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Growth inhibitory properties and antimicrobial evaluation of *Aloe schweinfurthii* (Baker) leaf rind extract

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Abstract
Cancer and infectious diseases combined are leading cause of death and public health concern. In developing countries, about 80% of the populace depends on medicinal plants for their general health care needs including treatment of infectious diseases and cancer. *Aloe schweinfurthii* (Aloaceae) is a small medicinal herb that is commonly used for the treatment of cancerous and infectious diseases in South-West Nigeria. The focus of this study was to evaluate the growth inhibitory and antimicrobial activities of the herb. The rind of the herb was collected, air dried, pulverized and extracted into distilled methanol by cold maceration. The dried extract obtained was subjected to growth inhibitory and antimicrobial assays. The extract displayed concentration dependent growth inhibitory activity with IC_{50} of 484.7±2.16 and 1188±2.32µg/mL compared to cyclophosphamide with IC_{50} of 174.3±0.19 and 834.5±0.84 µg/mL in *Sorghum bicolor* radical and *Allium cepa* root growth inhibitory assays, respectively. The extract displayed concentration dependent antibacterial and antifungal effects with the highest activity against *C. freundi* (18 mm zone of inhibition) at 50 mg/mL. The extract of *Aloe schweinfurthii* leaf rind displayed marked growth inhibitory and antimicrobial bioactivities. The extract maybe considered as a viable candidate for discovery of chemotherapeutic agent (s).

Keywords: Growth inhibition, Antimicrobial, *Aloe schweinfurthii*, Chemotherapeutic Agent

INTRODUCTION

The world is in need of new medicines for the treatment of new diseases and old re-emerging scourges. In recent times, there has been a very low output of new medicines despite advances in drug discovery techniques [1]. Hence, selection of neglected and under-studies plants has been shown to be effective in the discovery of new medicines from medicinal plants [2]. Cancer and infectious diseases are the most predominant therapeutic areas for which natural products drug discovery program is based, mainly because they are the most common cause of death globally [3].

Medicinal plants are widely accepted in several cultures for the treatment of various illnesses. Recently there has been an upsurge in the number of polyherbal mixtures that are sold as food supplements and remedies for the treatment various illnesses [4]. However, as at 2012, only 6 % of all higher plant species have

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been scientifically evaluated for biological activity and only 15% have been screened for phytochemical constituents [3]. *Aloe schweinfurthii* is indigenous to Nigeria and has not been well studied [5]. It is a small herb that belongs to Aloaceae family and among the *Yoruba* tribes of South-West Nigeria, the plant is described as an important ingredient in the preparation of recipe for the treatment of cancerous and infectious diseases. It is also used as laxative, stomachic and for internal parasites. In our earlier study we reported that *A. schweinfurthii* displayed some antiproliferative effects against human cancer cell lines and it also displayed mild DPPH radical scavenging antioxidant activities [6]. The focus of this present study is to evaluate the growth inhibitory and antimicrobial activities of *A. schweinfurthii* leaf rind extracts.

EXPERIMENTAL

Plant collection and preparation. *Aloe schweinfurthii* was collected from Idanre hill at Ondo State, South-West, Nigeria. The plant was authenticated at Herbarium section of the Forestry Research Institute of Nigeria (FRIN) and voucher number (FHI: 110105) was issued. The plant material was rinsed under running water. The leaf rind was obtained by scooping off the gel from the rind. The rind obtained were air-dried under shade, pulverized and extracted into distilled methanol. The extract was concentrated *in vacuo*, weighed and refrigerated at 4˚C until needed for analysis.

Qualitative phytochemical analysis. Methanol extract of *A. schweinfurthii* leaf rind was evaluated for the presence of different classes of secondary metabolites such as alkaloids, phenolics and glycosides using methods of phytochemical screening described by Trease and Evans [7].

***Sorghum bicolor* radicle growth inhibitory assay.** Viable seeds of *Sorghum bicolor* were used to estimate growth inhibitory potential of *A. schweinfurthii* extract. Ten milliliters (10 mL) of 39.06, 156.25, 625, 2500 and 10000 µg/mL were prepared by four-fold dilution from an initial twenty milligrams (20 mg) of the *A. schweinfurthii* extract was dissolved in 5% DMSO (Sigma-Aldrich, Germany). Also, 10 mL of the same concentrations as above were prepared for cyclophosphamide (positive control). The different concentrations of the extract (10 mL) were poured into different Petri-dishes lined with cotton wool and filter paper (Whatman No.1). Ten viable seeds were spread on each of the Petri-dishes and incubated in a dark cupboard at room temperature. The lengths of the radicle emerging from the seeds were measured after 96 hours incubation. The negative control seeds were treated with 10mL 5% DMSO in distilled water [8]. The experiment was repeated in three replicates for all concentrations and controls. The radicle lengths were measured to the nearest millimetre.

