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Antimicrobial activity of ipolamiide isolated from the stem-bark of *Stereospermum kunthianum* Cham (Bignoniaceae)

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Abstract
Stereospermum kunthianum (Bignoniaceae) plant is used in traditional ethnomedicine in treating bronchitis, venereal diseases, diarrhoea and dysentery. It is also used in the treatment of ulcers, leprosy, skin eruptions, respiratory ailments and gastritis. In continuation of our study of the activity of *Stereospermum kunthianum*, we subjected the compound ipolamiide, previously isolated and characterized, to antimicrobial study. The antimicrobial activity of ipolamiide was studied using agar cup plate and broth dilution methods against the following clinical isolates; *Escherichia coli, Staphylococcus aureus, Methicillin resistant Staphylococcus aureus, Shigella dysenteriae, Klebsiella pneumoniae, Candida albicans, Candida krusei, Candida tropicalis and Pseudomonas aeruginosa*. The results of antimicrobial screening showed that all the organisms tested were susceptible to ipolamiide and was found to be active against *Staphylococcus aureus, Streptococcus pneumoniae, Klebsiella pneumoniae, Escherichia coli, Salmonella typhi* for the bacteria and *Candida krusei* for the fungi. It was found to be inactive against Methicillin resistant *Staphylococcus aureus* (MRSA), *Shigella dysenteriae* and *Candida albicans* and *Candida tropicalis.* The compound showed zone of inhibition ranging from 21–29mm against all the tested microorganisms except MRSA and *Shigella dysenteriae*, standard antibiotic drugs erythromycin and fluconazole at concentrations of 5µg/ml each showed zones of inhibition ranging from 32 – 41mm for erythromycin except the *Candidas* while Fluconazole showed zones of inhibition ranging from 32 – 35mm for the *Candidas*.

Keywords: *Stereospermum kunthianum;* Antibacterial activity; Paper disc diffusion; Phytochemical screening.

INTRODUCTION

Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects [1]. The research into plants with alleged folkoric use should therefore be viewed as a fruitful and logical research strategy in the research for new drugs [2]. *Stereospermum kunthianum* (It is called Pink Jakaranda) from a family Bignoniaceae is a medicinal plant widely used by the people of Sudan-Guinea savanna region of Africa as remedy against bronchitis, venereal diseases, diarrhoea and dysentery. It is also used in the treatment of ulcers, leprosy, skin eruptions, respiratory ailments and gastritis. It is a remedy against cough and also found useful in treatment of hypertension [3]. We have reported the antidiarrhoeal activity of the ethanol portion of the leaf extract of the *Stereospermum kunthianum* [4]. The bioassay-guided fractionation of root bark of extract of *Stereospermum kunthianum* led to

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the isolation of four novel naphthoquinones (Sterekunthals A and B, pyranokunthones A and B) together with the known naphthoquinone pinnatal [5]. One iridoid and two phenylpropanoids were isolated from the stem bark of *S. kunthianum* together with mixtures of β-sitosterol and β-sitosterol glucoside [6]. In this paper, we report the antimicrobial activity of the previously isolated and characterized compound ipolamiide (an iridiod), from acetone fraction of the crude methanol extract of the stem-bark extract of *Stereospermum kunthianum*. [7]

EXPERIMENTAL

Ipolamiide previously isolated, characterized and elucidated was obtained pure and crystalline. The test microorganisms were clinical isolates obtained from the Medical Microbiology Department, Ahmadu Bello University Teaching Hospital, Zaria, Nigeria. These organisms included; Methicillin-resistant *Staphylococcus aureus, Escherichia coli, Salmonella typhi, Staphylococcus aureus, Shigella dysenteriae, Klebsiella pneumoniae, Candida albicans, Candida krusei, Candida tropicalis and Pseudomonas aeruginosa* All the isolates were checked for purity and maintained in slant of nutrient agar for the bacteria and Saboraud dextrose agar for the fungi.

Standardization of inoculum. McFarland's turbidity standard scale number 0.5 was prepared. Turbid suspension of the microorganisms was made using 100ml of normal saline. Dilution of the microorganism in the normal saline was carried out continuously until the turbidity matched that of the McFarland's scale by visual comparison. At this point, the microorganisms have a density of 1.5×10^8cfu/ml. The diffusion method was the method used for the screening. Ipolamiide (0.005 mg) was weighed and dissolved in 10ml of DMSO to obtain a concentration of 50µg/ml. This was the initial concentration of ipolamiide used to check its antimicrobial activity.

Antimicrobial screening. Mueller Hinton agar was the medium used as growth medium for the test microbes. The medium was prepared according to the manufacturer's instructions, sterilised at 121^{o}C for 15min, and was poured into sterile Petri dishes, the medium was allowed to cooled and solidified. The sterilised medium was then seeded with 0.1ml of the standard inoculums of the test microbes, the inoculums was spread evenly over the surface of the medium by the use of a sterile swab. A well was cut at the center of each inoculated medium by the use of a standard cork borer of 6mm in diameters. 0.1ml of the solution of ipolamiide of the concentration of 50µg/ml was then introduced into each well on the inoculated medium. Incubation was made at 37^0C for 24h, after which the plates of the medium was observed for the zone of inhibition. The zone was measured using a transparent ruler and the result recorded in millimeters.

