



Antifungal Effects of Methanolic Extract of Stem Bark of *Bridelia ferruginea* Benth. Leaves of *Aloe vera* L. and Stem Bark of *Alstonia boonei* De Wild

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Authors' contributions

This work was carried out in collaboration between both authors. The research project, the statistical analysis and the draft of the manuscript was carried out and written by author AMB and supervised by author EDF. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/MRJI/2019/v27i230094

Editor(s):

(1) Dr. Ng Zhi Xiang, Department of Biomedical Sciences, Faculty of Medicine, MAHSA University, Malaysia.

Reviewers:

(1) Dr. Patrick Valere Tsouh Fokou, University of Ghana, Ghana.

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(3) Francis Opoku, Department of Applied Chemistry, University of Johannesburg, Doornfontein Campus, South Africa.

Complete Peer review History: <http://www.sdiarticle3.com/review-history/24289>

Received 15 December 2015

Accepted 04 March 2016

Published 01 April 2019

Original Research Article

ABSTRACT

The antifungal activities, proximate and phytochemical constituents of *Aloe vera* leaves extract, stem barks of both *Alstonia boonei* and *Bridelia ferruginea* were investigated. The methanolic crude extracts of these plants parts were tested for antifungal activity at different concentrations of 50.0, 100.0, 150.0, 200.0 mg/mL, respectively against against *Aspergillus flavus*, *Absidia corymbifera* and *Aspergillus niger*. Radial mycelial growth and dry mycelial weight methods were used to test for the extract activities against these fungi. The results of antifungal activity showed that all the plant extracts exhibited strong antifungal activities at higher concentration of 200.0 mg/mL. The phytochemical results showed that all the three medicinal plants contained steroid, flavonoid, phytate, phenol and oxalate which were known to exhibit antimicrobial properties as well as physiological activities. The proximate analyses showed that all the three medicinal plants contained appropriate amount of ashes, crude fibre and high carbohydrate content which provide energy when consumed. The results of this study showed that these plants possess some antifungal properties therefore the medicinal plants could serve as a potential source of active ingredients for the production of antifungal drugs.

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Keywords: Medicinal plants; radial mycelia growth; dry mycelial weight; proximate; phytochemical.

1. INTRODUCTION

Medicinal plants have been a source of wide variety of biologically active compounds for many centuries and used extensively as crude material or as pure compounds for treating various disease conditions [1]. There are about 250,000 to 500,000 species of plants on earth in which 1-10 % of the plants species are used by humans [2] for medicinal purposes. Resistance to drugs especially antibiotics has become a major challenge facing the medical fraternity today. Most antibiotics that were once effective against pathogenic micro-organisms have now been rendered ineffective owing to resistance developed by these pathogen [3], and this has brought a renewed interest in plant medicinal drugs. Thus, a search for new drugs with better and cheaper substitute of plant origin is a natural choice and better alternative. Compounds from plant materials have been reported to possess in vitro activities against pathogenic microorganisms [4] and reduce or cure infections of microbial origin.

Aloe vera is a stemless or very short-stemmed succulent plant growing to 60-100 cm tall. The leaves are thick and fleshy, green to grey-green with some varieties showing white flecks on their upper and lower stem surfaces [5]. Gong et al. [6] reported that *Aloe vera* forms arbuscular mycorrhiza, a symbiosis that allows the plant better access to mineral nutrients in soil. *Aloe vera* leaves contain phytochemicals bioactivities such as; acetylated mannans, polymannans, anthraquinone C-glycosides, anthrones and anthraquinones [7] and [8]. Juice of *Aloe vera* is used in the treatment of dry and moist epidermis, prophylactic action and prevention of kraurosis, dermatitis, eczema, psoriasis, neurodermatitis, herpes and subcutaneous infections [9,10].

Bridelia is a plant genus of the family Phyllanthaceae. This genus comprises approximately between 60 and 70 species which are found in Africa, Australia and Asia. The tree is 6-15 m high, up to 1.5 m in girth and bole crooked branching low down. They usually a gnarled shrub which sometimes reaches the size of a tree in suitable condition [11] with a common names of Kirni Kizni (Hausa); Marehi (Fulani); Iralodan/Ira (Yoruba); Ola (Igbo); Kensange abia (Boki). Its habitat is the savannah, especially in the moisture regions extending from Guinea to Zaire and Angola. This plant is widely used in traditional African medicine in the treatment of arterial

hypertension or as diuretic agents and purgative [12].

