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Original Research

Antimicrobial Activities of the Alkaloidal Fractions of the Leaves of *Combretum zenkeri* Engl. & Diels (Combretaceae)

AJIBESIN, K.K. *, EKPO, B.A.J., BALA, D.N.
Department of Pharmacognosy and Traditional Medicine, Faculty of Pharmacy, University of Uyo, Uyo.

* correspondence

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The leaves of *Combretum zenkeri* are used in traditional medicine to treat skin diseases and other microbial forms of infection. The ethanol extract and the alkaloid fractions were evaluated for *in vitro* antimicrobial activities by agar-diffusion method to validate the ethnobotanical uses of the plant. Although the ethanol extract evoked reasonable activity against majority of the test organisms, the alkaloid fractions represented an improvement on the antimicrobial activity. The column alkaloid fraction coded C was the most active, while fraction E was the least. *Klebsiella aerogenes* gave the highest resistance, while *Aspergillus niger* showed the highest susceptibility. Alkaloid was found to be one of the bioactive compounds responsible for the antimicrobial activity of the plant.

Keywords: *Combretum zenkeri*, antimicrobial activity, ethanol extract, alkaloids.

INTRODUCTION

The genus *Combretum* comprises about 57 species [1]. *Combretum zenkeri* Engl. & Diels (Combretaceae) is a climbing shrub or scandent forest growing to 27m high in the savanna. It is widely distributed in Guinea, Cameroun and Southern Nigeria [2]. Some of these species are well known for their use in traditional medicine where they are used to treat various diseases, including microbial infections [3 - 5].

The leaves of *Combretum zenkeri* are used to treat skin diseases in Akwa Ibom State, while the Igbos use the leaves in worm infestation [5]. The Baule tribe of Central African Republic also use the leaves to cure male sterility [6]. The roots, together with that of *Aframomum melegueta*, are made into suppository to cure dysentery [7]. Some of the species of the genus *Combretum* have been reported to exhibit antimicrobial property [8 - 10].

This study aims at validating the ethnobotanical use of the leaves of *Combretum zenkeri* in the treatment of microbial diseases. It also aims at identifying the constituents responsible for such activity.

MATERIALS AND METHODS

Plant collection and authentication. The leaves of *Combretum zenkeri* were collected in June, 2003, in Ikot Ekpene, Akwa Ibom State of Nigeria.The plant was identified by Dr. (Mrs.) Essiett, a plant taxonomist in the Department of Botany, University of Uyo, Uyo. Voucher specimens (KKA 415) were deposited at the herbarium of the Department of Pharmacognosy and Traditional Medicine, Faculty of Pharmacy, University of Uyo, Uyo.

Extraction and fractionation. The leaves of the plant were air-dried at room temperature and powdered. The leaf powder (1 kg) was exhaustively extracted cold with 50 % ethanol. The liquid extract was filtered and concentrated *in vacuo* at 40 ^{0}C to dryness, to yield 42 g of the dry extract. Phytochemical screening was carried out on the dry ethanol extract to reveal the presence of secondary metabolites [11, 12].

Acid-base partitioning was carried out by dissolving the dry crude ethanol extract in 5 % HCL and shaken with $CHCl_3$. The aqueous fraction was neutralized with NH_4OH and shaken with $CHCl_3$ to obtain crude alkaloidal fraction. The alkaloidal fraction (20 g) was purified by chromatographing it on silica gel column (60 – 120 mesh size, 23 x 75 cm) and eluted with a gradient of n-hexane with ethyl acetate (9:1); ethyl acetate with methanol (9:1). The fractions collected in 25 ml test-tubes were pooled together on the basis of their TLC characteristics (silica gel G254, EtOAc:MeOH 4-1) under the UV (λ 366 nm) and with

the Dragendorff's spray reagent. Five pooled fractions coded A (4 g), B (3.5 g), C (4 g), D (2 g), E (3.2 g) were obtained.

ANTIMICROBIAL ASSAY

Microorganisms

The bacteria and the fungi used were clinical isolates sourced from St. Luke's Hospital stock culture unit, Anua, Akwa Ibom. The bacteria- *Staphylococcus aureus*, *Klebsiella aerogenes*, *Escherichia coli* and *Proteus mirabilis*- were sustained on blood agar slant at 4 ^{0}C before use, while fungi: *Aspergillus niger*, *Trichophyton mentagrophytes* and *Candida albicans* were maintained on Sabouraud's dextrose agar (Oxoid) slants at 4 ^{0}C prior to use for antimicrobial susceptibility testing.

Antimicrobial susceptibility testing

The dry crude ethanol extract and the fractions were separately redissolved in 50 % MeOH to obtain concentrations of 40 mg/ml and 80 mg/ml. The solutions of ethanol extract and fractions (150 μl) of these concentrations were separately added into equidistant wells (9 mm) bored on the surface of the agar plates, which had been previously and individually inoculated with one of the test organisms. A well contained a standard drug, gentamicin in the case of bacteria plates or nystatin in the case of fungi plates, while another well contained 50 % MeOH as the control. The bacteria were incubated at 37 ^{0}C for 18 h, while the fungi were incubated at 25 ^{0}C for 7 days. The presence of zones of inhibition encircling the wells was taken as an evidence of antimicrobial activity [13]. An inhibition zone diameter of 5 mm and above was taken as significant.

