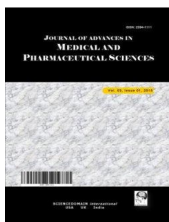

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Quantitative Phytochemical Analysis and Antifungal Susceptibility of *Vernonia amygdalina* against Some Strains of *Candida albicans*

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Authors' contributions

This work was carried out in collaboration among all authors. Author ADMO designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors SYD and NA managed the analyses of the study. Author NA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

This study was aimed at determining the quantitative phytochemical analysis and antifungal susceptibility of *Vernonia amygdalina* against some strains of *Candida albicans*. Reflux method of extraction was used for the successive extraction of the leaves of *Vernonia amygdalina*. Quantitative phytochemical screenings were done to determine the amounts of phytochemicals that are present in the crude extracts, the study revealed that phytochemicals which include flavonoids, tannins, alkaloids, saponins and phenol were present in the crude extracts. Three different strains (P37005, RM1000 and SC5314) were subjected for antifungal susceptibility test, the antifungal susceptibility test of the crude extracts against the strains were determined at different concentrations of 40,60, 80 and 100 mg/ml using agar well diffusion method. The highest zone of inhibition (ZOI) was 21.00 ±0.30 mm which was recorded for methanol leaf extract (MLE) at a concentration of 100 mg/ml against SC5314 (isolate:B3). The MIC and MFC values for the most active crude extracts were 12.5 mg/ml and 100 mg/ml for the n-hexane crude extract against strain P37005 (isolate B1), the value of

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12.5 mg/ml and 100 mg/ml was also revealed for the n-hexane crude extract against SC5314 (isolateB3) however, the methanol crude extract showed a value of 12.5mg/ml and 50mg/ml respectively against SC5314 (isolate:B3). The results from this study suggest that n-hexane and methanol crude extract have a better antifungal activity than the ethylacetate crude extract. This study also validate the claim of the local herbal practitioners of the use of the leaves of *Vernonia amygdalina* in curing candidiasis.

Keywords: Phytochemical; Vernonia amygdalina; antifungal; susceptibility; strains; crude extract.

1. INTRODUCTION

Vernonia amygdaline is a member of the Asteraceae family is a small shrub that grows in tropical Africa having a height of 2–5 m (6.6–16.4 ft). The leaves are elliptical and up to 20 cm (7.9 in) long. Its bark is rough [1] *V. amygdalina* is commonly called bitter leaf in English. "Oriwo'' in Edo "ewuro'' in Yoruba, "Shikaa'' in Hausa and olubu in Igbo [2]. The leaves are consumed as vegetable and condiments, after macerating and washing thoroughly to remove the bitterness. *Vernonia amygdalina* have been reported to have Antihelmitic antimalarial, antitumourigenic hypoglycemic and hypolipidaemic properties [3]. Athough, Medicinal plants are known to contain substances which could be used for treating purposes or used to produce drugs, many of such plants known to be used primitively to alleviate symptoms of illness have been screened to have medicinal importance, some of which include : *Vernonia amygdalina* (Bitter leaf), *Allium sativum* (Garlic), and *Zingiber officinale* (Ginger) These plants have been reportedly used in the treatment of ailment such as stomach disorder, fever, symptoms and cough traditionally [4] . *Vernonia amygdalina* is a valuable medicinal plant that is widespread in West Africa. It is known as bitter leaf due to its characteristics bitter taste and flavor, and can be used as an active anticancer, antibacterial, antifungal and anti-parasitic agent .This plant contains complex active components that are useful pharmacologically. In ethno medicine, the roots and the leaves are used to treat fever, Hiccup, Kidney problems, and stomach discomfort. Many West African countries such as Cameroon, Ghana and Nigeria use the stem and root as chewing sticks [5]. It is also documented that *Vernonia amygdalina* has been used traditionally in blood clothing and has elicited a substantial reduction in the level of glucose in the body [5].

2. MATERIALS AND METHODS

2.1 Plant Materials

The leaves of *Vernonia amygdalina* (bitter leaf) was collected from Maikunkele in Bosso Local Government, Niger state.

2.2 Collection and Identification of Test Organisms

Three strains of *Candida albican*: SC5314, P37005 and RM1000 were collected from Federal Istitute for Industrial Research Oshodi (FIIRO), Lagos, Nigeria. They were subjected to sugar fermentation test as described by John et al. [6]. Germ tube test was carried out to identify and differentiate them from other species which was done according to Cheesbrough [7] . Their molecular characterization was also carried out to confirm the identity of the strains [8].

