Evaluation of Cytotoxicity, Antimicrobial Activities and Minerals Composition of *Vigna subterranea* (L.) Verdc. (Bambara Groundnut) Extracts

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Summary

Introduction: Bambara nuts have phytochemicals noted to have different roles in nutrition, physiology and control of diseases.

Objectives: Nuts were investigated for their phytochemicals, cytotoxicity, antimicrobial activity and minerals presence.

Materials and Methods: The nuts were extracted with methanol–dichloromethane solvent. The extracts were qualitatively analyzed for the presence of phytochemicals. Cytotoxicity was evaluated against Hep 2, DU 145 and Vero cell lines using MTT assay. Bambara extract activity against *Escherichia coli, Staphylococcus aureus*, and *Pseudomonas aeruginosa* was done using diffusion test and micro dilution method to determine the inhibitory effect as well as minimum inhibitory concentration (MIC). Colony growth diameter for *Candida albicans* was measured and percent inhabitation of mycelial growth calculated. Mineral composition was determined through qualitative analysis.

Results: Alkaloids, saponins, terpenoids and tannins were identified. The dark brown cultivar cytotoxicity against Hep 2, Vero and DU 145 cell lines showed IC_{50}s of 15.5 μg/ml, 19.2 μg/ml, and 41.1 μg/ml respectively. MIC for *E. coli* was 7.72± 0.35 μg/ml, *S. aureus*, 12.5± 0.32 μg/ml, and *P. aeruginosa* was 7.95± 0.10 μg/ml. At 100 μg/ml; *E. coli, S. aureus* and *P. aeruginosa* showed zone of inhibition of 27± 0.74 mm, 25.3 ± 0.40 mm and 25.1± 0.24 mm respectively but these values were less as compared to those of ceftriaxone which were 37.0±0.5, 41.3±0.9 and 42.3±0.9 respectively The extract had the minimum inhibitory effect of 62.44% at 1 μg/ml but the greatest effect against mycelial growth was 91.12% at 4 μg/ml. The control drug clotrimazole however showed increased efficacy. The minerals present in the extracts were manganese, magnesium and potassium, sodium, iron and calcium.

Conclusion: The Bambara nuts extract showed appreciable efficacy against cell lines and microbes hence signifying their possible potential use as cytotoxic and antimicrobial agents as well as a plant with higher nutritional value to improve health.

Key words: Bambara nuts, antimicrobial activity, cytotoxic, minimum inhibitory concentration

1.0 Introduction

Neurodegenerative disorders, microorganisms and cancer are among the problems researchers are fighting with day in day out since they are among the main causes of mortality in the country [1, 2]. Change in life styles, drug factors, emergence of resistance, cost of medicines, polypharmacy, noncompliance to drugs, hygiene, drugs use, alcohol abuse, diet, immune-compromised cases, environmental changes and oxidants are some of the factors thought to contribute towards the rise of these disorders and diseases[3]. Unfortunately, attempts to use conventional medicines and even radiological procedures have left the patients being confronted with undesirable side effects worse than the disease itself [4, 5]. Complementary and alternative medicines are optional choices [6]. Since 60% of chemotherapeutic agents currently in use are in some way derived from natural sources [7], evaluating the potential of Bambara nut as a source of alternative drugs is imperative.

Furthermore, infectious diseases are the leading causes of death throughout the world, accounting for nearly one half of all deaths in the tropical countries, which are also becoming a serious problem in developed countries [8]. Failure of chemotherapy and multiple drug disease resistance that develops due to mutation or gene transfer have led to organisms that can be drug tolerant, drug destroying and showing drug impermeability, leaving questions on the some antibiotics efficacy. Furthermore, antibiotics are sometimes associated with several other problems on the host including toxicity, hypersensitivity reaction, suprainfection, nutritional deficiencies and masking of infections. This has forced scientists to search for new antimicrobial substances. Given the alarming incidence of antibiotic resistance bacteria of medical importance, there is a constant need for new and effective therapeutic agents. Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases from medicinal plants [9]. Antimicrobials of plant origin have enormous therapeutic potential. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials [10, 11].