RESULTS AND DISCUSSION

The result of phytochemical test indicated the presence of bioactive classes of compounds such as saponins, tannins, cardiac glycosides, flavonoids and alkaloids in the leaf of *Combretum zenkeri*. Anthraquinones and phlobatannins were absent, and terpenes were present in trace amounts (Table 1). The ethanol extract of the plant exhibited some reasonable antimicrobial activity against majority of the test organisms (Table 2), which was found to be statistically significant ($p<0.01$) with respect to the standard drugs. As a result, the crude alkaloid as one of the classes of compounds responsible for the observed activity was obtained from the ethanol extract by acid-base partitioning. The pronounced activity exerted by the crude alkaloid fraction stimulated its further purification by column chromatography. The pooled fractions from column elution elicited varying antimicrobial activities. Of note, however, are the activities of pooled fractions coded C and D, which gave positive test (orange) with Dragendorff's spray reagent on the TLC (silica gel, solvent system: EtOAc – MeOH 4:1). Their R_f values are 0.5 and 0.6 respectively. Of all the extract and fractions, fractions (C and D) yielded the broadest spectrum of activity which was statistically comparable ($p<0.01$) in activity to the standard drugs. However, fraction C had a slightly higher activity ($p<0.01$) than D (Table 2). Conversely, fractions B and E exhibited weak antimicrobial activity.

Table 1. Phytochemical screening result of the ethanol extract of *Combretum zenkeri* leaf

Metabolites	Conclusion
Alkaloids	
(a) Draggendorff's	++
(b) Mayer's	++
Flavonoids	++
Tannins	++
phlobatannins	-
Cadiac glycosides	
(a) Salkowski	++
(b) Keller-Kiliani	++
Antraquinones	
Free	-
Combined	-
Terpenes	+

Key: ++ = present; + = trace; - = absent

TABLE 2: Antimicrobial activity of the extract and fractions of *C. zenkeri* leaf Zone of inhibition in mm*.

Extract / Fraction	Conc. mg/ml	MICROORGANISMS						
		K. aerogenes	*E. coli*	*S. aureus*	*P. mirabilis*	*A. niger*	*T. mentagrophyte*	*C. albicans*
EtOH	80	-	8 ± 3.3	10 ± 3.7	5 ± 1.4	12 ± 3	14 ± 3.7	-
	40	-	5 ± 2.8	6 ± 1.4	3 ± 0	10 ± 2.8	7 ± 2.8	-
$CHCl_3$	80	6 ±2.4	10 ± 2.8	12 ± 2.4	11 ± 2.4	13 ± 3.7	12 ± 2.8	10 ±1.4
	40	2 ± 0	7 ± 4.2	8 ± 1.4	7 ± 1.4	9 ± 1.4	7 ± 1.4	5 ± 0
A	80	-	13 ± 3.6	12 ± 2.4	11 ± 4.1	7 ± 0	10 ± 1.4	14 ± 2.8
	40	-	9 ± 1.4	7 ± 2.8	6 ± 1.4	5 ± 1.4	5 ± 1.4	10 ±2.8
B	80	-	7 ± 2.6	-	-	4 ± 1.4	3 ± 1.4	-
	40	-	3 ± 1.4	-	-	1 ±10	2 ± 2.4	-
C	80	12 ± 3	19 ± 3.7	13 ± 2.8	14 ± 2.4	14 ± 3.7	14 ± 2.8	10 ± 3.7
	40	6 ± 4.2	14 ± 2.8	10 ± 1.4	10 ± 2.8	10 ± 2.4	7 ± 1.4	8 ± 0
D	80	13 ± 1.4	10 ± 2.4	11 ± 1.4	9 ± 2.4	7 ± 1.4	15 ± 4.2	12 ± 2.4
	40	9 ± 2.8	7 ± 2.8	4 ± 1.4	7 ± 4.9	2 ±0	11 ± 2.8	9 ±1.4
E	80	-	-	-	10 ± 2.4	5 ± 6.2	-	-
	40	-	-	-	8 ± 6.5	-	-	-
Gentamicin	4 μg/ml	20 ± 2.4	22 ± 6.5	21 ± 2.4	25 ± 5.1	NA	NA	NA
Nystatin	4μg/ml	NA	NA	NA	NA	17 ± 2.8	18 ±5.1	17 ± 3.7
50 % MeOH	150 μl	-	-	-	-	-	-	-

Key: * = mean of 3 plates; NA = Not applicable; - = absent ; Conc. = concentration; ± = Standard deviation values

Generally, the antimicrobial activity exhibited appears to be enhanced by purification. Some plants have also been demonstrated to show activity with purification [14 - 16]. The activity was also dose dependent. The pooled fractions, most especially the alkaloid fractions C and D, elicited more significant activity than the ethanol extract ($p<0.01$). Fungi showed more susceptibility than bacteria. However, *Klebsiella aerogenes* was the least susceptible bacterium, while *Aspergillus niger* was the most susceptible fungus. Alkaloids have been demonstrated as antimicrobial principles in certain plants. Emetin derived from *Cephalis ipecacuanha* and berberine from *Berberis vulgaris* L. are used to treat dysentery [17, 18].

CONCLUSION

This study has validated the use of *Combretum zenkeri* for the treatment of microbial infections. The antimicrobial activity of the plant was shown to be largely due to the alkaloid present in the plant.

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