***Allium cepa* root growth inhibitory assay.** The *A. cepa* root growth inhibitory assay was performed using a modified method described by Akinboro and Bakare [9]. Bulbs of *A. cepa* (50 ± 10 g) were washed with distilled water and grown in the dark over tap water at ambient temperature for 24-36 h until the roots have grown to approximately 2-3 cm length. Twenty (20) mL different concentrations of the extract (39.06, 1250, 2500, 5000 and 10000 µg/mL) were prepared by dissolving extract in 5 % DMSO. Different concentrations were poured into different Petri-dishes and the base of each of three *A. cepa* bulbs were placed on Petri-dishes each containing extract (39.06 - 10000 µg/mL). The same concentrations as above were prepared for cyclophosphamide (positive control), while the negative control bulbs were treated with 20 mL of 5% DMSO in distilled water. The root lengths were measured at 0 and after 96 hours for each concentration of extract and control. The

percentage root growth inhibition after treating with extract/cyclophosphamide at 96 hours was determined.

Determination of antimicrobial activity

Test organisms. Four clinical and typed strains of microorganisms (comprising three bacterial strains and one fungal strain) were used for the antimicrobial studies. All the organisms were obtained from the Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmaceutical Sciences in University of Ilorin, where stock cultures of the organisms were maintained at 4°C. The strains of organisms are *Pseudomonas aeruginosa*, *Escherichia coli*, *Citrobacter freundi* and *Candida albicans* cultures. They were diluted to achieve optical densities corresponding to 2.0×10^6 colony forming units (CFU/mL) for bacterial and 2.0×10^5 spore/mL for fungus strain (*Candida albicans*).

Antimicrobial susceptibility assay. The antimicrobial activity of the plant extract was screened using the agar-well diffusion method [10]. The inoculum suspension of each bacteria was swabbed uniformly to different solidified 20 mL Mueller-Hinton Agar (MHA) plates, and Sabouraud Dextrose Agar (SDA) for fungi. The inoculum was allowed to dry for 10 min and five (5) holes of 6 mm in diameter were made in the seeded agar using sterile cork borer. Four (4) different concentrations (100, 75, 50, and 25 mg/mL) of the extracts were prepared using DMSO. Aliquot of 100 µL of each concentration above was added into each well on the seeded plates, and 100 µL of DMSO, used to dissolve the plant extracts, was added to the fifth well of each to serve as negative control. The plates were prepared in duplicates, and were allowed to stand on the bench for 1 h for proper diffusion and thereafter the bacteria seeded plates were incubated at 37°C for 24 h. The same procedure was followed for the fungus *C. albicans* but incubated at 30°C. Similarly, these bacteria were also challenged with five (5) standard antibiotics (gentamicin, ciprofloxacin, imipenem, erythromycin and amoxicillin) as positive control using agar disc diffusion technique [11]. The resulting inhibition zones (diameter) were measured in millimeters (mm).

Data analysis. Data obtained was analyzed by Graphpad prism computer program. The concentration with 50% growth inhibition (IC_{50}) in *Sorghum bicolor* radicle growth inhibitory assay and *Allium cepa* root growth inhibitory assay was estimated from a dose-response inhibition curve using a non-linear regression curve data analysis. The results are displayed as mean ± SEM of three replicates. Statistical significance was evaluated using student's t-test and results with $p < 0.05$ were considered significant.

RESULTS

Preliminary phytochemical screening of *A. schweinfurthii* extract. Phytochemical investigation of *Aloe schweinfurthii* leaf extract led to identification alkaloids, flavonoid, saponins, free anthraquinone and cardiac glycoside as shown in table 1.

Antiproliferative activity of *A. schweinfurthii* extract. The extract displayed concentration dependent growth inhibitory activity as shown in table 2. In *Sorghum bicolor* radicle growth (SBRG) inhibitory assay, the extract displayed maximum inhibition (100 %) up to 5000 µg/mL and 58.94 % inhibition at 625 µg/mL compared with cyclophosphamide with 87.02 % inhibition at 625 µg/mL. The estimated IC_{50} value of the extract and cyclophosphamide are 484.7±2.16 and 174.3±0.19 µg/mL, respectively with the extract displaying only about one-third of SBRG inhibitory effect of cyclophosphamide. Similarly, the extract displayed *Allium cepa* root growth (ACRG) inhibitory effects with about 98.51 % inhibition at 10000 µg/mL and 39.45 % inhibition at 625 µg/mL. The estimated IC_{50}

value of the extract and standard drug was obtained as 1188±2.32 and 834.5±0.84 µg/mL, respectively. The extract displayed about three-quarter of ACRG inhibitory effect of cyclophosphamide. The extract displayed better SBRG inhibition than ACRG inhibition.

Antimicrobial activity of *A. schweinfurthii* extract. The antimicrobial activities of the extract and the standard antibiotics are concentration dependent antibacterial and antifungal effects as shown in Tables 3.

The extract displayed highest activity against *C. albican*>*C. freundi*> *E. coli*> *S. aureus* on both clinical and typed strains at 100 mg/mL.