Fig. 1. *Vernonia amygdalina*
Source: Field photograph

2.3 Identification, Authentication and Processing of Plant Materials

Fresh sample of the plant material (leaves) for this study were identified by the local herbal practitioners in Minna, Niger state while authentication of the plant sample was done by Mr. Lateef Akeem of the Herbarium Department of National Institute for Pharmaceutical Research and development (NIPRD), Idu, Abuja with the following voucher numbers: (NIPRD/H/6872) and the voucher specimens were deposited in the same Herbarium Department of the institute. The identified and authenticated medicinal plants parts were washed with distilled water to get rid of dirt. The washed leaves were air dried under shady environment (away from sunlight). The air-dried leaves of the medicinal plant was separately grounded with the aid of a mortar and pestle and subsequently pulverized into powdered using an electric blender. The pulverized samples was further sieved with a 150µm pore size filter to obtain a fine powdered-like texture, stored in amber bottles and kept in a cool dried environment under room temperature until it is required for usage.

2.4 Extraction Procedure

The grounded plant samples (Pulverized samples) were subjected to reflux extraction according to Hymete et al. [9] in order to obtain the crude extracts. The extraction was carried out beginning from non-polar solvent to polar solvent (n-hexane, ethyl acetate and methanol). 100 g of the plant samples was weighed and dissolve in 400 ml of the extracting solvent in a round bottom flask of 500 ml capacity. Starting with n-hexane (polarity index =0.1p') was gradually added until a ratio of 1:4 of the pulverized samples to the extracting solvent was attain. The flask containing the mixture was then placed on the heating mantle and the opening of the flask was connected to the condenser. The power source was then switched on to supply heat and the temperature was controlled (adjusted) to 30°C. The mixture was allowed to reflux for 2 hours. After refluxing, the mixture was filtered using muslin cloth and later with what man No 1 filter paper with pore size 20 µm to obtain a clear filtrate and further concentrated to a semi solid substance with the use of a rotary evaporator at a reduced temperature of (40°C) and then dried using water bath at 60°C. The extract was then stored in an air tight amber bottle and kept in the refrigerator for further analysis. The Marc (residue) was dried at room temperature for 45 minute and was extracted with the next solvent in increasing polarity (further in succession using ethyl acetate with polarity index=4.4p'). This procedure was repeated using the last solvent and the weight of the extracts for all the solvents used were measured and recorded accordingly. Percentage yield of each of the crude extract was calculated using the formulae below:

Percentage yield (%) = (Weight of extract / Weight of sample (dry plant material) × 100

2.5 Determination of Flavonoid

Aluminium chloride colorimetric method was used for flavonoid determination. A 0.5 ml (1 mg/mL) of the plant crude extract was mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminium chloride, 0.1 ml of 1 M sodium acetate and 2.8 ml of distilled water and kept at room temperature for 30 minute. The absorbance of the reaction mixture was taken at 415 nm with a double beam Shimadzu UV spectrophotometer, UV -1800. The calibration curve was prepared by using quercetin solutions at concentration of 12.5 to 100 g/ml in methanol [10].

2.6 Determination of Total Phenol

The total phenol content of the crude extract was determined according to the method described by Singleton et al. [11]. A 0.5 ml (1 mg/ml) was oxidized with 2.5 ml of 10% Folin- Ciocalteau's reagent (v/v) and neutralized by 2 ml of 7.5% sodium carbonate. The reaction mixture was incubated for 40 minutes at 45^{0}C and the absorbance was taken at 765 nm using the double beam Shimadzu UV spectrophotometer, UV-1800. The total phenol content was subsequently calculated using Gallic acid as standard.

2.7 Determination of Total Alkaloid

A 0.5 g of the crude extract was mixed with 5 ml of 96% ethanol -20% H_2SO4 in ratio (1:1) and filter. 1 ml of the filtrate was added to 5 ml of 60% H2SO4, the mixture was allowed to stand for 5 minute and 5 ml of 0.5% of formaldehyde solution was added and allowed to stand for 3 hours. The absorbance was taken at a wave length of 565 nm using Shimadzu UV spectrophotometer, UV- 1800. The concentration of alkaloid in the sample was calculated using

the molar extinction coefficient of vincristine, ε=15136 mol/cm [12].

2.8 Determination of Saponin

A 0.5 g of the crude extract was mixed with 20 ml of 1 M HCL and the mixture was boiled for 4 hours and allowed to cool. After cooling and filtered, 50 ml of petroleum ether was added to the filtrate, for ether layer and evaporated to dryness. 5 ml of acetone- ethanol (1:1) were added to the residue, 6 ml ferrous sulphate reagent and 2 ml of concentrated H2SO4. The mixture was homogenized and allowed to stand for 10 minutes before the absorbance was taken at 490 nm using Shimadzu UV spectrophotometer UV- 1800 [12].

