SJIF Impact Factor: 5.464

WORLD JOURNAL OF ADVANCE HEALTHCARE RESEARCH

ISSN: 2457-0400
Volume: 4.
Issue: 3.
Page N. 147-155
Year: 2020

Original Article www.wjahr.com

THE PHYTOCHEMICAL CONTENT AND ANTIMICROBIAL ACTIVITY OF MUNG BEAN (*VIGNA RADIATA* L.) EXTRACT AGAINST SOME SELECTED PATHOGENS OF MEDICAL IMPORTANCE

Adeoye John Kayode[1,2]*, Adewole Sunday Awe[4], Folasade Banji-Onisile[2], Adebanke Adeorite[3] Agboola and Olusegun Victor Oyetayo[2]

[1]Department of Microbiology, University of Fort Hare, South Africa.
[2]Department of Microbiology, Federal University of Technology, P. M. B., 704 Akure, Nigeria.
[3]Department of Medical Microbiology and Parasitology, Afe Babalola University, Ado-Ekiti, Nigeria.
[4]Department of Science Laboratory Technology, Federal Polytechnics Offa, Kwara State.

Received date: 03 April 2020 **Revised date:** 24 April 2020 **Accepted date:** 14 May 2020

***Corresponding author: Adeoye John Kayode**

Department of Microbiology, University of Fort Hare, South Africa.

ABSTRACT

The current study investigated the phytochemical and antimicrobial characteristics of unsprouted mung bean seed obtained at Igasi Akoko, in Akoko North West Local Government Area of Ondo State, Nigeria. The seed (*Vigna radiata* L.) was grounded into powder and divided into two equal parts (A and B). Sample A was used as a positive control for bioactive components present in the seed. The crude extract of sample (B) was obtained by soaking in ethanol to extract the bioactive components. Qualitative and quantitative phytochemical analyses of samples (A) and extract of sample B were assessed. The phytochemical assay revealed that alkaloid, saponin, terpenoid, steroid, and cardiac glycosides were present, ranging from 12.47 to 47.25 mg/g and 8.05 to 27.73 mg/g respectively. The antimicrobial activity of the extract was tested against referenced microbial cultures using appropriate microbiological techniques. The antimicrobial activities of the extract vary with different zones of inhibition ranging from 2.85 to 3.10 mm against *Salmonella typhi*, ATCC6539 (3.05 mm) *Pseudomonas aeruginosa* ATCC9027 (3.10 mm) and *Citrobacter freundi* ATCC8090 (2.85mm) but *Klebsiella pneumoniae*, and *Enterobacter aerogene*s were resistant. The extract did not exhibit any antifungal effect against *Candida albicans*, *Cryptococcus neoformans* and *Aspergillus fumigatus.* In light of this finding, mung bean seed could be considered as a potential source of natural antimicrobial agent.

KEYWORDS: Extract, bioactive components, antimicrobial, phytochemical analyses.

INTRODUCTION

Over the three decades, there has been an increase in the necessity to examine natural sources for new biologically active agents as a result of the increase in microorganisms developing resistance against a large number of antibiotics (Awala and Oyetayo 2015; Mitchell *et al.*, 2008; Stadler and Keller, 2008). Therefore, there is a necessity for a rapid approach to developing alternative antimicrobial drugs. One way to achieve this is screening native plants of medicinal importance that symbolize a wealthy source of new antimicrobial agents (Tateda and Ishii, 2003; Altaf *et al.*, 2019). Natural bioactive materials from plants have customarily supplied industries producing pharmaceutical drugs with one the of major sources of compounds which forms about 40% of the natural derivative of modern drugs, using either the synthesized version or the natural substance (Gautam *et al.*, 2007; Altaf *et al.*, 2019).

Legumes are among the plants capable of producing a high diversity of secondary metabolites which usually serve as the compounds for defense against microbes and herbivores, these compounds could be used as a structural pillar for synthetic drug modifications (Anjana *et al.*, 2011; Wink, 2013; Martín-Cabrejas, 2019). The genus *Vigna*, formerly known as *Leguminosae* now *Fabaceae* (Martín-Cabrejas, 2019) is made up of more than 200-member species originating from the tropical regions of the world. *Vigna* genus is closely related to the genus *Phaseolus*, which is made up of more than 20 species that are native to the tropical regions (Baudoin *et al.*, 2001).