Alstonia boonei (De Wild) also known as devil tree can be found in tropical and sub-tropical Africa, Central America and Australia. *Alstonia boonei* belong to the family Apocynaceae is a large deciduous evergreen tree usually up to 45 m tall and 1.2 m in diameter consist of about 40-60 species [13]. *Alstonia boonei* known as Ahun in Yoruba, Egbu-ora in Igbo, Ukhu in Edo and Ukpukunu in Urhobo, the plant is widely distributed in the lowlands and rain-forest areas of Nigeria [14]. Parts of the plant are employed for the treatment of a variety of ailments in Africa and the stem bark has been listed in the African Pharmacopoeia as an agent useful for treatment of malaria, intestinal helminthic, muscular pain, insomnia, hypertension, fever, painful micturition, chronic diarrhoea, rheumatic pains, as anti-venom for snake bites and in the treatment of arrow poisoning [15]. Ogiangbe et al. [16] reported insecticidal properties of an alkaloid from *Alstonia boonei* De Wild. Olajide et al. [17] established anti-inflammatory, antipyretic and analgesic properties of *A. boonei* stem bark extract.

The aim of this study was to determine the proximate, phytochemical constituents and the antifungal activities of the methanolic crude extract of the three medicinal plants on some pathogenic fungi.

2. MATERIALS AND METHODS

2.1 Collection of Plant Materials

The leaves of *A. vera*, stem barks of *A. boonei* and *B. ferruginea* were obtained within the vicinity areas of Olorunda Area, Ado Ekiti, Ekiti State Nigeria. The three plants were identified and authenticated at the Department of Plant Science, Ekiti State University, Ado Ekiti.

2.2 Preparation of Plant Extracts

The leaves of *A. vera*, stem barks of both *A. boonei* and *B. ferruginea* were washed in tap water and cut into small pieces of about 2 mm x 2 mm in size. The plants pieces were air dried for 15 days under room temperature (28°C). They were then grounded into fine powder using an electric blender (Model M 20 IKA Universal Mill, IKA Group Japan). About 800 g each of the finely grounded powdered of *A. boonei*, *A. vera* and

B. ferruginea were carefully weighed and soaked separately in 1000 ml of 95% redistilled methanol according to the method of [18]. The extracts were kept in a refrigerator at 4°C until used.

2.3 Sources of Fungi

The fungi cultures *Absidia corymbifera*, *Aspergillus flavus* and *Aspergillus niger* used in this work were obtained from the stock cultures maintained on slants of Potato Dextrose Agar (P.D.A) in the Department of Microbiology, Ekiti State University, Ado Ekiti Nigeria. The cultures were kept at 4°C until when required for use.

2.4 Determination of Antifungal Activities

The standard methods as described by Fagbohun and Faleye [19] were used for the determination of antifungal activities whereby the following concentrations (50.0, 100.0, 150.0 and 200.0 mg/ml) each of the extract were aseptically dispensed into different sterile Petri-dishes. About 15 ml of sterilized molten malt extract agar was added to the plates and then swirled gently to mix the content evenly. Mycelial discs (6 mm diameter) taken with a sterile cork-borer from the advancing edges of 3-5 days old of each cultures of the test fungi were placed centrally on the cooled seeded plates and incubated at 28°C for 7 days. The control experiments were carried out as described above, but only 1ml of the extracting solvent was added to each of the plates. The experiment were done in duplicate and repeated twice. The radial mycelial growth of both test and control plates were measured using an electronic calliper. The net growth was obtained by subtracting the diameter of the inoculum plugs from the test.

2.5 Dry Mycelial Growth Assay

The antifungal properties of each of the extracts were tested using the dry mycelial growth assay as described by Fagbohun and Faleye [19]. Five mycelial discs were introduced into 120 ml Erlenmeyer flasks containing 25 ml of sterile malt extract broth. Different concentrations (50.0, 100.0, 150.0 and 200.0 mg/ml) of each extracts were introduced into each of the conical flask containing inoculum plugs of the test fungi. The flasks were incubated at 28°C for 7 days. For control, only 1 ml of the extracting solvent was added to each of the flasks and treated as described above. At the end of the 7th day of incubation, the content of each flask was

decanted into a funnel containing pre-dried and pre-weighed Whatman filter paper. The content and the filter paper was dried at 50-60°C in an oven until constant weight was obtained for each test fungi.