2.9 Determination of Tannin

A 0.2 g of the extract was weighed into a 50 ml beaker; 20 ml of 50% methanol was added, covered with para film and placed in a water bath at 80°C for one hour. The mixture was shaken thoroughly and the content was transferred into a 100 ml volumetric flask. 20 ml of water, 2.5 ml of 10% Folin Denis reagent and 10ml of 17% Na_2 CO_3 was added and mixed thoroughly. The mixture was allowed to stand for 20 minute. Observation for bluish green colouration was done at the end of range 12.5-100 ug/ml of Tannic acid. The absorbance of tannic acid standard solution as well as sample was taken after colour development on a spectrophometer at wave length of 760 nm using Shimadzu UV-spectrophometer, UV-1800 [13]

2.10 Phytic Acid Content

The phytic acid content was determined using a modified indirect colorimetric method of [14]. The method depends on an iron phosphorous ratio of 4:6 and is based on the ability of standard ferric chloride to precipitate phytate in dilute HCL extract of the sample. 5 g of the sample was extracted with 20 ml of 3% trichloroacetic acid and filtered. 5 ml of the filtrate was used for the analysis; the phytate was precipitated as ferric phytate and converted to ferric hydroxide and soluble sodium phytate by adding 5 ml of 1 M NaOH. The precipitate was dissolved with hot 3.2 M HNO_3 and the absorbance was taken immediately at 480 nm. Preparation of standard curve for phytic acid was done as follows: standard curve of different Fe $(NO_3)_3$ concentration was plotted against the corresponding absorbance of spectrophotometer to calculate the ferric iron concentration. The phytate phosphorous was calculated from the concentration of ferric iron assuming 4:6 Iron: phosphorous molar ratio.

2.11 Determination of Oxalate

Oxalate was determined by permanganate titrimetric method as described by Oke [15]. Two gram (2 gram) of the crude extract was suspended in 190 ml of distilled water in 250 ml volumetric Flask, 10 ml of 6 M HCL was added and the suspension digested at 100^{0}C for 1 hour, cooled, then made to the mark before Filtration. Duplicate portion of 125 of the filtrate were measured into beakers and four drops of methyl red indicator added. This is followed by the addition of concentrated NH_4OH solution drop wise until the test solution changes from salmon pink colour to a faint yellow colour (pH 4-4.5). Each portion is then heated to 90^{0}C and 10 ml of 5% $CaCl_2$ solution added while being stirred constantly. After heating, it was cooled and left overnight at 5°C. The solution was then centrifuged at 2500 rpm for 5 minutes, the supernatant decanted and the precipitate completely dissolved in 10 ml of 20% (v/v) H_2SO_4 solution. The total filtrate resulting from the digestion was made up to 300 ml aliquots of 125 ml of the filtrate was heated until near boiling and the titrated against 0.05 M standardized $KMno_4$solution to a faint pink colour which persisted for 30 seconds. The calcium oxalate content is calculated using the formula below:

$$T\times (Vme) (Df) \times 10^5 \ (mg/100g) / (ME) \times Mf$$

Where T is the titre of $KMnO_4$ (ml), Vme is the volume- mass equivalent (1 cm^3 of 0.05 M $KNnO_4$ solution is equivalent to 0.00225 anhydrous oxalic acid), Df is the dilution factor V_T/A (2.5 where V_T is the total volume of titrate (300 ml) and A is the aliquot used (125 ml), ME is the molar equivalent of $KMnO_4$ in oxalate ($KMnO_4$ redox reaction) and Mf is the mass of extract used.

2.12 Antifungal Activity of the Crude Extracts

2.12.1 Preparation of 0.5Mcfarland standard

The populations of the clinical isolate were determined from the McFarland turbidity standard [16]. Zero point zero five milliliter (0.05

ml) of 1% $BaCl_2$ was mixed thoroughly with 9.95 ml of 1% H_2SO_4 in a test tube. The absorbance of the mixture (white precipitate) was determined at 530 nm.

2.12.2 Standardization of the test organisms

The Standardization of the test organisms was carried out as described by Magaldi et al. [17] with slight modification. The test organism was cultured on sabouraud dextrose agar at room temperature for 24-72 hours. A loopful of the cultured organism (*Candida albicans*) was transferred into 9 ml of sterile sabouraud dextrose broth. Serial dilutions of $10^{-1} – 10^{-7}$ was made and the absorbance was determined at 530 nm using Shimadzu UV spectrophotometer. The optical density (absorbance) was compared with the optical density (absorbance) of 0.5 Mcfarland standard previously obtained. The dilution corresponding to that of the 0.5 Mcfarland standard was used as the standard organism which gives a population of 1.5×10^3 cfu/ml .