Additionally, minerals have been appreciated by scientists and researchers in their role of decreasing incidences of neurodegenerative disorders, microorganisms and cancer. Of interest is the postulation that mineral deficiency may activate certain procarcinogens and thereby influence oncogenesis. Elevated zinc levels have been observed in bronchogenic and colonic cancer. In contrast, lowering of zinc levels is seen in a variety of infective and other disease states. Zinc has an important role in cell-mediated immune functions, and zinc deficiency has been reported to be associated with increased risk of cancer [12–13]. Magnesium (Mg) deficiency can paradoxically increase the risk of, or protect against oncogenesis [14]. It has been proposed that Mg is central in the cell cycle, and that its deficiency is an important conditioner in precancerous cell transformation [15–16]. In addition, immunocompetence (that eliminates transformed cells) is Mg-dependent [11, 17]. Magnesium supplementation of those who are Mg

89

deficient, like chronic alcoholics, might decrease emergence of some malignancies [13]. Magnesium and zinc cations are required for synthesis of nucleic acids and enzymatic activity, and their deficiency causes impaired immunosurveillance. Zinc deficiency has increased oncogenicity of some chemical agents and decreased that of others [15–16]. Extensive studies investigating the role of copper in cancer have been carried out [14, 16]. Copper levels are elevated in Hodgkin's disease; a fall was noted in patients who responded to therapy. Copper levels have been found to be elevated in several types of tumors, and the significance is at present unknown. Correlation of nickel levels with oral cancer, arsenic with laryngeal carcinoma, and lead with leukemia, lymphomas, and ovarian cancer

has been attempted in order to define the role of these trace metals in oncogenesis and diagnosis [13]. Selenium is another potent mineral that has been shown to be effective against breast cancer as well as other cancers. The relationship between selenium status and intake among breast cancer patients was studied by scientists in Kuala Lumpur [18].

In Kenya, Bambara groundnut is a minor crop and is used as a traditional food only by the Luhya, Giriama and Kambe at the coast, and to a lesser extent by the Luo [19]. The groundnut is a leguminous drought tolerant crop which has food, feed, nutrition and medicinal value [20]. The groundnut is grown in a number of countries [21] as shown in the table 1 below.

Table 1: Presents a list of vernacular names that are assigned To Bambara groundnut according to the countries it is grown.

Country	Common names used by different communities in Africa
Kenya(Luhya)	Dzimbande
Kenya (Swahili)	Njugu mawe
	Madagascar groundnu, earth pea, baffin pea, Jugo, njugo bean
South Africa (Sepedi)	Ditloo
South Africa(Tshivenda)	Phonda voandzou, nzama
Malawi	Indhlubu and underground bean
Congo	Groundnut, Congo goober,

Potentially valuable treasures in this nut remain extensively unexplored as the groundnut six cultivars (dark brown, cream spotted, brown, black, and dark–brown spotted, red and light spotted) are among such treasures as their compounds have not been fully investigated with few reports being put forward. Report [22] indicate that the seeds of Bambara are nutritious and contain phytochemicals such as alkaloids (0.40 %), saponins (0.43 %) and flavonoids (0.29 %). These results of the phytochemical screening and quantitative estimation compared favorably with those reported from some medicinal plants found in Nigeria [23]. Similar report shows that the yields of the alkaloids which are low are useful in prolonging the action of several hormones and acting as stimulants. Bambara nut can also be fermented with lactic acid bacteria to make a probiotic beverage that not only increase the economic value of the nutritious legume but also help in addressing malnutrition and human health [24].

Research has also shown that Bambara groundnut landraces with low levels of condensed tannins have beneficial health effects on human nutrition [25]. For instance, landraces containing relatively high levels of soluble fibers are believed to reduce incidences of heart disease, colon cancer and diarrhea [26]. Black seeded landraces are reported to be used traditionally cancer, cure impotence in men, nausea and morning sickness in women in Botswana [21]. However, the use of Bambara nut extracts for the management of microbes and toxicity towards cancer has not been validated. Therefore the study was designed to determine the cytotoxicity and antimicrobial properties and also establish the minerals profile in Bambara nuts obtained from Kenya.

2. Materials and Methods

2.1. Plant material

Bambara nuts under study were collected from Bungoma County, Western Kenya and Kenya agriculture and livestock research organization (KALRO) in April, 2014 where six cultivars: dark brown, cream spotted, brown, black, and dark–brown spotted, red and light spotted were obtained. The phytochemical analyses and cytotoxic activity were done at the Centre for Traditional Medicine and Drug Research laboratories, KEMRI, Nairobi while antibacterial and antifungal activities of the extracts were done at Kenya Medical Training College in Nyeri County, Central Kenya.