The net dry mycelial weight was determined by subtracting the constant weight of the filter papers and the test fungi from weight of the filter papers.

2.6 Phytochemical Analysis

Phytochemical screenings were carried out on the powdered samples to identify their major constituents such as alkaloids, saponins, tannins, flavonoid, steroid, phytate, phenols and oxalate using standard qualitative methods as described by Sofowora [1] and Trease and Evans [20].

2.7 Proximate Analyses

The proximate analysis of the samples for moisture, ash, fibre, carbohydrate and fat was determined using the method of A.O.A.C. [21]. The nitrogen was determined by micro-kjeldahl method as described by Pearson [22] the percentage nitrogen was converted to crude protein by multiplying 6.25. All determinations were performed in triplicates.

2.8 Statistical Analysis

A statistical analysis was carried out using statistical package for social science SPSS version 17.0 to determine the mean, ANOVA, Standard error and Standard deviation.

3. RESULTS AND DISCUSSION

The results of phytochemical analyses are presented in Table 1. Steroid, flavonoid, phytate, phenol and oxalate were present in the three medicinal plants. Steroids are a class of lipids with a particular molecular ring structure called the cyclo-pentanoperhy-drophonanthrene ring system [1]. Steroids are common in both plants and animals. In human ovaries and testes, the adrenal cortex and the placental secrete steroids. Iniaqhe et al. [23] reported the presence of steroids in *Acalypha hispida*. Flavonoid was moderately present in *B. ferruginea* and *A. vera*, while saponin and alkaloid were moderately present in *A. vera* and *A. boonei* but were absent in *B. ferruginea*. Flavonoids are hydroxylated phenolic substances synthesized by plants in

response to microbial infection [24]. Flavonoids have been found to be effective antimicrobial substances against a wide array of microorganisms. More, lipophilic nature of flavonoids may also disrupt fungal membranes [25]. Saponin showed a positive result in *A. vera* and *A. boonei*. Saponin is one of the secondary metabolites found in a wide range of plant species [6]. They are stored in plant cells as inactive precursors but are readily converted into biological active antibiotics by enzymes in response to pathogen attack [7]. The results obtained in this study thus suggest the identified phytochemical compounds may be the bioactive constituents and these plants proving to be an increasingly valuable reservoir of bioactive compounds of substantial medicinal merit.

Table 1. Quantitative phytochemical analyses of the three medicinal plants

Test	Medicinal plants		
	<i>Bridelia ferruginea</i>	<i>Aloe vera</i>	<i>Alstonia boonei</i>
Steroids	+	+	+
Alkaloid	-	++	++
Flavonoid	++	++	+
Saponin	-	++	++
Tannin	+	+	-
Phytate	+	+	+
Phenol	+	+	+
Oxalate	+	+	+

KEY - = Absence of constituents, + = Trace of constituents, ++ = Moderate constituents

The results of proximate analyses (g/100 g) of leaves *A. vera*, stem barks of *B. ferruginea* and *A. boonei* showed that the three medicinal plants contained high carbohydrate content of 73.08, 60.7 and 79.00 respectively (Table 2). These results are similar to what was reported for *P. fistulosus* with 62.39 (g/100 g) [26] and sweet potatoes leaves 82.8 g/100 g [27]. However, the result of present study was higher than the reported values for *Senna obtusifolia* with 23.70 (g/100 g) and *Amarathus incurvatus* with 39.05 (g/100 g) [28].

Carbohydrate constitutes a major class of naturally occurring organic compounds which are essential for the maintenance of plant and animal life, it also provide raw materials for many industries [29]. The Recommended Dietary Allowance (RDA) values are children (40%), adults (40%), pregnant women (30%) and

lactating mother (25%) [30]. This implies that the three medicinal plants are good sources of carbohydrate when consumed.