Mung bean which is equally known as golden gram or green gram is the seed of *Vigna radiata* (Guriqbal *et al.*, 2011; Wilczek, 2008), native to the Indian subcontinent (Walshaw, 2010; Venkidasamy *et al.*, 2019). It is a leguminous plant which could be an erect or climbing (Fuller, 2007). The seeds are small, ovoid in shape and mostly green but could also show other colours (Adeoye and Oyetayo, 2016).

Recent claims on the antimicrobial activities of sprouted mung bean seed have aroused global interest in mung bean. There has been a dearth of vital information on the antimicrobial properties and phytochemical of the unsprouted mung bean seed. Therefore, the present study investigated the phytochemical and antimicrobial prospects of unsprouted mung bean seed extract indigenous to Nigeria using simple conventional microbiological techniques of antimicrobial screening through disc diffusion assay.

MATERIALS AND METHODS

Collection of mung bean

Mung bean (*V. radiata* L.) seed used for this study were obtained from Oba market, Igasi Akoko, in Akoko North West Local Government Area of Ondo State, Nigeria. The seeds were taken to the microbiology laboratory, Federal University of Technology, Akure (FUTA), Ondo State, Nigeria for analysis.

Collection of Test microorganisms

Typed cultures (*Klesiella pneumoniae* ATCC 1883, *Enterobacter aerogenes* ATCC 3048, *Salmonella typhi* ATCC 6539, *Pseudomonas aeruginosa* ATCC 9027, *Citrobacter freundii* ATCC 8090) were procured at the Microbiology Laboratory, Federal Institute of Industrial Research, Oshodi (FIIRO) Lagos, Nigeria. *Aspergillus fumigatus*, *Cryptococcus neoformans* and *Candida albicans* were obtained from the culture bank of the Department of Microbiology, Federal University of Technology, Akure (FUTA). The isolates were resuscitated in peptone water, tested for viability and were further purified on nutrient and Sabouraud dextrose agar (SDA) and were incubated at 37°C for 24 h and 27°C for 48 - 72 h, respectively for bacteria and fungi. The organisms were preserved on slant agar in a refrigerator (Haier Thermocool HR-137 Quinda, China), at 4°C, for further studies.

Sample preparation

Mung bean seeds (800 g) were weighed using an electronic weighing balance (Electronic Balance, MT-301 Model) and sorted to remove unwanted materials from the seeds. The seeds were grounded into coarse particles using a mortar and pestle and the particles were further reduced to a fine powder using attrition milling machine and stored in grease free, airtight container to avoid absorption of moisture. The powdered sample was divided into two equal parts (A and B). Sample A was served as a positive control for bioactive components present in the seed. The qualitative and quantitative phytochemical components were determined on sample A. Bioactive components of sample B were extracted with ethanol and phytochemical analysis was done on the extract.

Preparation of mung bean seed extract

Preparation of seed extract was done as described by Jayaraman *et al.* (2014) with modifications. The mung bean flour (400 g) was extracted in an Erlenmeyer flask containing 97% ethanol covered with aluminium foil for 72 hours, at laboratory temperatures (25±2) and stirred occasionally by gently shaking the flask. The mixture was then filtered using three-fold sterile muslin cloth and the filtrates were allowed to air dry to recover the crude extract. The extract was put into a sterile container, and preserved at 4°C for further studies.

The percentage yield of the extract was estimated based on dry weight as follows:

Where,

$$\text{Yield (\%)} = \frac{W_1 \times 100}{W_2}$$

W_1= weight of extract after solvent evaporation W_2 = Weight of the grounded mung bean powder.

Phytochemical screening of unsprouted mung bean seed and seed extract

Qualitative and quantitative phytochemical analysis was carried out on the samples of the finely grounded unsprouted mung bean seed as well as the crude extract obtained from mung bean seed to determine its bioactive components and its extract through standard protocols earlier described (Harborne, 2005).

Qualitative phytochemical screening of mung bean extract

Test for tannins

Mung bean samples were gently mixed in purified water followed by filtration after which solution of Ferric chloride reagent (0.1 % $FeCl_3$) was added to the filtrate. Blue, blue-green or black precipitate was recorded as a proof for the presence of tannin (Trease and Evans, 2005).