2.2. Extraction and screening for phytochemicals

Nuts were air dried at room temperature to a constant weight, milled, ground (each cultivar 800g) and stored in labeled airtight bags prior to use. The powdered materials from the six cultivars were extracted separately with methanol–dichlomethane (1:1, 0.5 L) for 48 hours at room temperature. The filtrates were dried *in vacuo* using rotary evaporator to yield yellowish

Sticky solid (80 g each). The six extracts were qualitatively analyzed for the presence of phytochemicals. Drangendoff's reagent was used to determine the presence of alkaloids [27, 28]. The extract were dissolved in 2N –hydrochloric acid on a water bath, then shaken and filtered. The filtrates were extracted with chloroform to remove undesirable matters. Finally the pH of the acidic aqueous layer was adjusted with ammonia followed by extracting the alkaloid bases
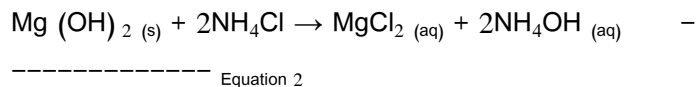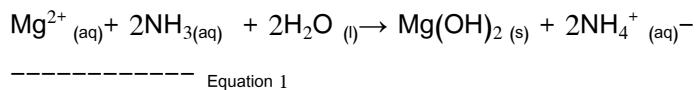
91

with chloroform. Thin layer chromatography was carried out and then sprayed with Drangendoff's reagent. The persistent frothing test [29] was used to determine presence of saponins. Briefly, to 1g of each extract, 30mL of tap water was added. The mixture was vigorously shaken for 30 seconds and left to stand for 30 minutes, and heated to boil. Tannins were also determined as described by Trease and Evans [30]. Briefly, 0.5 g powdered extract was dissolved in 5 mL of distilled water, then boiled gently and cooled. 1mL of this solution was put in a test tube and 3 drops of Ferric Chloride solution added. Determination of flavonoids in the nut was done according to the method described by Ujowundu(31). In brief, 5 mL of diluted ammonia solution was added to a portion of the aqueous filtrate of the extract, followed by addition of concentrated sulphuric acid. The Salkowski test was used to determine terpenoids (32) where 5mL/5g of extract was mixed in 2mL of chloroform, and 3mL concentrated sulphuric acid was carefully added to see color changes.

2.3. Minerals composition analysis

Qualitative analysis of macro and micro cations from all the extracts were done. The cations included K^+, Na^+, Ca^{2+} Fe^{3+}, $Zn2^+$, Mn^{2+}, and Al^{3+} ions were analyzed to verify their presence (33). The presence of Na^+ and K^+ ions in the extracts was confirmed by the flame test. Presence of Mg^{2+} ions required diluting the extracts with ammonia solution.

$$Mg^{2+}_{(aq)} + 2NH_{3(aq)} + 2H_2O_{(l)} \rightarrow Mg(OH)_{2 (s)} + 2NH_4^+_{(aq)} - \text{------------} \quad \text{Equation 1}$$

$$Mg(OH)_{2 (s)} + 2NH_4Cl \rightarrow MgCl_{2 (aq)} + 2NH_4OH_{(aq)} - \text{------------} \quad \text{Equation 2}$$

When disodium hydrogen phosphate solution was added to the product ($2NH_4OH$) above, with cooling and scratching the inside of the test tubes with a glass rod, a white crystalline precipitate of magnesium phosphate was formed as follows.

$$Mg^{2+}_{(aq)} + 2NH_{3(aq)} + HPO_4^{2-}_{(aq)} \rightarrow Mg(NH_4)PO_{4(s)} - \text{----------------------} \quad \text{Equation 3.}$$
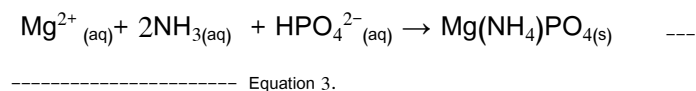
Ca^{2+} ions present in extracts formed precipitate with ammonium carbonate in the presence of ammonium chloride in neutral medium. The precipitating solution was buffered at pH 9.5 with NH_4^+ ions and NH_3 molecules. $(NH_4)_2CO_3$ was used at 0.2 M with a neutral or slightly basic pH. Confirmation of cations of Al^{3+}, Fe^{3+} and Mn^{2+} in the extracts was also carried out. They initially formed sulfide precipitate in the presence of high S^{2-} ion concentration in basic solution. Furthermore, Al^{3+} ions precipitated as hydroxides rather than as sulfides together with Fe^{3+} which formed both hydroxides and sulfides. These ions precipitated in 0.1 M H_2S solution at pH 8-9. A buffer was used to maintain this pH. In these conditions, H^+ ion concentration was reduced to a very low concentration (10^{-9}M) and the solution saturated with H_2S. Under these conditions, the equilibrium shifted to the right.

$$H_2S_{(aq)} \rightleftharpoons 2H^+_{(aq)} + S^{2-}_{(aq)} \text{----------------} \quad \text{Equation 4}$$
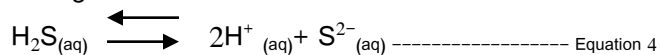
The concentration of S^{2-} was high enough to precipitate FeS and MnS. The presence of ammonia converted Mn^{2+} into the amine complexes. The cations of Al^{3+}, Fe^{3+} and Mn^{2+} formed sulfide which precipitated in the presence of high S^{2-} ion concentration in basic solution.