The crude protein content (g/100 g) of *A. vera* leaves, stem barks of *B. ferruginea* and *A. boonei* were 7.84, 4.35 and 3.00 respectively. The values are compared favourably with that of *Telfaria occidentalis* with a protein content of 6 g/100 g [31] but are lower than those reported for *Piper guineense* 29.78 and *T. triangulare* 31.00 (g/100 g) [32]. Proteins are biological macromolecule consisting of one or more long chain of amino acid residue which serves as enzymatic catalyst. A plant that provides more than 12 g/100 of caloric value from protein is considered good sources of protein [30]. Therefore, the three medicinal plants whose protein values were 4.35, 7.84 and 3.00 respectively (g/100 g) are not good source of protein.

The ash content (g/100 g) of stem bark of *B. ferruginea*, leaves of *A. vera* and stem bark of *A. boonei* were 6.53, 2.36 and 3.00 respectively. The ash content in these medicinal plants is lower when compared to the values reported for the leaves of *A. viridus* 22.84 (g/100 g) [33], *Ipomea batatas* with 11.10(g/100 g) and *Moringa oleifera* with 15.09 (g/100 g) [34]. They are however, higher than that of *A. sativum* with 2.30 (g/100 g) [26]. The ash content is a reflection of the amount of mineral elements presents in a sample, which is the non-combustible residue left after plant is burnt.

The moisture content values (g/100 g) for the stem bark of *B. ferruginea*, leaves of *A. vera* and stem bark of *A. boonei* were 7.33, 7.72 and 5.30 respectively. The moisture content of these plants is low when compared to that of *Xylopi aethiopica* (16.06 g/100 g) [35] and *Acalypha hipsida* (11.91 g/100 g) [23].

These values were relatively low, therefore it would hinder the growth of microorganisms and enhance the shelf life. Water activity is a measurement of the availability of water for biological reaction, it determines the ability of microorganism to grow, if water activity decreases, the growth of microorganisms on the samples will decrease. However, the water activity values for growth of most pathogens in food ranged from 83-98 g/100 g [30]. This is good for the long preservation and will prevent early spoilage by growth of microorganism.

The value of the crude fat (g/100 g) for the stem bark of *B. ferruginea*, leaves of *A. vera* and stem bark of *A. boonei* were 5.45, 4.27 and 6.00 respectively. These values are moderate in amount when compared to those of *Talium triangulare* (5.09 g/100 g), *Amarathus hybridus* (4.80 g/100 g) [32] and *Gnetum africanum* (3.15 g/100) [35]. Dietary fats increase the palatability of food by absorbing and retaining flavours [36]. A diet providing 1-2% of its caloric of energy as fat is said to be sufficient for human being as excess fat consumption is implicated in certain cardiovascular disorder such as atherosclerosis, cancer and aging [34].

The crude fibre values (g/100 g) of the leaves of *A. vera*, stem barks of *B. ferruginea* and stem bark of *A. boonei* were 4.34, 7.84 and 3.00 respectively. These values are low when compare to that of *A. esculentus* with 14.71, *M. charantia* with 16.62 [26], *P. thonningii* with 35.05 g/100 g [36] and *Blighia sapida* with 44.09 [35] but higher than that of *Gnetum africanum* 0.80 and *Telfaria occidentalis* 2.90 [37]. Adequate intake of dietary fibre can lower the serum cholesterol level, risk of coronary, heart disease, hypertension, constipation, diabetes, colon and breast cancer [38]. Therefore, the medicinal plants are not a good source of crude fibre since the values are below those recommended by Dietary Allowance (RDA) e.g the RDA values of fibre for children (19-25 g/100), adults (21-38 g), pregnant women (28 g) and lactating mothers (29 g) [30].