2.13 Preparation of Extract Concentration

Two hundred milligram (200 mg), 300 mg 400 mg and 500 mg of the normal hexane, ethyl acetate and methanol extract was weighed and dissolved in 5 ml each of 10% Dimethyl sulfoxide (DMSO) to give a concentration of 40 mg/ml, 60 mg/ml,80 mg/ml and 100 mg/ml concentrations respectively [18] .

2.14 Determination of the Antifungal Activity of the Crude Extracts

The susceptibility test was carried out using Agar Well Diffusion Method as described by Magaldi et al. [17]. Sabouraud dextrose agar (SDA) was prepared according to the manufacturer's instruction. The prepared SDA was then inoculated with a loop full of the standardized test organism by the spread plate method using a sterile rod spreader to obtain uniform growth, wells was made using 6 mm sterile cork borer and labelled accordingly. 100 µl (0.1 ml) of the prepared crude extract of varying concentration (40, 60, 80 and 100 mg/ml) was transferred into each of the wells with a micropipette and allowed to stand for 30 minutes to 1 hour for pre-diffusion and then incubated at room temperature for 24-72 hours.100 µl of 10% DMSO (free from extract) was transferred into a freshly prepared SDA containing the test organism to serve as negative control while Fluconazole (1 mg/ml) was used as the positive control. This was achieved by transferring 100 µl of the prepared standard antibiotics into the well and cultures were allowed to stand for 30 minute after which they were incubated at room temperature for 24-72 hours. The zone of inhibition (ZOI) was measured using a meter scale rule. The experiment was done in triplicates and the mean values with the corresponding standard deviation of the inhibition zone diameter (IZD was recorded. Crude extract that measured zone of inhibition ≤10 was recorded as resistant while >10 was recorded for sensitivity [19].

2.15 Determination of Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) of the Most Active Crude Extract

The MIC was determined using the Tube Dilution Method as described by Ewansiha et al. [18]. A four fold serial dilution of the most active plant extract was carried to give a decrease in concentration from 100, 50, 25, 12.5, 6.25, 3.125, 1.25 and 0.78 mg/ml. The stock solution was prepared by dissolving 800 mg of the extract in 4 ml of 10% dimethyl sulphoxide (200 mg/ml). Two millilitres (2 ml) of the stock concentration was transferred to a test tube labeled a containing fresh 2 ml Sabouraud Dextrose Broth (SDB) to give a concentration of 100 mg/ml. From tube a, 2 ml will be transferred into a second tube labeled B containing 2 ml SDB to give a concentration of 50 mg/ml. This procedure continues until a concentration of 0.78 mg/ml was obtained in the last test tube labeled H. They were properly shaken to obtain a homogenous mixture and all test tubes were inoculated with 0.1ml of the standardized test organism. Two separate test tubes containing sterile broth plus 10% DMSO and sterile broth plus test organism was prepared for negative and positive controls respectively. All test tubes were incubated at room temperature for 24 to 72 hours, after which the test tubes were compared with each control tubes. The concentration/tube without visible turbidity was taken as the MIC. The MFC was determined by subculturing from the MIC tube and other tubes that showed no turbidity onto freshly prepared SDA and incubated at room temperature for 24 to 72 hours. The concentration that showed no visible growth after incubation was taken as the MFC.

2.16 Statistical Analysis

The data are presented as Mean ±standard deviation; all data were analyzed by one way

ANOVA. Differences were considered significant at P≤0.05 .The analysis were carried out using Statistical Package for Social Science (SPSS) version 20.

3. RESULTS

3.1 Biochemical Characteristics and Germtube Test of Fungal Strain (*Candida albicans*)

The result showed the gram reaction, biochemical and germ tube tests that was carried out to confirmed the identity of the three fungal strains as shown in Table 1. This was done in accordance with [7].

3.2 Molecular Characterization Conducted for the Fungal Strains (*Candida albicans*)

The results from the molecular analysis confirmed the identity of the strains which is represented in Table 2. The sequenced BLAST results revealed the following Identities of the fungal strain (*Candida albicans*) and their accession numbers which were determined from the GENE bank through the NCBI web site [8].Their corresponding ascension numbers were as follows: Isolate B1: P37005 (AP023893.1), Isolate B2: RM1000 (AB_017634.2) and Isolate B3: SC5314(CP025163.1).

3.3 Percentage Yield of the Leaf Crude Extracts of *Vernonia amygdalina*

Table 3 represents the percentage yields of the leaf crude extracts of *Vernonia amygdalina* .The milled plant samples were extracted with n – hexane, ethyl acetate and methanol. The leaves of *Vernonia amygdalina* had a percentage yield of 11.42%, 0.39% and 8.28% which were obtained in NHLE, EALE and MLE respectively. NHLE had the highest percentage yield of 11.42% and the lowest yield was obtained in EALE 0.39%.