92

## 2.4. Cytotoxicity activity

2.4.1. Cell culture

The mammalian cells used included human cancer cell line Hep-2 (human hepatic carcinoma) and DU 145 (prostate cancer cell line) and noncancerous Vero cell line (African green monkey kidney fibroblast cells). The cells were cultured in 75-cm^3 culture flasks in Eagles Minimal essential medium (MEM, Gibco) supplemented with Fetal Bovine serum GiBco BRL (10%) and amikacin (60 mg/liter), at 37°C in an atmosphere of 95% humidity, 5% CO_2. The culture medium was changed twice a week.

2.4.2. Cytotoxicity assay

The cytotoxic activities of Bambara extract was determined using rapid colorimetric assay based on the fact that mitochondrial oxidoreductase enzymes are capable of reducing the tetrazolium dye MTT 3-(4,5-dimethylthiazol- 2-yl)-2,5-diphenyltetrazolium bromide to its insoluble formazan, which has a purple color. The cellular oxidoreductase enzymes may, under defined conditions, reflect the number of viable cells present (34). A cell density of 2.0×10^4 cells per well in 100 μL were seeded on 96-well plates and incubated for 12 h at 37 °C and 5% CO_2 to attach to the surface. Extracts were added to the cultured cells in rows H–B over a concentration

range of 0.14 to 100 μg/mL, whereas wells 1–8 of row A served as untreated controls and wells 9–12 as blank (1% Dimethyl sulfoxide (DMSO), v/v). The plates were incubated for 48 h at 37 °C and 5% CO_2, followed by an addition of 10 μL MTT viability indicator reagent. The plates were then incubated for additional 4 h at the same conditions. All the media was removed from the plates and 100 μL DMSO was added to dissolve the formazan crystals (figure 1–3).

| Figure 1: Vero cell line | Figure 2: Hep 2 cell line | Figure 3: DU 145 cell line |
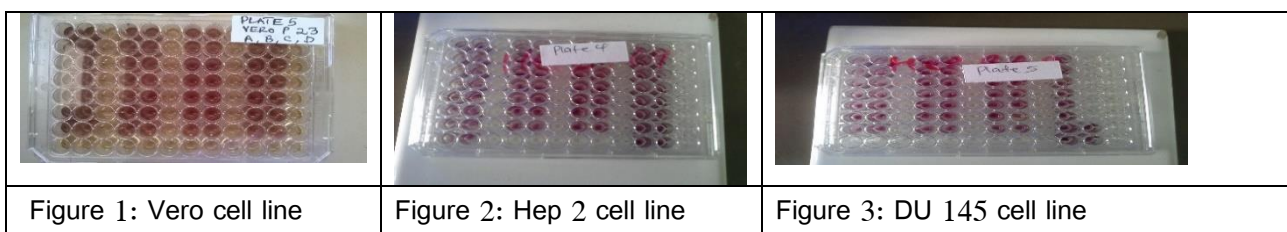
The plates were read on a scanning multi-well spectrophotometer at 562 nm. The results were recorded as optical density (OD) per well at each drug concentration. Results were expressed as the mean ± standard error. The percentage of cell viability was calculated by the formula: % Viability = Corrected OD of sample /Control OD ×100 %; while the growth inhibition = 100 − % viability. The concentration of the extract causing 50% inhibition of cancer cell growth was considered as IC$_{50}$. Statistical analyses were performed using a two-tailed Student's *t*-test and P < 0.05 was considered to be statistically significant.

2.5 Antimicrobial activities

2.5.1 Antibacterial activity

93

The extracts from the Bambara nuts with varied concentration were evaluated for antibacterial agents against the growth of *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* using the Kirby−Baurer diffusion test (35). MICs were determined by serial dilutions (5 μl, 7 μl, 8 μl, 12.5 μl, 25 μl, 50 μl and 75 μl) of extracts in test tubes with standard amount of broth (1 ml). Following a period of incubation (24 hours), the test tubes were examined for growth. Nutrient agar plates were prepared and inoculated with test organisms by a spread plate method [35]. The sterilized filter paper disc of 5 mm diameter (Whatmann's No. 1 filter paper) was used. Furthermore, sterile impregnated disc with extracts were dried and placed on the agar surface with forceps and pressed gently down to ensure complete contact of the disc on the agar surface. All the plates were incubated at 37°C for 24 hours. Clear inhibition zone around the well indicated the antimicrobial activity of the corresponding extracts and the diameters of the zone were measured in millimeters. Ceftriaxone was used as positive control against the tested bacteria. The experiment was done in triplicate, and the means +/− standard deviations were reported.