Test for alkaloids

Five mL of HCl (10 % v/v) were introduced to 0.5 g of the specimen in test tubes and were placed in a water bath for a period of 2 mins, and the mix was filtered. Three drops of Dragendroff's reagent was added to 1 mL of the filtrates to treat it in order to separate it into portions. A reddish-brown colouration confirmed alkaloids presence (Adegoke *et al.*, 2010)

Test for steroids

About 2 mL of acetic anhydride was added to 0.5 g to each sample; 2 mL of H_2SO_4 was also added. Colour violet turning to green or blue revealed steroids presence (Boxi *et al.*, 2010).

Test for saponins
The capability of saponins to form an overflowing mass of small bubbles in aqueous solution was used as a confirmatory test for saponins. About 0.5 g of the specimen was boiled in distilled water using a water bath and the content was agitated for a steady continuing frothing. The froth was homogenized with three drops of olive oil and vigorously shaken, it was then checked for emulsion formation (Adegoke *et al.*, 2010).

Test for phlobatannins
Aqueous extract of mung bean seed was boiled with 1% HCl(aq). Formation of deposit of red precipitate was taken as preliminary proof for the presence of phlobatannin.

Testing for anthraquinones
Five mL of benzene and 0.2 g of the seed samples were shaken together. About 5 ml of 10% (NH_3) solution was added to the filtrates and the mixes were swayed. Presence of pink-red, red or violet colour in ammonium medium (lower) phase shows the presence of free hydroxyl-anthraquinone.

Testing for flavonoids
Mung bean samples were heated alongside ten mL of ethyl acetate for 30 minutes using a water bath. The mixtures were separated by filtration and 4 mL each, of the filtrates, were mixed with 1 mL of dilute (NH_3) solution in a conical flask. Formation of a yellow colouration reveals flavonoids presence (Shahid-Ud-Duaula and Anwarul, 2009).

Testing for terpenoids
The Salkowski test was used which involves mixing 5 mL of each extract in 2 mL of Chloroform, and 3 mL conc. (H_2SO_4) was cautiously added which then form a layer. A reddish-brown coloration confirms terpenoids the presence.

Testing for cardiac glycosides
Legal test
The samples were dissolved in pyridine. Few drops (20%) of Sodium nitro preside alongside with 20% Sodium hydroxide (NaOH) was added. Presence of glycosides was confirmed by a change in colour from violet to blue to green (Obianime and Uche, 2008).

Lieberman's test
A 0.2 g of mung bean samples were dissolved in 2 mL of Acetic anhydride. The mixture was cooled in ice. H_2SO_4 was then carefully added. Confirmation of the presence steroidal nucleus was by looking out for a change in colour from violet to blue to green, i.e. aglycone fragment of cardiac glycoside.

Salkowski's test
A 0.2 g of mung bean samples were dissolved in 2 mL of Chloroform. Acid (H_2SO_4) was meticulously added which formed a lower layer. Formation reddish-brown colour at the interface confirms terpenoid presence (Trease and Evans, 2005).

Keller - Kiliani's test
A 0.2 g of mung bean samples were dissolved in glacial acetic acid (2 mL) comprising a drop of $FeCl_3$ solution. The mixture was under layered with 1 mL of conc. H_2SO_4. A brown ring that formed at the interface indicates the presence of a deoxy-sugar, cardenolides characteristic. There may be the formation of brown ring below the violet ring and in the acetic acid layer as well, a greenish ring may form exactly above the ring and slowly spread around this layer (Trease and Evans, 2005).

Quantitative phytochemical screening of mung bean seed and mung bean seed extract
Cardiac glycosides Determination
Buljet's reagent as outlined by El-Olemy *et al.* (1994) was used for evaluating cardiac glycoside content in mung bean samples. They were further refined using lead acetate and di-sodium hydrogen phosphate (Na_2HPO_4) solution before adding fresh preparation of Buljet's reagent (aqueous picric acid (95 mL) + 5 mL of 10% aqueous NaOH). The dissimilarity between the strength of the colours of the blank specimens and experimental (distilled water and Buljet's reagent) shows the absorbance and it corresponds to the glycosides concentration.