3.4 Quantitative Phytochemical Determination of *Vernonia amygdalina* Leaf Crude Extracts Obtained Using Different Solvents

Table 4 showed the results obtained from the quantitative determination of n-hexane leaf extract (NHLE), ethyl acetate leaf extract (EALE) and Methanol leaf extract (MLE) of *Vernonia amygdalina*. The n-hexane leaf extract (NHLE) of *Vernonia amygdalina* had phytic acid (160.78±0.58) as the highest in amount while oxalate (5.65± 0.58) was the lowest. Others were flavonoid (60.88±0.58), phenols (113.46±0.58), tannin (68.32±0.58), alkaloid (132.11±0.58) and saponins (40.22±0.58). Likewise, the ethyl acetate leaf extract (EALE) of *Vernonia amygdalina* also had phytic acid (45.63±0.58) as the highest in amount while

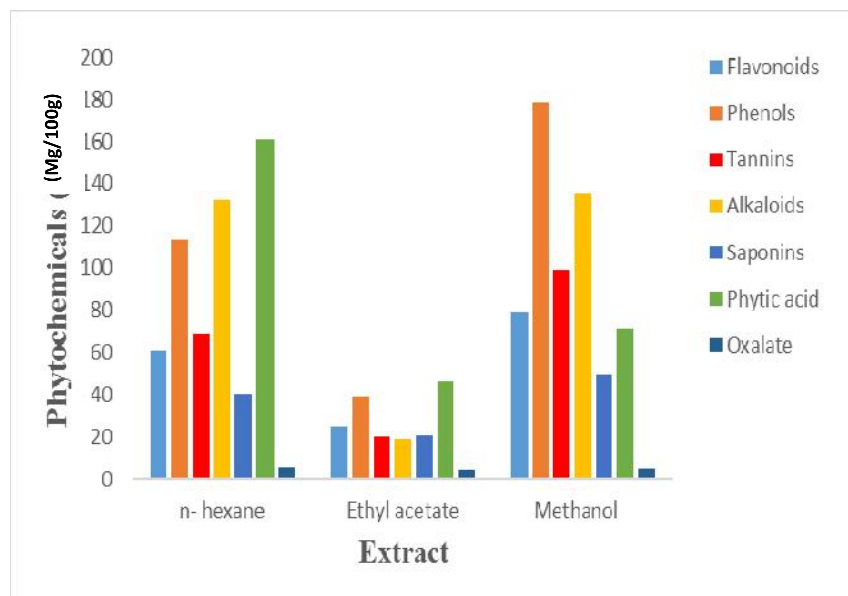

Fig. 2. Quantitative phytochemical determination of *Vernonia amygdalina* leaf crude extracts obtained using different solvents

Table 1. Biochemical characteristics and germtube test conducted for the fungal strains (*Candida albicans*)

				Fermentation									Assimilation										
S/N	**Isolate code**	**Gram reaction**	**Shape**	**Glucose**	**Fructose**	**Sorbitol**	**D-Mannito**	**Lactose**	**Sucrose**	**Mannose**	**Arabinose**	**Galactose**	**Glucose**	**Fructose**	**Sorbitol**	**D-Mannito**	**Lactose**	**Sucrose**	**Mannose**	**Arabinose**	**Galactose**	**Germtubetest**	**Inference**
1	B1	+	OVAL	+	+	-	-	-	-	-	-	+	+	+	-	-	-	+	+	-	+	+	*Candida albican*
2	B2	+	OVAL	+	+	-	-	-	-	-	-	+	+	+	-	-	-	+	+	-	+	+	*Candida albican*
3	B3	+	OVAL	+	+	-	-	-	-	-	-	-	+	+	-	-	-	+	+	-	+	+	*Candida albican*

Table 2. Molecular characterization conducted for the fungal strains (*Candida albicans*)

Isolated code	Strains	Max score	Total score	Query cover	Expected value	Identity	Ascension number
B1	P37005	7542	5850	100%	0.0	100%	APO23893.1
B2	RM1000	700	8811	100%	0.0	100%	AB_017634.2
B3	SC5314	234	5243	100%	0.0	100%	CP025163.1

Table 3. Percentage yield of *Vernonia amygdalina* leaf crude extract

Plant sample	Leaf			
	WS/DP(g)	NHLEg(%)	EALEg(%)	MLEg(%)
Vernonia amygdalina	100	11.42(11.42)	0.39(0.39)	8.28(8.28)

Key: WS/DP: Weight of sample/ weight of dried powered material, NHLE: n-hexane leaf extract, EALE: Ethyl acetate leaf extract, MLE: Methanol leaf extract

oxalate (4.55±0.58) was the lowest. Others present in amounts were flavonoid (24.96.±0.58), phenols (38.69±0.58), tannin (19.88±0.58), alkaloid (18.66.±0.58) and saponins (20.44±0.58). Methanol leaf extract had phenols (178.92±0.58) as the highest in amount while oxalate (5.30±0.58) was also present in trace amount. Others were flavonoid (79.28±0.58), tannin (98.92±0.58), alkaloid (135.65±0.58), saponins (49.16±0.58) and phytic acid (70.59±0.58).