2.5.2 Antifungal activity

The antifungal activity was assayed against the growth of *Candida albicans*. The diffusion method [8] in sabourand dextrose agar (SDA) was used to determine the antifungal activity of the extract at 1,2 and 4 μg/ml concentrations. Extract was dissolved in Dimethyl sulfoxide (DMSO) and added to saboraund dextrose agar media medium immediately before it was poured into petri dishes (9 cm diameter) at 40–45°C to obtain a series of concentrations above. Negative control plates were treated with DMSO alone, and three replicates per treatment were used. Ten milligrams of Clotrimazole (British pharmacopoeia, batch number 15–03023; Candistat®, Elys, Pessaries) was used as a positive control. Plates were incubated at 25°C. Colony growth diameter was measured after the fungal growth in the control treatment had completely covered petri dishes. The percent inhibition of mycelial growth, in terms of fungitoxicity of the extracts, was calculated [34] % inhibition= {(Mc−Mt)/Mc} x 100 Where Mc is the average increase in the mycelial growth in control and Mt is the average increase in the mycelial growth in treatment. The experiment was performed in triplicate.

2.6. Statistical analysis

Data were expressed as mean ± standard error of mean (SEM) for the three−independent experiments. The significant difference between means was determined using one−way analysis of variance (ANOVA). Statistical significance was established at $p < 0.05$.

3. Results

3.1 Phytochemical analysis results

3.2. Cytotoxic activity

Cytotoxicity of the extracts was studied by means of a colorimetric assay (MTT assay) against three cell lines; Hep 2, DU 145 and Vero, yielding varying IC_{50} values. The concentration−effect curve of most potent cultivar against vero is depicted in figure 1 as IC_{50} values and for the other cultivars presented in Table 3.

94

Table 2 shows the preliminary qualitative phytochemical screening of Bambara nuts extract.

Test	Compounds tested	Results	Inference
Liebmans–Burchard reagent	Terpenoids	Reddish–brown precipitate	Terpenoid present
Dragendorff's reagent	Alkaloids	Orange colouration of spot on TLC	Alkaloid present
Froth foam test	Saponins	Stable froth	Saponins present
Magnesium chloride reagent	Flavonoids	White precipitate	Flavonoids absent
Ferric chloride reagent	Tannins	Blue Black precipitates	Tannins present

Figure 4: Cytotoxic activity of dark brown cultivar against vero cell line.
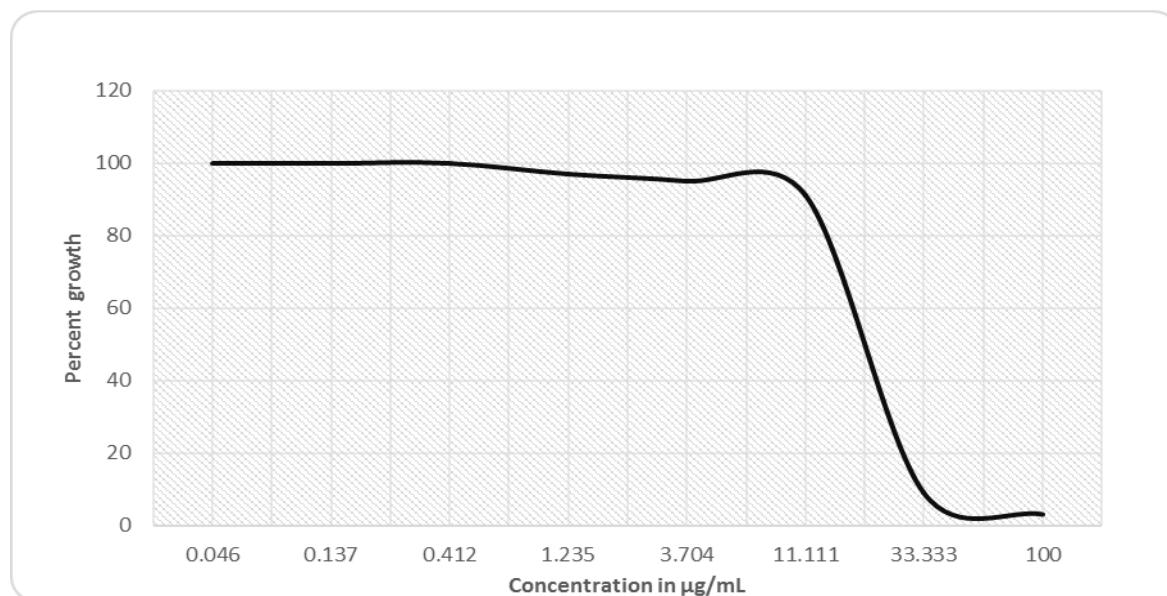

Figure 4 shows brown cultivar extracts cytotoxic activity against vero (African green monkey kidney fibroblast) cell line. Inhibitory concentration at 50% of this cultivar against Vero cell line is 19.2 μg/ml. The nut extract showed concentration dependent cytotoxic effect against the cell lines.