The antifungal activities of the methanolic extract of *A. vera* leaves exhibited weak antifungal activity on the radial mycelial growth of the test fungi as shown in (Table 3). At 50 mg/ml the methanolic extract of *A. vera* had a weak antifungal activity on radial mycelial growth of *A. flavus*, *A. corymbifera* and *A. niger* with radial mycelia growth of 18.00, 11.00 and 9.00 mm respectively, when compare with the controls of 39.00, 19.00 and 16.00 mm respectively. The extract had a moderate antifungal activity on radial mycelial growth of *A. flavus*, *A. corymbifera* and *A. niger* at 100 mg/ml and above with radial mycelia growth of 8.00, 6.00 and 7.00 mm respectively. However, the extract had a strong antifungal activity on radial mycelial growth of *A. flavus*, *A. corymbifera* and *A. niger* at 200 mg/ml with radial mycelia growth of 2.00, 3.00 and 2.00 mm respectively, while their controls were 39.00, 19.00 and 16.00 mm respectively. The result of this work showed that the radial mycelial growth of the test fungi decreased as the concentration

of methanolic extract from *A. vera* increased with antifungal effect on the test fungi, this might be due to the presence of alkaloid. Alkaloid was reported by [38] to exhibit antifungal activities against a broad spectrum of pathogenic fungi. The result of this work is in agreement with the findings of [39] who reported that the extract of neem plant (*Azadirachta indica*) caused a significant delay in sporulation and inhibited mycelial elongation in *Cercospora cruenta*, *Collectrichum truncatum* and *Fusarium oxysporium*. Similarly, the result of this work also agreed with the findings of [40] who reported that the ethanolic extract of *Luffa cylindrical* fruit inhibited the mycelia growth of *A. fumigatus*, *A. niger* and *Candida albicans* as the concentration of the extract increased from 50 mg/ml to 150 mg/ml. Also, Khan and Omotosho [41] reported that crude methanolic extracts of *Barringtonia asiatica* (leaves, fruits, seeds, stems and root barks) exhibited a very strong antifungal activity against *A. niger* as the concentration of the extracts increased.

The methanolic extract of the stem bark of *A. boonei* showed a strong antifungal activity on the radial mycelial growth of the test fungi (Table 4). At 200 mg/ml the crude extract had strong antifungal activities on radial mycelial growth of *A. flavus*, *A. corymbifera* and *A. niger* with radial mycelia growth of 6.00, 4.00 and 2.00 mm respectively. Also, the extract at 100 mg/ml had a moderate antifungal activity on radial mycelial growth of *A. flavus*, *A. corymbifera* and *A. niger* with radial mycelia growth of 18.00, 10.00 and 7.00 mm respectively, while their controls were 34.00, 29.00 and 16.00 mm respectively. The extract at 150 mg/ml, had a strong antifungal activities on radial mycelial growth of *A. corymbifera* and *A. niger* with radial mycelial growth of 5.00 and 4.00 mm respectively. Similarly, the extract at 50 mg/ml had a weak antifungal activity on radial mycelial growth of *A. flavus* and *A. niger* with radial mycelia growth of 29.00 and 10.00 mm respectively, while their controls were 34.00 and 16.00 mm respectively. The result of this work showed that increasing concentration of extract of *A. boonei* inhibited the radial mycelial growth of the test fungi. This might be due to flavonoid present in the plant. Flavonoids have been found to be effective antimicrobial substances against a wide array of microorganisms. Their activity is probably due to their ability to compete with extracellular, soluble proteins and to compete with fungal cell walls [24]. The result of this work is in agreement with the findings of [42] who reported that increased

concentrations of the methanolic crude extracts of *Tectona grandis*, *Shilajit* and *Valeriana cajani*, progressively decreased the spore germination of *Alternaria cajani*, *Helminthosporium* spp, *Bipolaris* spp. *Curvalaria lunata* and *Fusarium* spp. Similarly, Effiong and Sanni [43] reported that increasing concentration of aqueous and ethanolic extracts of *Lemma pauciscostata* inhibited the growth of fish spoilage fungi such as *Fusarium oxysporium*, *Penicillium digitatum*, *A. niger*, *A. fumigatus*, *A. flavus*, *Rhizopus oryzae* as the concentration was increased.

The methanolic extracts of the stem bark of *B. ferruginea* exhibited strong antifungal activity on the radial mycelial growth of the test fungi (Table 5). At 200 mg/ml the extract had a strong antifungal effect on *A. flavus*, *A. corymbifera* and *A. niger* with radial mycelial growth of 7.00, 4.00 and 1.00 mm respectively while their controls were 32.00, 20.00 and 18.00 mm. Similarly, the extract had a moderate antifungal activity on *A. flavus* at 100mg/ml with a radial mycelial growth of 20.00 mm while the control was 32.00 mm. The methanolic extract at 50 mg/ml had weak antifungal activities in radial mycelial growth of *A. flavus*, *A. corymbifera* and *A. niger* with radial mycelia growth of 25.00, 17.00 and 13.00 mm respectively. While their controls were 32.00, 20.00 and 18.00 mm. respectively. The result showed that increasing the concentration of the methanolic extract of *B. ferruginea* inhibited the radial mycelial growth of the test fungi. This

might be due to phenolic compounds present in the plant.

