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Antifungal activity of Nilobamate isolated from *Acacia nilotica* Willd

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Abstract

The seed-pod of *Acacia nilotica Willd* was selected on the basis of its popular use in the Hausa folk medicine to treat some fungal diseases such as foot and mouth cow diseases in northern Nigeria. Plant material was extracted with 95 % ethanol that provided ethanol extract. Ethanol extract was subjected to column chromatography followed by thin layer chromatography, which afforded a compound (AN-5). The test compound AN-5 showed significant and slightly better antifungal activity against *Aspergillus fumigatus* as compared to the standard drug ketoconazole. Spectroscopic analysis revealed AN-5 to be a carbamate derivative (octyl 2-hydroxy phenylcarbamate). Further studies are recommended for development of octyl 2-hydroxyphenylcarbamate (Nilobamate) as a new lead compound for better treatment of fungal infections.

Keywords: Seed-pod; ketoconazole; *Acacia nilotica; Aspergillus fumigatus*

Introduction

Acacia nilotica Willd (Mimosaceae) is widely used tree from Africa and the Indian subcontinent (Fagge and Greaves, 1990). Decoctions prepared from the stem-bark and fruits of the plant are used to combat dysentery, leprosy, pneumonia and meningitis in Guinea Bissau and Sudan (Watt and Breyer-Brandwij, 1962; Kokwano, 1977), while in Nigeria the seed-pod, gum and roots are used against skin diseases and malaria fever (Etkin, 1997). Water extracts of the stem-bark and fruits have demonstrated anti-bacterial and anti-malarial activities on Pseudomonas aeruginosa and the larvae of Culex mosquito respectively (El-hamidi, 1970; Taura *et al.,* 2004). Acetone and aqueous extracts of the stem-bark of *A. nilotica* have shown some inhibition on the enzyme prolygalacturanase and the yeast *Pyricularia oryzae* (Prasad and Gupta, 1967; Gupta and Bilgrami, 1970). Dichloromethane and methanol extracts of the leaves of plant have shown activity on *Trypanosoma brucei* and *Typanosoma rhodensiense* (Watt *et al.,* 1962). Seed-pod extracts of the plant have demonstrated antibacterial activity on some β-lactamase producing bacteria (Mbatchou and Adoum*,* 2011). Column chromatogr-

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aphed fractions of the seed-pod have demonstrated mortalities of *Artemia salina* larvae by Brine Shrimp Lethality Assay technique which is indicative of cytotoxic, pesticidal and pharmacological properties (Mbatchou and Adoum, 2011).

This research work is aimed at isolation and characterization of antifungal compounds from the seed-pod of *A. nilotica* and comparing their activity with ketoconzole (a standard drug) against *Aspergillus fumigatus and Candida albicans*.

Materials and methods

General procedures

Solvents (Torteil Chemical Limited, Kano, Kano state Nigeria) were redistilled before use. Column chromatography was carried out with silica gel (60-120 Merck, net surface area $500m^2$/g, pore volume 0.75 cm^3). Filter agent (celite) was mixed with the seed-pod extract of Acacia nilotica Willd before loading on the column. Thin layer chromatography (TLC.) was performed on 20 x 5 cm glass plates coated with silica gel (Merck, TLC grade, with gypsum binder and fluorescent indicator) of 0.5mm thickness. TLC bands were viewed under ultraviolet (UV) lamp (254-365 nm) and by exposure to iodine. The silica gel plates were prepared by coating slurry of silica gel with distilled water (1g: 3ml) on plates, and activating them at 120 °C for at least 24 hours before use. Agar diffusion method was employed to test sample (AN-5) obtained from the column against fungi. *Aspergillus fumigatus* and *Candida albicans* obtai-ned from Murtala Mohammed Hospital, Kano, were identified according to the method described by Cheesbrough (2000).

Plant Material

The seed-pod of A. nilotica Willd was collected at random from Worno Borno, about 6 km south of Azare town, Bauchi State of Nigeria. The plant was identified by Baba Ali and authenticated by B.S. Aliyu both of the Department of Biological Sciences, Bayero University Kano, Nigeria. The plant was air dried at room temperature and then milled.