It was observed that the dark brown extracts was most potent with IC_{50} of 15.5 μg/ml closely followed by cream spotted cultivar with IC_{50} of 17.9 μg/ml against Hep 2 cell lines. Hep 2 cancer cell lines were more sensitive to the extract in comparison to DU 145 cell lines which showed varied values to various Bambara varieties as indicated by the IC_{50} against the cell line.

95

Table 3. Cytotoxic activity of Bambara cultivar against two cancer cell lines and one non-cancerous cell line (n=3) IC_{50} values (µg/ml).

Drug variety	Hep 2 IC_{50}	DU 145 IC_{50}	Vero IC_{50}
Black	50.5	60.1	35.2
Dark brown	15.5	41.1	19.2
Cream spotted	17.9	34.0	28.9
Brown	39.8	35.0	33.7
Dark brown spotted	44.4	40.2	40.1
Red	39.7	39.9	43.3

The extract of Bambara nut inhibited all the bacterial strains as in (Table 4). The results showed that increase in concentration of extract increased the zone of inhibition against all the tested microbial strains. At 100 µg/ml for example, the displayed inhibitory effect towards the strains gave zone of inhibition as *E. coli* = 27.0± 0.7 mm, *S. aureus* = 25.3± 0.4 mm and *P. aeruginosa* = 25.1± 0.2 mm compared to 37.0±0.5, 41.3±0.9 and 42.3± 0.9 respectively for ceftriaxone. The MIC obtained against *E. coli* at 7.0± 0.35 µg/ml, *S. aureus* at 12.5± 0.32 µg/ml and *P. aeruginosa* at 7.0± 0.10 µg/ml (Table 4).

3.2.2 Antibacterial activity

Table 4: Antibacterial activity of dark brown cultivar extract of Bambara groundnut

Tested Microorganism	Concentration and Zones of Inhibition (mm) of extract					MIC (in µg/ml) for the extracts
	25 µg/ml	50 µg/ml	75 µg/ml	100 µg/ml	Ceftriaxone at 100 µg/ml	
Escherichia coli	12.5±0.1	20.0±0.4	21.0±0.3	27.0±0.7	37.0±0.5	7.72± 0.35
Pseudomonas aeruginosa	12.0±0.7	12.6±0.5	21.0±0.3	25.1±0.2	41.3±0.9	7.95± 0.10
Staphylococcus aureus	10.3±0.2	13.3±0.6	23.3±0.8	25.3±0.4	42.3±0.9	12.5± 0.32

Values are represented as mean ± SD of triplicate experiments; MIC means Minimum Inhibitory Concentration (µg/ml)

3.2.3 Antifungal activity

Table 5: Effect of Methanol−Dichlomethane extract on mycelial growth of tested fungi (mean ± S.D) using disc diffusion method

	Percent Reduction In Mycelial Growth for *Candida albicans* at Three Concentrations		
	1 µg/ml	2 µg/ml	4 µg/ml
Bambara extract	62.44± 4.3	71.6± 2.4	91.12±0.1
Cotrimoxazole	71.2 ± 3.2	81.4± 7.1	95.3± 9.1
Dimethylsulfoxide	−	−	−

The extract of Bambara nuts led to reduced mycelial growth of Candida albicans by 62.44%, 71.6%, and 91.12% at the concentrations of 1, 2, and 4 µg/ml compared to clotrimazole whose inhibition was 71.2%, 81.4%, and 95.3% respectively at the same concentrations. Statistical differences ($p \leq 0,05$) with negative controls indicated that DMSO did not influence the results of biological evaluation but clotrimazole had impact. The extracts showed significant antifungal activity in vitro that was comparable to the standard drug clotrimazole.

3.4 Macro /Micro Minerals

The flame test gave characteristic yellow flame color for Na+ with a pale –violet flame color for K+. Furthermore, Mg2+ ions formed a white gelatinous precipitate of Mg $(OH)2$ in dilute ammonia solution, where then on ammonium chloride solution addition to the precipitate, the pH was lowered and the Mg $(OH)_2$ precipitate dissolved. Ca^{2+} ions formed precipitates with ammonium carbonate in the presence of ammonium chloride in neutral medium. A white precipitate indicated the presence of Ca^{2+}. Cations of iron (III), manganese (II) and aluminium (III) on analyzing of the extract did not react with dilute hydrochloric acid. However they formed precipitates with ammonium sulphide in neutral medium i.e. they formed sulfide precipitates in the presence of high S^{2-} ion concentration in basic solution. Also, Al^{3+} ions precipitated as hydroxides rather than as sulfides together with Fe^{3+} which formed both hydroxides and sulfides. Al $(OH)_3$ formed gelatinous solids where Al $(OH)_3$ was white. The concentration of S^{2-} became high enough to precipitate FeS and MnS. FeS was black as MnS, like Mn^{2+} ion was pale pink