A phenolic compound has been reported by [44] to be toxic to microorganisms by enzyme inhibition and possibly by reacting with sulfhydryl groups or through more nonspecific interactions with the proteins. The result of this work is in agreement to the findings of [18], who reported that the radial mycelial growth of *A. flavus*, *Epidermophyton floccus*, *Penicillium italicum*, *Scopulariopsis brevicalis* and *Trichoderma* spp, were progressively inhibited by increasing concentration of diethyl ether and ethyl acetate extracts from the peels of *Dioscorea dumetorum*. Similarly, Shalini et al. [45] reported the strong antifungal activity observed on germination of spore of *Alternaria solani*, *Helminthosporium* spp, *Bipolaris* spp, *Curvalaria lunata* and *Fusarium* spp as the concentration of methanolic extract of roots and aerial parts of *Acorus calamus*, *Tinospora cardifolia* and *Celestrus paniculatus* increased.

Using dry mycelial weight assay, the methanolic extract of *A. vera* leaves at different concentrations showed a weak antifungal activity on the test fungi (Table 6). At 50 mg/ml, the methanolic extract of *A. vera* had a weak antifungal activity on *A. flavus*, *A. corymbifera* and *A. niger* with dry mycelial weight of 90.00, 133.00 and 135.00 mg respectively while, the control was 123.00, 198.00 and 210.00 mg

Table 2. Proximate analyses of the three medicinal plants (g/100 g)

Test	Medicinal Plants		
	<i>B. ferruginea</i>	<i>A. vera</i>	<i>A. boonei</i>
Ash content	6.54±0.05	2.36±0.01	3.00±1.00
Moisture content	7.33±0.04	7.72±0.02	5.30±5.20
Crude protein	15.7±0.30	4.73±0.01	3.33±1.00
Crude fat	5.45±0.05	4.27±0.01	6.00±4.25
Crude fibre	4.35±0.06	7.84±0.02	3.00±10.00
Carbohydrates	60.7±0.71	73.08±0.04	79.00±2.00

Table 3. Effect of methanolic extract of *Aloe vera* on radial mycelia growth of the test fungi (mm)

Test fungi	Concentration of crude extract (mg)				
	Control	50	100	150	200
<i>Aspergillus flavus</i>	39.00 ^d	18.00 ^c	8.00 ^b	5.00 ^{ab}	2.00 ^a
<i>Absidia corymbifera</i>	19.00 ^c	11.00 ^b	6.00 ^a	6.00 ^a	3.00 ^a
<i>Aspergillus niger</i>	16.00 ^b	9.00 ^a	7.00 ^a	6.00 ^a	2.00 ^a

Means for each treatment with the same alphabet in each row are not significantly different at 5% level of significance ($p < 0.05$).

Table 4. Effect of methanolic extract of *Alstonie boonie* on radial mycelia growth of the test fungi (mm)

Test fungi	Concentration of crude extract (mg)				
	Control	50	100	150	200
<i>Aspergillus flavus</i>	34.00 ^c	29.00 ^c	18.00 ^b	13.00 ^{ab}	6.00 ^a
<i>Absidia corymbifera</i>	29.00 ^c	17.00 ^b	10.00 ^a	5.00 ^a	4.00 ^a
<i>Aspergillus niger</i>	16.00 ^b	10.00 ^{ab}	7.00 ^{ab}	4.00 ^a	2.00 ^a

Table 5. Effect of methanolic extract of *Bridelia ferruginea* on radial mycelia growth of the test fungi (mm)

Test fungi	Concentration of crude extract (mg)				
	Control	50	100	150	200
<i>Aspergillus flavus</i>	32.00 ^d	25.00 ^c	20.00 ^b	12.00 ^a	7.00 ^a
<i>Absidia corymbifera</i>	20.00 ^b	17.00 ^b	9.00 ^a	7.00 ^a	4.00 ^a
<i>Aspergillus niger</i>	18.00 ^d	13.00 ^c	7.00 ^b	4.00 ^{ab}	1.00 ^a

Means for each treatment with the same alphabet in each row are not significantly different at 5% level of significance ($p < 0.05$).