Extraction and Isolation

The plant sample (500g) was soaked in 95% ethanol (1.5 L) for two weeks. The percolate was evaporated to dryness at 40°C with the aid of a rotary evaporator (R110). A brown solid residue (F_{001}) of 57.98 g was obtained. A slurry of 1kg of silica gel prepared with petroleum ether was packed on a column (38'' length, 2''id), and then washed five times with petroleum ether and chloroform to remove any oily materials present. The washed silica gel was allowed to dry for 4 days. 25.0g of the ethanol extract (F_{001}) of seed-pod of *Acacia nilotica Willd* was mixed with equal portions of celite and silica gel until a homogenous solid mixture was formed. The sample mixture was then loaded on the column that has been packed with the washed silica gel. An additional portion of silica gel (10.0g) was added to form a protective layer on top of the adsorbent. The column was then eluted in the following order with petroleum ether (1.5 liters), petroleum ether/chloroform (1:1, 1.5 liters), chloroform (1.5 liters), chloroform/ethyl acetate (1:1, 1.5 liters), ethyl acetate (1.5 liters), ethyl acetate/methanol (1:1, 1.5 liters) and methanol (1.5 liters) solvents. Eluents were collected in 250 ml porti-

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Table 1. Column Chromatography Fractionation of the ethanol extract (F_{001}) of *A. nilotica.*

Column Eluents	Single or Pooled portions	Pooled Fraction	Weight (g)	% Recovery
100 % Petroleum ether	1, 2, 3 & 4	-	-	-
Petroleum ether/CHCl3	-	-	-	-
(1:1)	-	-	-	-
100 % CHCl3	6	-	-	-
CHCl3/EtOAc (1:1)	7	AN-1	0.006	0.0024
"	8	AN-2	0.0077	0.0308
"	9	-	-	-
"	10	AN-3	0.1018	0.4072
"	11	AN-4	2.0793	8.3172
"	12	AN-5	2.2891	9.1584
"	13	AN-6	2.2277	8.9108
"	14	AN-7	2.2896	9.1584
100 % EtOAc	15 & 16	AN-8	2.4749	9.8996
"	17, 18 & 19	AN-9	2.2325	8.93
EtOAc/MeOH (1 : 1)	20	AN-10	0.1728	0.6912
,,	21 & 22	AN-11	0.0945	0.378
"	23	AN-12	0.1568	0.6272
"	24	AN-13	0.4621	1.8484
,,	25	AN-14	0.9765	3.906
100 % MeOH	26, 27 & 28	AN-15	2.8798	11.5172
,	29	AN-16	0.1991	0.7964
,,	30 & 31	AN-17	4.1325	16.53

ons, with each portion separately concentrated using rotary evaporator at 40 degrees celcius and analyzed on TLC plates. Similar portions of eluents were combined on the basis of their TLC patterns (Table 1). Fractionation process provided 17 sub-fractions (AN1 to AN17). Fraction AN-5 was further subjected to spectroscopic analysis (Table 1), which resulted in structural determination of the isolated compound.

Antifungal activity

Filter paper discs (Whatman no. 1) of 5mm diameter were produced using a paper punch. They were sterilized in an oven (Compestat Brit) at 160 °C for an hour (Andersom, 1970). Sensitivity discs of column chromatographed fraction AN-5 were prepared by dissolving 0.10, 0.20, 0.30, 0.40 and 0.50g of it in 1ml portions of sterile distilled water. From each suspension, 0.1 ml was transferred to a vial containing 20 filter paper discs to give concentrations of 500, 1,000, 1,500, 2,000 and 2,500 µg/disc. Antifungal discs of ketoconazole were also prepared in the same way. The impregnated discs were dried using oven at 45°C and stored in closed vials in a deep freezer prior to use.

Agar diffusion method used in the experiment was adapted from the proposed methods of Bauer *et al.,* (1966) and Matsen (1979). However, malt extract agar was used instead of Mueller-Hinton medium. Plates were inoculated by introducing 0.10ml of an overnight nutrient broth (Oxoid) culture of the test organism into malt extract agar contained in a sterile Petri dish, and seeded agar plates were swirled clockwise and counter clockwise before being allowed to set a gel at room temperature. Impregnated discs (five per plate) were firmly pressed to the agar surface. The plates were then incubated at 26°C for 4-7 days. Diameters of zones of inhibition were measured manually in millimeters for each disk (Table 2).