Table 1: Phytochemical Analysis of *Aloe schweinfurthii* Extracts

Bioactive constituent	Chemical Test	Extract
Alkaloids	Wagner's	+
	Meyer's	+
Flavonoids	Lead acetate	++
Tannins	$Fecl_3$	++
Saponins	Frothing	
	Emulsifying	+++
Anthraquinone	Combined	+
	Free	+
Cardiac glycoside	Kedde's	+

- Absence of component; + Trace presence of component; ++ moderate amount of component, +++ Copious amount of component

Table 2: Percentage inhibition and IC_{50} of *A. schweinfurthii* leaf rind Extract on *Sorghum bicolor* radicle and *Allium cepa* root growth

Conc. (µg/mL)	SBRG Inhibitory Assay		ACRG Inhibitory Assay	
	ASL	CTZ	ASL	CTZ
10000	100.00±0.00	100.00±0.00	98.51±1.28	100.00±0.00
5000	100.00±0.00	100.00±0.00	97.01±0.56	100.00±0.00
2500	84.46±3.56	97.97±0.57	56.72±3.82	97.01±1.01
1250	75.00±1.46	95.27±1.73	47.76±1.08	50.75±2.57
625	58.94±3.33	87.02±1.58	39.45±3.31	46.15±1.43
IC_{50} (µg/mL)	484.7±2.16	174.3±0.19	1188±2.32	834.5±0.84

CLS= *Aloe schweinfurthii* leaf extract; CTZ = Cyclophosphamide; SBRG = *Sorghum bicolor* radicle growth; ACRG = *Allium cepa* root growth

Table 3. Mean zone (diameter) of inhibition (mm) of *A. schweinfurthii* extract on test organisms

Conc. (mg/mL)	Clinical isolate				Typed strain (ATCC No)			
	S. aureus	*E. coli*	*C. freundi*	*C. albican*	*S. aureus* (25913)	*E. coli* (00726)	*C. freundi* (8090)	*C. albican* (3147)
100	20.0	22.0	25.0	30.0	16.0	25.0	27.0	28.0
75	14.0	18.0	20.0	22.0	14.0	20.0	25.0	20.0
50	0.0	12.0	18.0	16.0	0.0	16.0	15.0	14.0
25	0.0	0.0	0.0	0.0	0.0	12.0	0.0	0.0
Positive control	18.0^a	0.0^a	1.2^a		0.0^a	25.0^a	35.0^a	
	0.0^b	0.0^b	30.0^b		10.0^b	8.0^b	28.0^b	
	28.0^c	14.0^c	18.0^c		14.0^c	30.0^c	40.0^c	
	0.0^d	1.0^d	0.0^d		0.0^d	0.0^d	0.0^d	
	0.0^e	1.2^e	1.0^e		0.0^e	0.0^e	0.0^e	

a = Zone of Inhibition of gentamicin at 10 µg/mL; b = Zone of Inhibition of ciprofloxacin at 5µg/mL
c = Zone of Inhibition of imipenem at 10 µg /mL; d = Zone of Inhibition of erythromycin at 15µg /mL
e = Zone of Inhibition of amoxicillin at 10µg /mL

At concentration of 25 mg/mL, only typed *E. coli* was susceptible to the extract. On the other hand, the extract also displayed concentration dependent antibacterial and antifungal similar antimicrobial activities against both clinical and typed strains. Typed strains of *S. aureus, E. coil* used in this study were most susceptible to imipenem while *C. freundi* was most susceptible to ciprofloxacin. On the other hand, all the typed bacterial strains were most susceptible to imipenem.

DISCUSSION

Biological activities of natural products are determined by their phytochemical constituents [12]. Phytochemical test of the extract revealed the presence of high amount of saponins, flavonoids and tannins, while alkaloids, anthraquinone and cardiac glycoside are present in trace amount as shown in table 1. The results obtained from this study corroborate earlier report of phytochemical constituents of some aloe species including *A. vera* were reported to contain similar phytochemical constituents as observed in *A. schweinfurthii* [12, 13]. Plants of Aloaceae family are commonly known for their laxative activity which is primary due to the anthracene derivatives phytoconstituents [15].

The leaf rind extract of *A. schweinfurthii* displayed growth inhibitory activities with much higher inhibitory activity against *S. bicolor* radicle growththan *A. cepa* root growth. Similarly, the positive control (cyclophosphamide) displayed higher *S. bicolor*radicle growth than *A. cepa* root growth. This may however implies that the extract is more effective in inhibiting initiation of cell proliferation than slowing down the rate cell proliferation. In a previous study by Salawu *et al*., 2017, extracts of various parts of *A. schwenfurthii* including the leaf rind, were observed to display antiproliferative activities against human cancer cell lines [6]. The results from this study and previous studysuggest that *A. schweinfurthii* maybe a vaible candidates for anticancer drug discovery [6].

The extractalso displayed postive antimicrobial activities as shown in table 3. The extract was observed to displayed broad spectrum antimicrobial activities against clincal and typed microbial strains with no significant differences in antimcrobial effects of the extract against clinical and typed strains orgamisms. To the best of the knowledge of available literature, this is the first reports of the antibacterial activity of *A. schwenfurthii* and it support the claims of traditional healer who use the extract for the treament of various infectious diseases.

This study observed that extract of *Aloe schwenfurthii* leaf rind displays growth inhibitory and antimicrobial activity. The extract maybe considered as a positive candidate for the development of chemotherapeutic agent(s).

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