Table 6. Effect of methanolic extract of *Aloe vera* on dry mycelia weight of the test fungi (mg)

Test fungi	Concentration of crude extract (mg)				
	Control	50	100	150	200
<i>Aspergillus flavus</i>	123.00 ^c	90.00 ^b	80.00 ^{ab}	69.00 ^{ab}	63.00 ^a
<i>Absidia corymbifera</i>	198.00 ^c	133.00 ^b	95.00 ^{ab}	70.00 ^a	55.00 ^a
<i>Aspergillus niger</i>	210.00 ^c	135.00 ^b	90.00 ^{ab}	72.00 ^a	40.00 ^a

Means for each treatment with the same alphabet in each row are not significantly different at 5% level of significance ($p < 0.05$).

respectively. The extract had a moderate antifungal effect on all the tested fungi at the concentration of 100 mg/150 mg respectively. Also, the extract at concentration of 200 mg/ml had a strong antifungal activity on *A. flavus*, *A. corymbifera* and *A. niger* with dry mycelial weight of 63.00 mg, 55.00 and 40.00 mg respectively. While, the control were 123.00, 198.00 and 210.00 mg respectively. This result showed that the dry mycelial weight of the test fungi decreased progressively as the concentration of methanolic extract of *A. vera* increased. This might be due to the presence of tannin present in the plant. The presence of tannin in the medicinal plant suggests the ability of these plants to play major roles as antifungal, antidiarrheal, antioxidant and anti-hemorrhoidal agent [46]. The result of this work is in agreement with the findings of [47] who reported that ethanolic extract of *Digera muricata* had a significant antifungal activity against *Candida albicans*, *A. flavus*, *A. terreus* and *A. niger* as the concentration of the extract increased. Similarly Okemo and Bais [48] reported that the methanolic stem bark extract of *Masea lanceolata* inhibited the mycelial growth of certain

fungal plant pathogens such as *phytophora cryptogea*, *Trichoderma viren*, *A. niger*, *Phoma spp.*, *Fusarium oxysporium*, *Cochliobolus heterostrophus*, *Sclerotium rolfsii* and *Pyrenophora teres*.

Using dry mycelial weight assay, the methanolic extracts of the stem bark of *A. boonie* at different concentrations exhibited strong antifungal activity on the test fungi (Table 7). At 200 mg/ml, the methanolic extract of *A. boonie* had a strong antifungal activity on dry mycelial weight of *A. flavus*, *A. corymbifera* and *A. niger* with dry mycelial weight of 45.00, 38.00 and 68.00 mg respectively, while their control was 125.00, 145.00 and 215.00 mg respectively. Similarly, *A. boonie* exhibited a moderate antifungal effect on all the tested fungi at 100 mg/ml and 150mg/ml respectively. However, at 50 mg/ml the extract had a weak antifungal effect on *A. flavus*, *A. corymbifera* and *A. niger* with dry mycelial weight of 85.00, 105.00 and 156.00 mg respectively while the control was 125.00, 145.00 and 215.00 mg respectively. The result of this work showed that dry mycelial weight of all the test fungi decreased with increased in the concentration of

the extract of *A. boonie*. This might be due to the presence of saponin in the medicinal plants. Saponin appears to act by disrupting the membrane integrity of fungal cells [27]. The result of this work is in agreement with the findings of [49] who reported that the aqueous extracts of *Alchornea cordifolia* (bark), *Annona muricata*, *Garcinia kola*, *Zingiber officinale* and *Allium sativum* inhibited the mycelial weight of *Fusarium solani*, *Fusarium oxysporium*, *A. niger* and *Rhizopus stolonifer* at higher concentration of 200 mg/ml. Similarly, Anita et al. [34] reported that ethanol, methanol, ethylacetate and water extract of roots and aerial parts of *Rubia tinctorium* L. progressively reduced the mycelial weight of *Aspergillus flavus*, *F. oxysporium*, *Geotrichum penicillatum*, *Candida albicans* and *Saccharomyces cerevisiae* as the concentration of extract increased with complete inhibition at a concentration of 100 mg/ml. The result of this work is also similar to the findings of [50] who reported the significant antifungal activities exhibited by the roots extract of *Rubia tinctorum* on *Trichoderma viridile*, *Doratomyces stemonitis* and *Penicillium vermucosum*.