3.5 Antifungal Susceptibility of the Leaf, Crude Extracts of *Vernonia amygdalina* against Strain P37005 (Isolate: B1)

Antifungal susceptibility of the leaf, crude extracts of *Vernonia amygdalina* against strain P37005 (Isolate: B1) is presented in Table 5. The n-hexane leaf extract (NHLE) showed no activity at 40 mg/ml but at 60, 80 and 100 mg/ml concentration there was inhibitory activity and the mean zone of inhibition (MZI) was 8.00±0.20 mm, 9.33±0.80 and 13.00±0.70 mm respectively. Ethyl acetate leaf extracts (EALE) showed no inhibitory activity for all the varying concentration used at 40, 60, 80 and 100 mg/ml .The methanol leaf extract (MLE) had no inihibitory activity at 40 and 60 mg/ml but at 80 and 100 mg/ml concentrations there was inhibitory activity and the mean zone of inhibition (MZI) was 7.00±0.20 mm and 9.00±0.70 mm, respectively.

3.6 Antifungal Susceptibility of the Leaf Crude Extracts of *Vernonia amygdalina* against Strain RM1000 (Isolate: B2)

Antifungal susceptibility of the leaf crude extracts of *Vernonia amygdalina* against strain RM1000 (Isolate: B2) is presented in Table 6. The n-hexane leaf extract (NHLE) showed no activity at 40 mg/ml concentration but at 60, 80 and 100 mg/ml concentration there was inhibitory activity and the mean zone of inhibition (MZI) was 6.00±0.20 mm, 8.67±0.67 mm and 10.00±0.60 mm respectively. Ethyl acetate leaf extracts (EALE) showed no inhibitory activity for all the varying concentration used at 40, 60 80 and 100 mg/ml. The methanol leaf extract (MLE) had no inhibitory activity at 40, 60 mg/ml but at 80 and 100 mg/ml concentration there was inhibitory activity and the mean zone of inhibition (MZI) was 6.00±0.20 mm and 8.00±0.20 mm respectively.

3.7 Antifungal Susceptibility of the Leaf Crude Extracts of *Vernonia amygdalina* Against Strain SC5314 (Isolate: B3)

Antifungal susceptibility of the leaf crude extracts of *Vernonia amygdalina* against strain SC5314 (Isolate: B3) is presented in Table 7. The n-hexane leaf extract (NHLE) showed no activity at 40 mg/ml concentration but at 60, 80 and 100 mg/ml concentration there was inhibitory activity and the mean zone of inhibition (MZI) was 8.00±0.76 mm, 12.67±0.76 mm and 14.00±0.60 mm respectively. Ethyl acetate leaf extracts (EALE) showed no inhibitory activity at varying concentration used at 40, 60 80 and 100 mg/ml. The methanol leaf extract (MLE) had no inhibitory activity at 40 and 60 mg/ml concentration but at 80 and 100 mg/ml concentrations there was inhibitory activity and the mean zone of inhibition (MZI) was 16.00±0.70 mm and 21.00±0.30 mm.

3.8 Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) of the Most Active Crude Extracts

The result of the Minimum inhibitory concentration (MIC) and Minimum fungicidal concentration (MFC) of the most active crude extracts is shown in Table 8. The MIC and MFC values for the most active crude extracts were 12.5 mg/ml and 100mg/ml for the n-hexane crude extract against strain P37005 (isolate B1), the value of 12.5 mg/ml and 100 mg/ml was also revealed for the n-hexane crude extract against SC5314 (isolateB3) however, the methanol crude extract showed a value of 12.5 mg/ml and 50 mg/ml respectively against SC5314 (isolate :B3).