4. Discussion

4.1 Cytotoxicity

The Bambara nuts extract showed appreciable degree of cytotoxicity against Vero cell line (Figure 1) at IC_{50} = 19.27 µg/ml. This could be attributed to saponin, tannins, terpenoids, and alkaloids that were demonstrated to be present in the extract. This observation was in agreement with previous cytotoxicity

reports for other extracts against cancerous cells [36 & 37]. The dark brown Bambara cultivar was more active at IC $_{50}$ = 15.5 µg/ml followed by the cream spotted cultivar at IC $_{50}$ = 17.9 µg/ml against the Hep 2 cell line (Table 3). The activity of the extracts against the DU 145 human cancer cell line was assessed and all the six cultivars showed lower activity of IC50 = 34 µg/ml as compared to their activity against Hep 2 cell lines. Hep 2 cancer cells were more sensitive to the extract in comparison to DU 145 cell line. United States National Cancer Institute (US-NCI) establishes that a crude extract that shows an IC$_{50}$ value of less than 100 µg/mL is considered active [38]. When the IC$_{50}$ value is lower than 30 µg/mL, the US-NCI considers a crude extract promising for purification and a biological activity study. IC50 values below this stringent point were noted with two extracts in at least two of the three studied cell lines, the lowest (15.5 µg/ml and 17.9) being obtained with dark brown and cream spotted Bambara nut extracts in Hep 2 cancer cell lines and 19.2 µg/ml in vero cell lines (Table 3). These data suggests that the dark brown and cream spotted Bambara nut extracts are more toxic to cancer cells than vero normal cells lines. Therefore, the extracts from dark brown and cream spotted could be considered as better candidates for potential anticancer herbal drugs. Bambara nut extracts have phytochemicals that include terpenoids, saponins, flavonoids, and alkaloids that may be responsible for the cytotoxic effects against all these cell lines. Phytoprinciples in medicinal plants such as alkaloids and terpenes and fats have been used to cure human ailments like cancer [41]. Phytochemicals from medicinal extracts have been shown to exhibit cytotoxicity through the succeeding down-regulation of numerous toxicity and anti-apoptosis gene products [40].Several cytotoxicity reports on cell lines reveals the importance of chemical constituents as potent cytotoxic agents against different cell lines. *In vitro* studies on phytochemicals suggested that they exhibit cell growth arrest probably at different cell cycle phases [42].

4.2 Antibacterial Activity

The extract of Bambara nut inhibited growth in all the tested bacterial strains as shown in Table 4. The results showed that increase in concentration of extract caused an increase in the zone of inhibition [20]. The activities of extracts were less on comparison to those of ceftriaxone (Table 4). The zone margin was taken as the area showing no obvious, visible growth that can be detected with the unaided eye. Faint growth of tiny colonies, which was detected only with a magnifying lens at the edge of the zone of inhibited growth, were ignored. The sizes of the zones of inhibition were interpreted by referring to zone diameter interpretative standards and equivalent minimum inhibitory concentration breakpoints of the National Committee for Clinical Laboratory Standards (NCCLS) M100-S12: Performance Standards for Antimicrobial Susceptibility Testing: Twelfth Informational Supplement. The extract was found to be most effective against *Escherichia coli* showing the maximum zone of inhibition (27.0±0.7 mm) followed by *Staphylococcus aureus* (25.3±0.4) whereas low activity for *pseudomonas auriginosa* (25.1±0.2mm) at 100 µg/ml of the extract. All the tested organisms were affected moderately hence intermediate inhibition

98

with the MeOH−CH$_2$Cl$_2$ nut extracts. The extract also showed good activity against both gram positive and gram negative tested microorganisms though with varied zones of inhibition at the tested concentrations. This effect is in agreement with the antibacterial studies and may be attributed to the presence of phytochemicals in the nut [13, 44 & 45]. In the study, a micro dilution test, a quantitative test that allows for higher accuracy and reproducibility, was used to determine MIC value [9&11]. MIC (in μg/ml) for the extracts indicated too, the presence of the activity in the nut extract. MIC was expressed as the lowest dilution, which inhibited growth judged by lack of turbidity in the tube.