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Table 2. Antifungal activity of Nilobamate and Ketoconazole against *A. fumigatus* and *C. albicans.*

Fungi	Treatment	Concentration (µg/disc)				
		500	1000	1500	2000	2500
A. fumigates	Nilobamate	9	10	10.20	10.30	10.40
	Ketoconazole	0	0	9	10	10.10
C. albicans	Nilobamate	0	0	0	0	0
	Ketoconazole	10.30	15.30	15.60	20.40	20.60

Table 3. ^{1}H (400 MHz, $CDCl_3$) and ^{13}C (100 MHz, EtOH) NMR data of the isolated compound from the seed-pod of *A. nilotica.*

Position	^{1}H δH (ppm)	J value(Hz)	^{13}C δC (ppm)
1'	-		145.6
2'	7.72, d		131 CH
3'	7.21, t		124 CH
4'	7.16, t		123 CH
5'	7.62, d		128 CH
6'	-		145.3
1	-		138
2	4.3-4.4, t		39 CH_2
3	4.3-4.4, t		30 Hz
4	4.3-4.4, t		29 CH_2
5	4.3-4.4, t		24 CH_2
6	4.3-4.4, t	7.2	23 CH_2
7	4.3-4.4, t		17 CH_2
8	3.3-3.7, sextet		16CH_2
9	2.0-2.2, t		11 CH_3
O-H	5.3, s		
N-H	2.8, s		

Results and Discussion

Phytochemical studies provided the isolated compound from AN-5 subfraction. Molecular formula of the compound was determined to be $C_{15}H_{23}NO_3$. According to absorption frequencies in IR spectroscopy revealed is a broad band of O-H at 3372.79cm-1 which occured more to the right and the presence of ester is indicated by $v(\mathrm{CO})$ 1705.97 cm^{-1}. The compound is aromatic, $v(\mathrm{C{=}C})$ 1628.64cm^{-1}, $v(\mathrm{CH})$ 2943.20cm^{-1} and the absence of some bands suggests no conjugation of ester carbonyl with the ring. Aliphatic absorption $v(\mathrm{CH})$ 1456.8cm^{-1} were also shown. The aromatic ring was detected via its further indication in ^{1}H NMR: AB quartet at δ 7.68. The singlet at δ 8.10 must be due to OH proton of the phe-nol. The aromatic pattern in the ^{1}H NMR is too complex for analysis. The ^{1}H NMR of comp-ound AN-5 indicated a triplet at δ 2.1 for the methyl protons of C-8, singlet at δ2.8 for the nitrogen proton, sextet at δ3.65 for the methylene protons of C-9 and C-7. The quintet at δ4.26 is as a result of C-1 and C-3 protons. The triplet of methylene protons at δ4.302 is due to C-2 protons, whereas the triplets at δ7.21 and δ7.6 are due to C-2$^{/}$, C-4$^{/}$, C-3$^{/}$, and carbon 5$^{/}$ protons respectively. At δ5.3 a pronounced singlet of OH proton of a phenol (4.0-7.0 ppm) is observed. The summary of ^{1}H and ^{13}C NMR spectral data (Table 3) further supported that compound (AN-5) is a carbamic acid derivative, octyl 2–hydroxylphenylcabamate (Figure 1). Finally, fraction AN-5 isolated from the seed pods of *Acacia nilotica Willd* was characterized as octyl 2-hydroxyph-enylcarbamate (nilocarbamate).