Table 4. Quantitative phytochemical determination of *Vernonia amygdalina* leaf crude extracts obtained using different solvents

	Phytochemicals(mg/100g)						
Extracts	**Flavonoids**	**Phenols**	**Tannins**	**Alkaloids**	**Saponins**	**Phytic acid**	**Oxalate**
n- hexane	60.88±0.58[b]	113.46±0.58[b]	68.32±0.58[b]	132.11±0.58[b]	40.22±0.58[b]	160.78±0.58[a]	5.65±0.58[a]
Ethyl acetate	24.96±0.58[c]	38.69±0.58[c]	19.88±0.58[c]	18.66±0.58[c]	20.44±0.58[c]	45.63±0.58[c]	4.55±0.58[a]
Methanol	79.28±0.58[a]	178.92±0.58[a]	98.92±0.58[a]	135.65±0.58[a]	49.16±0.58[a]	70.59±0.58[b]	5.30±0.58[a]

Values are means ± standard deviation of triplicate values. Means with dissimilar letter (s) differ significantly according to the least significant different at $p \leq 0.05$

Table 5. Antifungal susceptibility of the leaf of *Vernonia amygdalina* 40-100mg/ml against strain P37005 (Isolate: B1)

Conc. (mg/ml)	Leaf		
	n-hexane	Ethyacetate	Methanol
40	0.00±0.00^{d}	0.00±0.00^{b}	0.00±0.00^{d}
60	8.00±0.00^{c}	0.00±0.00^{b}	0.00±0.00^{d}
80	9.00±0.20^{c}	0.00±0.00^{b}	7.00±0.20^{c}
100	13.00±0.70^{b}	0.00±0.00^{b}	9.00±0.70^{b}
Fluconazole (1 mg/ml)	40.00±0.80^{a}	40.00±0.80^{a}	40.00±0.80^{a}
DMSO(100 ul)	0.00±0.00^{d}	0.00±0.00^{b}	0.00±0.00^{d}

Values are means ± standard deviation of triplicate values. Means with dissimilar letter (s) differ significantly according to the least significant different at p≤0.05

Table 6. Antifungal susceptibility of the leaf crude extract of *Vernonia amygdalina* 40-100mg/ml against strain RM1000 (Isolate: B2)

Conc. (mg/ml)	Leaf		
	n-hexane	Ethyacetate	Methanol
40	0.00±0.00^{e}	0.00±0.00^{b}	0.00±0.10^{d}
60	6.00±0.20^{d}	0.00±0.00^{b}	0.00±0.10^{d}
80	8.67±0.67^{c}	0.00±0.00^{b}	6.00±0.20^{c}
100	10.00±0.60^{b}	0.00±0.00^{b}	8.00±0.20^{b}
Fluconazole (1 mg/ml)	37.00±0.40^{a}	37.00±0.40^{a}	37.00±0.40^{a}
DMSO(100 ul)	0.00.±0.00^{e}	0.00±0.00^{b}	0.00±0.00^{d}

Values are means ± standard deviation of triplicate values. Means with dissimilar letter (s) differ significantly according to the least significant different at p≤0.05

Table 7. Antifungal susceptibility of the leaf of *Vernonia amygdalina* 40-100mg/ml against strain SC5314 (Isolate: B3)

Conc. (mg/ml)	Leaf		
	n-hexane	Ethyacetate	Methanol
40	0.00±0.00^{e}	0.00±0.00^{b}	0.00±0.00^{d}
60	8.00±0.76^{d}	0.00±0.00^{b}	0.00±0.00^{d}
80	12..67±0.76^{c}	0.00±0.00^{b}	16.00±0.70^{c}
100	14.00±0.60^{b}	0.00±0.00^{b}	21.00±0.30^{b}
Fluconazole (1 mg/ml)	38.00±0.60^{a}	38.00±0.60^{a}	38.00±0.60^{a}
DMSO(100 ul)	0.00±0.00^{e}	0.00±0.00^{b}	0.00±0.00^{d}

Values are means ± standard deviation of triplicate values. Means with dissimilar letter (s) differ significantly according to the least significant different at p≤0.05

Table 8. Minimum inhibitory concentration (MIC) and Minimum fungicidal concentration (MFC) for the most active crude extract

S/N0	Isolated code	Strain	Plant part	Crude extracts	MIC	MFC
1	B1	P37005	Leaf	n – hexane	12.5 mg /ml	100 mg/ml
2	B3	SC5314	Leaf	n – hexane	12.5 mg/ml	100 mg/ml
3	B3	SC5314	Leaf	Methanol	12.5 mg/ml	50 mg/ml

4. DISCUSSION

The result of the biochemical, germ tube test and molecular characterization confirmed the three isolates collected. The use of biochemical and molecular characterization in identifying isolates is in agreement with a similar work of Ewansiha et al. [18] as shown in Tables 1-2. Three different solvents namely (n- hexane, ethyl acetate and methanol) with different polarity index were used for the extraction of the leaves of *Vernonia amygdalina*. The percentage yield of *Vernonia amygdalina* is shown in Table 3. The n-hexane leaf extract (NHLE) of *Vernonia amygdalina* had the highest percentage yield of 11.42(%), Ethyacetate extract (EAE) was the lowest with a