4.3 Antifungal Activity

Nut extracts showed antifungal activity against the fungal *Candida albicans*. However, fungal sensitivity did not vary according to the nut cultivars. The extract significantly reduced the mycelial growth of the tested fungi from 62.44% to 75.6% to 91.12% at the concentration of 1 μg/ml through 4 μg/ml as compared to control drug (clotrimazole) which showed inhibition at 71.2% to 81.4% to 95.3%. The concentration of the extract was directly proportional to the activity on mycelial growth. The observation is in agreement with the antifungal studies reported for extracts previously prepared from other plants [8, 10, 22 &33]. In general, there was a positive relationship between the concentrations of the MeOH−CH$_2$Cl$_2$ extracts and the inhibition rate on mycelia growth of the tested fungi activities. This shows that the nuts are the reservoirs of valuable phytochemicals and fats/oils signifying that they possess a good activity against *Candida albicans*.

Previous studies reported that other plant extracts inhibit the Ras−cyclic AMP (cAMP)−protein kinase A (PKA) cascade, thereby inhibiting hyphal growth and inducing the expression of the catalase−encoding gene *CAT1*. In addition to its effect on *C. albicans* signaling pathways, extracts can induce ROS accumulation within *C. albicans* cells, which may protect against subsequent OX stress. The cause of ROS generation in response to extracts is poorly understood though [10, 46].

4.4 Macro and micro minerals

Bambara nuts contains both micro and macro minerals. This qualitative study was in agreement with qualitative studies on Bambara nut to determine sodium and potassium by flame photometry [31, 47]. Qualitative analysis towards micronutrients revealed the presence of aluminium, manganese and iron was still in agreement with early reports [31, 48 & 49] on microminerals (aluminium, manganese and iron) which were determined using atomic absorption spectrophotometer. The existence of certain elements in diet is as imperative as the choice of diet for reducing the incidence of cancer and even in the management of the cancer patient. Magnesium deficiency may thus result in a variety of metabolic abnormalities and clinical consequences even cancer development [50]. Researchers [51] in Poland in their work had a conclusion that scantiness of magnesium (Mg) and other antioxidants are significant risk factors in the predisposition to various forms of leukemia. Other researchers [52] found out that 46% of patients admitted to an ICU in a tertiary cancer center presented low (Mg)

99

levels. They concluded that the incidence of hypomagnesaemia in critically ill-cancer patients is high. This shows magnesium link with cancer. Some researchers [53] reported high dietary intake of magnesium may decrease risk of colorectal cancer in Japanese men. They studied and examined the association between dietary intake of magnesium and CRC risk in Japanese men and women aged 45–74 y and they concluded that higher dietary intake of magnesium may decrease the risk of colorectal cancer (CRC) in Japanese men. It's seen that Magnesium deficiency would lead to a functional imbalance in cells, ultimately setting the stage for cancer. Anything that weakens cell physiology will lead to the infections that surround and penetrate tumor tissues. These infections are proving to be a fundamental part of cancer. Magnesium deficiency poses a direct threat to the health of our cells. Without appropriate amounts our cells calcify and lead to death. A study of rats surviving magnesium deficiency sufficient to cause death and convulsions during early infancy in some, and heart/kidney lesions weeks later in others, disclosed that some of survivors had thymic nodules or lymphosarcoma which also shows a link between magnesium and cancer [54]. It is known that carcinogenesis induces magnesium distribution disturbances, which cause its mobilization through blood cells and depletion in non-neoplastic tissues. Magnesium deficiency appears to be carcinogenic, and in the case of solid tumors, a high level of supplemented magnesium inhibits carcinogenesis [55]. Epidemiologic and experimental data suggested that adequate magnesium might protect against initiation of precancerous cellular changes. Other elements with some anticancer activity include molybdenum, zinc, magnesium, and germanium [56].

5.0 Conclusions

Phytochemical analysis indicated that Bambara nuts contained terpenoids, saponins, alkaloids and tannins. The major secondary metabolites were present in all cultivars. The extract showed more activity on Hep 2 and Vero cell lines but less on DU 145. Specifically, cytotoxic activity against Hep 2 cells might affirm its traditional use to treating liver cancer. It may be worthwhile to investigate antimetastasis and other anticancer activities together with exploring the specific chemical compounds, which are responsible for cytotoxic action and their mechanism of actions. Furthermore, demonstration of antimicrobial activity against the test isolates is an indication that there is possibility of sourcing alternative antibiotic substances in this nut for the development of newer antibacterial agents. Based on the above observations, we recommend further evaluation of this nut on other normal and cancerous cell lines and their efficacy as antimicrobial agents. Although *in vitro* toxicity cannot be directly extrapolated to *in vivo* toxicity, the results obtained portrays a low risk of the indigenous application of Bambara nut extracts.

100

cooperation and assistance when carrying out antimicrobial activity.

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