The methanolic extracts of stem bark of *B. ferruginea* at showed a strong antifungal effect on dry mycelial weight of the test fungi (Table 8). At 200 mg/ml, the extract had a strong antifungal activity on dry mycelial weight of *A. flavus*, *A.*

corymbifera and *A. niger* with dry mycelial weight of 43.00, 65.00 and 54.00 mg respectively while their control was 108.00 mg, 125.00 mg and 174.00 mg respectively. Also, *B. ferruginea* exhibited a moderate antifungal effect on all the tested fungi at concentration of 100 mg/ml and 150 mg/ml. However, the extract at 50 mg/ml had a weak antifungal effect on *A. flavus*, *A. corymbifera* and *A. niger* with dry mycelial weight of 88.00, 113.00 and 150.00 mg respectively while the control was 108.00, 125.00 and 174.00 mg respectively. The result of this work showed that the dry mycelial weight of the test fungi decreased as the concentration of plant extract increased. This might be due to the presence of flavonoid in the medicinal plant. Flavonoids have been found to be effective antimicrobial substances against a wide array of microorganisms. Their activity is probably due to their ability to compete with fungal cell walls [24]. This work is in agreement with the findings of [51] who reported that the plantain ash, bitter leaves extract and neem seed oil significantly reduced the dry mycelial weight of *Rhizopus stolonifer* a prevalent fungus associated with yam rots as the concentration increased. Hussain et al. [26] reported the significant antifungal activity exhibited by the methanolic extract of leaf of *Barringtonia racemosa* on certain phytopathogenic fungi such as *Fusarium* spp and *Aspergillus* spp.

Table 7. Effect of methanolic extract of *Alstonia boonei* on dry mycelia weight of the test fungi (mg)

Test fungi	Concentration of crude extract (mg)				
	Control	50	100	150	200
<i>Aspergillus flavus</i>	125.00 ^c	85.00 ^b	68.00 ^{ab}	63.00 ^{ab}	45.00 ^a
<i>Absidia corymbifera</i>	145.00 ^d	105.00 ^c	82.00 ^{bc}	73.00 ^b	38.00 ^a
<i>Aspergillus niger</i>	215.00 ^d	156.00 ^c	110.00 ^b	90.00 ^{ab}	68.00 ^a

Means for each treatment with the same alphabet in each row are not significantly different at 5% level of significance ($p < 0.05$)

Table 8. Effect of methanolic extract of *Bridelia ferruginea* on dry mycelia weight of the test fungi

Test fungi	Concentration of crude extract (mg)				
	Contro	50	100	150	200
<i>Aspergillus flavus</i>	108.00 ^c	88.00 ^{bc}	77.00 ^{bc}	68.00 ^{ab}	43.00 ^a
<i>Absidia corymbifera</i>	125.00 ^c	113.00 ^{bc}	90.00 ^{ab}	79.00 ^a	65.00 ^a
<i>Aspergillus niger</i>	174.00 ^c	150.00 ^{bc}	110.00 ^{abc}	75.00 ^{ab}	54.00 ^a

Means for each treatment with the same alphabet in each row are not significantly different at 5% level of significance ($p < 0.05$)

5. CONCLUSION

Plants have contributed immensely to the medical field. It has been the source of most drugs used for combating infections. The three medicinal plants used in this study were found to contain the important constituent needed to combat various kinds of infection in human and plants. The proximate and phytochemical components of the medicinal plants may have been responsible for the antifungal activities of the plants. However, this study showed that the plants extract at a concentration (150 and 200 mg/ mL) had a strong antifungal effect on the test fungi. The quantification and determination of the active ingredients of the studied medicinal plants on the test organisms should be the object of further investigation with the mechanism(s) of action of their photochemistry.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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