percentage yield of 0.39(%) while methanol leaf extract (MLE) had a percentage yield of 8.28%. The differences in percentage (%) might be as a result of the different solvents used, solubility of the different components in them and as well the availability of the different extractable components in them [18,20]. Table 4 shows the quantitative phytochemical analysis of *Vernonia amygdalina* leaf crude extract obtained from different solvents. The quantitative phytochemical was done to determine the amount of constituents that are present in each of them. Several of them which include flavonoid, phenols, tannins and alkaloids was determined. A study conducted by Oloyed [12] on *Vernonia amygdalina* (bitter leave) revealed that the plant contain flavonoids, saponins, alkaloids, tannins and anthraquinones. Similarly, [21] also detected the presence of alkaloids, tannins, saponins and Flavonoids. Flavonoids generally are also reported to be present in glycosylated forms in plants and the sugar moiety has been found to be an important factor in determining their bioactivtity. Flavonoids have antioxidants potentials hence could offer protection against heart disease and cancer probably by enhancing the body defense against pathology induced free –radicals [22]. As reported by Jones et al. [23] phenolic compounds are some of the most widespread molecules among plant secondary metabolites which are known to act as natural antioxidants, antiulcer, anti–inflammatory, antispasmotic and antidepresssnt activities [24]. The tannins containing plant extracts can be used against stomach and duodenal tumors and as anti-inflammatory, antiseptic, antioxidant and homeostatic pharmaceuticals [25]. Furthermore it have been reported by Jisika et al. [26] that alkaloids potentials which tend to be organic and natural ingredients that have nitrogen and are also physiological active together with sedative and analgesic roles. Oxalates and phytic acid are said to be antinutrients but they have proven potentials, especially phytic acid which was found to be anticancer against bone, prostate, ovarian, breast, liver, cholesterol, leukemia, sarcoma and skin cancers. They are also important in protection of the gut from toxins and may have a positive impact on cholesterol and blood sugar [27]. Tables 5-7 shows the antifungal activity of the leaf of *Vernonia amygdalina* against the three different strains (P37005, RM1000 and SC5314).There was inhibitory activity for n- hexane leaf extract (NHLE) and Methanol leaf extract (MLE) of *Vernonia amygalina* at different concentration of the crude extracts for all the strains but the highest value 21.00 ±0.30 mm was recorded for methanol leaf extract (MLE) at a concentration of 100 mg/ml against SC5314 (isolate: B3). The zone of inhibition (ZI) increased as the concentrations were increased. According to Prescott et al. [28] reported that the activity of antimicrobial agent is concentration dependent in addition, Edeoga et al. [19] reported that the inhibitory zones varies with the type of solvent used for extraction. This result is in agreement with the work of Gamba et al. [29] and also in conformity with the work of Okigbo and Mmeka [30] who reported inhibitory activity of the leaf of *Vernonia amygdalina* against *Candida albicans*. The activity of n-hexane and methanol crude extracts of *Vernonia amygdalina* could be due to the presences of higher concentrations of bioactive substances that were found in both of them. The concentration of the bioactive substance eg saponin, alkaloids might have been in sufficient concentration for both the n-hexane and methanol crude extract compared to that of the ethyl acetate. Concentration of bioactive substance is one of the factors that affect microbial susceptibility, the higher the concentration the higher the activity of the chemical substances [31] these bioactive substances are mostly the antimicrobial agents that carry out inhibitory activity for both bacterial and fungal. There importance in medicinal plant extracts cannot be overemphasized. The result of the antifungal activity of the standard drug (fluconazole1 mg/ml) used in this study showed a better zone of inhibition as compared to the crude. However, the negative control dimethylsulphur oxide (DMSO) showed no inhibitory activity against the standardized test strains. This suggests that DMSO does not contain any antimicrobial agent. Table 8 shows the values of the minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) that were obtained. The MIC and MFC values were different from a similar work done by Edeoga et al. [19]. The differences might be due to geographical location of the plant, differences in laboratory procedures and reagent used [32]. The season of the plant, age of the plant and method of extraction may also affect the yield and the bioactive components of the plant [33]. The result from this study confirms the claims by local marketers and consumers of the use of the leaf either singly or in combination for the treatment of candidiasis.

5. CONCLUSION

The leaves of *Vernonia amygdalina* used in this study have shown promising pharmacological prospect. The crude extracts of n-hexane and methanol had inhibitory activity against the tested strains of *Candida albicans* (P37005, RM1000 and SC5314). However, the results from this study also indicate that the leaves of *Vernonia amygdalina* might have contained sufficient amount of bioactive substance that have antifungal potentials against the tested strains. The extracts from the leaves of *Vernonia amygdalina* should be further subjected to *in vivo* trials in order to evaluate their toxicity profile on organ system, which would ascertain their safety to humans. Active components should be characterized as well as elucidation of structure of the components should be done.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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