Minimum inhibitory concentration (MIC). The minimum inhibitory concentration of ipolamiide against the test microorganisms were determined using broth dilution technique [8, 9]. Nutrient broth was prepared according to manufacturer's instruction; 10mls of the broth was dispensed into screw-capped tubes. Mueller Hinton broth was prepared, 10ml was dispensed into test tubes and was sterilized at 121^0C for 15mins, and the broth was allowed to cool. Mc-Farland's turbidity standard scale number 0.5 was prepared to give turbid solution. Normal saline was prepared, 10ml was dispensed into sterile test tubes and the test microbes was inoculated and incubated at 37^0C for 6 h. Dilution of the microbes was carried out in the normal saline until the turbidity marched that of the Mc-farland's scale by visual comparison. At this point the test microbes has a concentration of about 1.5×10^8cfµ/ml. Two-fold serial dilution of the ipolamiide in

the sterilized broth was made, to obtained the concentrations of 50, 25, 12.5, 6.25 and 3.125 µg/ml. The initial concentration was obtained by dissolving 0.005mg of ipolamiide. 0.1 ml of the test microbes in the normal saline was then inoculated into different concentrations of ipolamiide. Incubation was made at 37^0C for 24 h, after which the test tubes of the broth was observed for turbidity (growth). The lowest concentration of the compound in the broth, which showed no turbidity, was recorded as the minimum inhibition concentration.

Minimum bactericidal/ fungicidal concentration (MBC/MFC). The minimum bactericidal concentration was determined using the method described by [10] by assaying test tubes content from MIC determination. A loopful of the content of each tube was inoculated by streaking on solidified agar plate and then incubated at 37^0C for 24 h for bacteria and for fungi respectively after which the plates were observed for microbial growth. The concentration of the sub-culture with no growth was considered as MBC/MFC. [10].

RESULTS

Antimicrobial study of ipolamiide. The antimicrobial activities of Ipolamiide is summarized in (Table 1). Ipolamiide was found to be active against *Staphylococcus aureus, Streptococcus pneumoniae, Klebsiella pneumoniae, Escherichia coli, and Salmonella typhi* for the bacteria and *Candida krusei* for the fungi. It was found to be inactive against *MRSA, Shigella dysenteriae* and *Candida albicans* and *Candida tropicalis.*

Table 1: Result of sensitivity test of microorganisms against ipolamiide

Test Organism	Ipolamiide	Erythromycin	Fluconazole
MRSA	R	S	R
Staphylococcus aureus	S	R	R
Strept. pneumoniae	S	R	R
Klebsiella pneumoniae	S	S	R
Escherichia coli	S	S	R
Shigella dysenteria	R	S	R
Salmonella typhi	S	S	R
Candida albicans	R	R	S
Candida krusei	S	R	S
Candida tropicalis	R	R	S

Key: S = Sensitive, R = Resistant

Table 2: Susceptibility results of the zone of inhibition of microorganisms against ipolamiide

Test organisms	Zone of inhibition (mm)		
	Ipolamiide	Erythromycin	Fluconazole
MRSA	0	37	0
Staphylococcus aureus	24	35	0
Strept. pneumoniae	29	0	0
Klebsiella pneumoniae	27	40	0
Escherichia coli.	22	35	0
Shigella dysenteriae	0	41	0
Salmonella typhi	24	32	0
Candida albicans	0	0	35
Candida krusei	21	0	34
Candida tropicalis	0	0	32

Table 3: Determination of MIC/MBC (µg/ml) of ipolamiide on the test organisms

Test organism	MIC	MBC
Staphylococcus aureus	12.5	25
Streptococcus pneumoniae	6.25	12.5
Klebsiella pneumoniae	6.25	25
Escherichia coli.	12.5	50
Salmonella typhi	12.5	25
Candida krusei	12.5	50

The compound showed zone of inhibition ranging from 21 – 29mm against all the tested microorganisms except *MRSA* and *Shigella dysenteriae*. Standard antibiotic drugs, erythromycin and fluconazole, at concentrations of 5µg/ml, each showed zones of inhibition ranging from 32 – 41mm for erythromycin except the *Candidas* while fluconazole showed zones of inhibition ranging from 32 – 35mm for the *Candidas* as shown in table 2.

DISCUSSION

The results of antimicrobial screening showed that all the organisms tested were susceptible to compound of Ipolamiide and was found to be active against *Staphylococcus aureus, Streptococcus pneumoniae, Klebsiella pneumoniae, Escherichia coli, and Salmonella typhi* for the bacteria and *Candida krusei* for the fungi. However, it was found to be inactive against MRSA, *Shigella dysenteriae* and *Candida albicans* and *Candida tropicalis.* The compound showed zone of inhibition ranging from 21–29 mm against all the tested microorganisms except *MRSA* and *Shigella dysenteriae*. Standard antibiotic drugs erythromycin and fluconazole, at concentrations of 5 µg/ml, each showed zones of inhibition ranging from 32-41 mm for erythromycin except the *Candidas* while fluconazole showed zones of inhibition ranging from 32-35 mm for the *Candidas*. The low MIC (6.25 µg/ml) of ipolamiide showed activities against the microorganisms which are associated with different type of infections which include urinary tract infections, notably *Staphylococcus aureus* and typhoid fever *Salmonella typhi. Staphylococcus aureus* is noted to be responsible for a wide range of diseases, such as pneumonia, skin and soft tissue infections, and diabetic foot infections [12]. Ipolamiide showed activity against *E. coli, which* is the common cause of urinary tract infection and accounts for approximately 90% of first urinary tract infection in young women [13].

The very low MIC and MBC (Table 3) of this compound indicates its potential as antimicrobial agent. It is important to note that, the activity of ipolamiide against *Candida krusei* indicates that the plant can serve as a source of an antifungal agent.

Conclusion. Based on the findings of the present study, the compound ipolamiide serves as a good antimicrobial as well as antifungal agent. The activity exhibited by the compound against the tested organisms that are associated with various infectious diseases, have provided scientific justification for the ethno medicinal uses of the plant in Zaria, Kaduna State, Nigeria.

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