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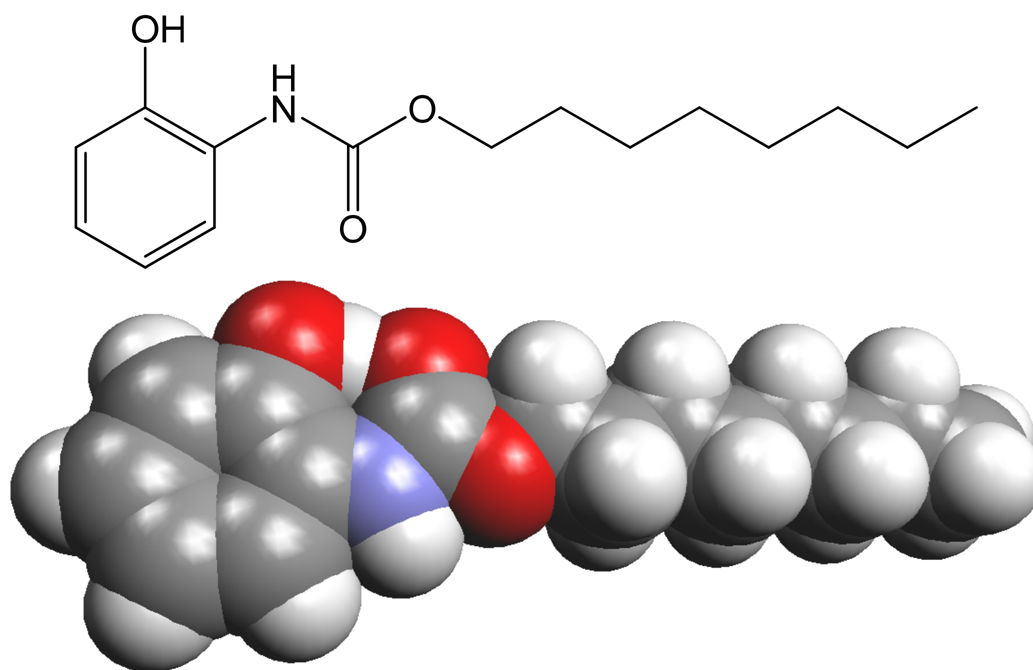

Figure 1. Chemical structure of octyl 2-hydroxyph-enylcarbamate (nilocarbamate).

Nilobamate was evaluated for antifungal activity against two fungal strains (*A. fumigatus* and *C. albicans*), according to protocols described by Bauer *et al.,* (1966) and Matsen (1979). Experimental results showed significant activity against *A. nilotica* in comparison to *Ketoconazole* (standard drug)in all tested concentrations. However, Ketoconazole (standard drug) exhibited growth inhibition only at concentrations higher than 1500 µg/disc (Table 2). Apart from *A. fumigatus, Candida albicans* was found to be non-susceptible or resistant to nilobamate but highly sensitive to Ketoconazole at the tested concentrations. Preliminary investigations revealed significant potential of nilobamate to be further developed as a new lead compound against fungal infections.

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Conflict of interest

The author declares that there is no conflict of any competing interest.

References

Bauer AW, Kirby WMM, Sherris JC, Turck M. (1966). Antibiotic susceptibility testing by a standardized single disc method. *American Journal of Clinical Pathology 45, 493-496.*

Cheesbrough M. (2000). District laboratory practice in tropical countries(part 2). Cambridge University press, Cambridge, pp. 235-238.

El-hamidi A. (1970). Drug plants of the Sudan Republic in native medicine. *Planta medica, 18: 278.*

Etkin NL. (1997). Antimalarial drugs used by Hausa in northern Nigeria. *Nig. Top. Doc. 27, 21-24.*

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Fagge CW, Greaves A. (1990). *Acacia nilotica* 1869-1988. Annotated bibliography. CAB *Int. F. 42, 1-81.*

Gupta SC, Bilgrami RS. (1970). Inhibitory effect of some decoctions on the production and activity of cellulolytic enzyme of three pathogenic fungi. *Proceedings of the National Academy of Sciences of India* 40, 6-8.

Kokwano JO. (1977). Medical plants of East African literature bureau. Nairobi press.

Matsen JM. (1979). Bacterial susceptibility testing and assays. In: Clinical diagnosis and management by laboratory methods(ed). Henry, J.B. 16th ed. Vol. 2 W.B. Saunders and Co., Philadelphia. pp. 190-1938.

Mbatchou VC, Adoum OA. (2011). Antibacterial activities of seed-pod extract of *Acacia nilotica Willd* on *Artemia salina* larvae. *J. of Applied Biosciences, 40: 2738-2745.*

Prasad V, Gupta SC.1967. Inhibitory effect of pectic enzymes of *Alternaria tenius. Indian Journal of .Experimental Biology.*5, 192.

Taura DW, Mukhtar MD, Adoum OA. (2004). Lethality of the aqueous extracts of *Acacia nilotica, Gueira senegalensis, Kigelia Africana* and *Securidaca longepedunculata on Culex mosquito* larva. *Journal of Sciences* 6, *115-118.*

Watt JM, Breyer-Brandwij MG. (1962). The medicinal and poisonous plants of Southern and Eastern Africa. 2nd edition, Liningstone Ltd, London. pp.12-34.

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