseases such as ringworm, scabies, eczema and wounds [3, 4, 5]. The leaves and pods are normally used.

Cassia nigricans is a woody annual herb or undershrub up to 1.2 – 1.5 m high with small yellow flowers. It is widespread in India, Arabia, Northern Nigeria and other tropical African countries, especially in cultivated ground or old clearings by roadsides and open grassy areas [3, 6]. The leaves of *Cassia nigricans* are used for treating gastro-intestinal problems by traditional healers in Nigeria. It is also used for the treatment of peptic ulcer, diarrheoa, rheumatoid pains and stomach ache [7].

The phytochemical and cytotoxic screening of the plant have been carried out earlier and the four crude extracts showed strong cytotoxic activity. The extracts were found to be positive for carbohydrate, anthracene derivatives, cardiac and saponin glycosides as well as alkaloids [8].

The aim of the present study is to investigate the antimicrobial activity of the various extracts of the leaves of *Cassia nigricans* in different solvents.

MATERIALS AND METHODS

Plant Material

The leaves of *Cassia nigricans* were collected from Ja'ama village near Ahmadu Bello University (ABU) Dam, Zaria. The plant was identified at the Herbarium, Department of Biological Sciences, ABU, Zaria by Mallam Mohammed Musa and given Voucher Specimen No 613.

Extraction Procedure

The leaves (300 g) were air dried, powdered and exhaustively extracted by Soxlet extraction using each of the following solvents: Petroleum ether (60 – 80 ^{0}C), ethyl acetate, chloroform and methanol, respectively. Each extract was concentrated and evaporated to dryness on a rotary evaporator.

Test Microorganisms

Standard strains of *Staphylococcus aureus* ATCC 13709, *Corynebacterium pyogenes* ATCC 10242,

Table 1: Result of zone of inhibition of *Cassia nigricans* extracts against microorganisms

	Diameter of zone of inhibition (mm)			
Test organisms	CNME	CNET	CNCH	CNPE
Staphylococcus aureus	26	22	27	0
Streptococcus pyogenes	22	23	25	0
Corynebacterium pyogenes	21	18	24	4
Salmonella typhi	20	20	18	0
Escherichia coli	18	17	20	0
Pseudomonas aeruginosa	21	20	24	0

Table 2: Minimum inhibition concentration of *Cassia nigricans* Vahl extracts

Test Organism	Zone of Inhibition at different concentration of ***Cassia nigricans*** extract (ug/ml)																			
	CNME					CNET					CNCH					CNPE				
	$1x10^4$	$2x10^4$	$3x10^4$	$4x10^4$	$5x10^4$	$1x10^4$	$2x10^4$	$3x10^4$	$4x10^4$	$5x10^4$	$1x10^4$	$2x10^4$	$3x10^4$	$4x10^4$	$5x10^4$	$1x10^4$	$2x10^4$	$3x10^4$	$4x10^4$	$5x10^4$
S. aureus	-	o+	+	+	+	-	o+	+	+	+	+	o+	+	+	+	-	-	-	-	-
S. pyogenes	-	o+	+	+	+	-	o+	+	+	+	+	o+	+	+	+	-	-	-	-	-
C. pyogenes	-	o+	+	+	+	-	-	o+	+	+	+	o+	+	+	+	-	-	-	-	o+
S. typhi	-	o+	+	+	+	-	o+	+	+	+	+	-	-	+	+	-	-	-	-	-
E. coli	-	-	o+	+	+	-	-	o+	+	+	+	o+	+	+	+	-	-	-		-
P. aeruginosa	-	o+	+	+	+	-	o+	+	+	+	+	o+	+	+	+	-	-	-	-	-

Salmonella typhi ATCC 9184, *Escherichia coli* ATCC 10418, *Pseudomonas aeruginosa* NCTC 6750 and *Streptococcus pyogenes* (local strain), obtained from the Department of Medical Microbiology, ABU Teaching Hospital, Zaria were used for the study.

Antimicrobial Screening

The paper disc diffusion method was used to determine the antimicrobial activities of the crude extract of petroleum ether (CNPE), ethyl acetate (CNEA), chloroform extract, (CNCH) and methanol extract (CNME) using standard procedure [9, 10]. Solutions of varying concentrations ranging from 1.0 x 10^4 to 5.0 x 10^4 µg/ml were prepared for CNPE, CNET, CNCH and CNME. They were also prepared using the pure extruding solvent for each extract.

Nutrient agar was prepared, sterilized and used as the growth medium for the microorganisms. 20 ml of the sterilized medium was poured into each sterilized Petri dish, covered and allowed to solidify. This afforded what is known as plate of Mueller – Hinton Sensitivity Agar (Oxoid). The Oxoid plate was then seeded with he test microorganism by the spread plate technique and were left for about 30 min to dry. The sterilized paper discs were soaked in the prepared solutions of the extracts with varying concentrations and were dried at 50 ^{0}C. The dried paper disc was then planted on the nutrient agar seeded with the test microorganisms. The plates were incubated at 37 ^{0}C for 24 h, after which they were inspected for the zones of inhibition of growth. The zones of inhibition of growth produced by the minimum inhibitory concentrations (MICs) were determined and recorded. MIC is the lowest concentration that inhibited the visible growth of the microorganisms, expressed in µg/ml. A control experiment was also set up using only extruding solvent for each of the test organisms.

RESULTS AND DISCUSSION

Table 1 shows the results of the anti-microorganisms recorded in mm of the diametrical sections of the respective zones of inhibition for each crude extract. Table 2 shows the results of MICs. The results demonstrated that the CNME, CNET and CNCH had very high growth inhibitory effects on all the microorganisms. The MIC value for the CNME on *Staphylococcus aureus, Streptococcus pyogenes, Corynebacterium pyogenes, Salmonella typhi* and

Pseudomonas aeroginosa was found to be 2 x 10^4 µg/ml, while for *Escherichia coli* the value was 3 x 10^4 µg/ml. For CNET, the MIC was observed to be 2 x 10^4 µg/ml. against *Staphylococcus aureus, Streptococcus pyogenes, Salmonella typhi* and *Pseudomonas aeruginosa,* while for *Corynebacterium pyogenes* and *Escherichia coli* the MIC value was 3 x 10^4 µg/ml. The MIC for the CNCH was found to be 2 x 10^4 µg/ml for all the test microorganisms, except for *Salmonella typhi,* which was 3 x 10^4 µg/ml. The CNPE extract did not show any inhibition against the test microorganisms.

The results of the antimicrobial activity of the various crude extracts were in agreement with the uses of the extract of the leaves of *Cassia nigricans* in traditional medicine for the treatment of wounds, ulcers and skin diseases. The leaves of the plant appeared to be a potential source of broad spectrum antibiotics. Studies are in progress to purify and characterize the active principles in the leaves.

In conclusion, active principles possessing antimicrobial activity may be extracted from the leaves of *Cassia nigricans* Vahl by methanol, ethyl acetate and chloroform.

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