Alkaloid determination
Five grams of mung bean samples were transferred into 250 mL beaker each and 200 mL of 10 % acetic acid in ethanol was added and covered. The solution was left to stay for four hours after which they were filtered and the extract was evaporated using a water bath to a-quarter of the original volume. Conc. Ammonium hydroxide (NH_4OH) was added in drops to the extract until complete precipitation was formed. The solution was allowed to stay and the precipitate was recovered and washed in dilute (NH_4OH) and then filtered. The (Alkaloid) residue was then dried and weighed.

Terpenoids determination
Ten gram of powdered mung bean samples were dissolved in alcohol for 24 h. They were filtered and the filtrates were extracted in petroleum ether and the ether extract was regarded as total terpenoids (Obianime and Uche, 2008).

Determination of steroid
The method outlined Trease and Evans (2005) was adopted for determining the steroid content of mung bean samples. A portion of 2 mL was taken from a 2.5 g solution of powdered mung bean samples prepared in distilled water (50 mL) after shaking vigorously for one hour, it was then washed in 3 mL of 0.1M NaOH (pH 9). It was later mixed with 3 mL of ice-cold acetic anhydride and 2 mL of chloroform after which two drops of conc.H_2SO_4 was carefully added. Measurement of the

samples absorbance and the blank were read at 420 nm using Gulfex Medical and Scientific England, (Spectrum Lab 23A, model number 23A08215) spectrophotometer.

Saponins determination

The method of Obadoni and Ochuko (2001) was adopted for the quantitative assay of saponins. Twenty (20 g) grams of the powdered mung bean sample were put in 100 mL of 20% liquid ethanol and agitated in a shaker for 30 min after which the samples were heated over a water bath at 55°C for four hours. Filtration of the mixture was done and the residue were re-extracted with another 200 mL of 20% liquid ethanol. They were evaporated at 90°C to about 40 mL, using the water bath. After this, the concentrates were transferred into a separatory funnel (250 mL) and extraction was done twice with 20 mL of diethyl ether. After this, the aqueous layer was maintained and 60 mL n-butanol was added while the ether layer was discarded. The n-butanol extracts were washed two times with 10 mL of 5% aqueous sodium chloride and remaining solutions were heated to evaporate using a water bath and were dried in the oven at 40°C to a constant weight.

Determination of antimicrobial sensitivity of mung bean extract

Antimicrobial activity of mung bean extract in this study was done by agar well diffusion method (Schinor *et al.* 2007). Preparation of fresh cultures for the experiments were done by transferring a loopful of cells from the stock cultures to test tubes on the nutrient broth (NB) for bacteria and were incubated for 24 hours at 37°C to 5 mL of NB 0.2 ml of culture was inoculated and incubated to a turbidity that equals that of 0.5 Mc Farland standard which is the equivalent of 10^6 – 10^8 CFU/ml. Cooled (45°C) molten Mueller Hinton agar (20ml) was poured into sterile petri-dishes and were left to solidify. A portion of the cultures were spread on the surface of the solidified agar plates. About 8 mm wells were bored in the agar with sterile cork borer (8 mm).

Preparation of Fungi spores was done from fresh matured cultures that grown at 25 ± 1°C on an SDA (Sabouraud dextrose agar). The suspensions were adjusted to by microscopic enumeration to 10^6 spores per ml with a hematocytometer cell counter. About 20 mL of cool (45°C) molten Mueller Hinton agar was dispensed into sterile plates to solidify. The cultures were superficially spread on the agar plates and wells were bored in the agar with sterile cork borer (8 mm). Crude extract initially dissolved in 30% dimetheylsufoxide (DMSO) of different concentrations (50, 100, 200, 300 and 400 mg/mL) were passed through 0.22 µl membrane filter were introduced to each wells using micropipette. Commercialized antibiotic discs were used as a positive control for bacteria (cloxacillin (5µg), ofloxacin (5 µg), augumentin (30 µg), ceftazidin (30 µg), cefuroxin (30 µg), gentamycin (10 µg), chlorotetracyclin (30 µg), and erythromycin (10 µg) while clotrimazole was used for fungi.

Negative control (30%) DMSO was used and the plates were left for 1 h at room temperature (25 ± 1°C) to give room for the complete spread of the seed extracts into the media. The plates were incubated at 37^{o}C for 24 hours for bacteria and fungi were incubated at (25 ± 1°C) for 48 to 72 h. The experiment was done in triplicates and inhibition zones were taken with a ruler. The diameters of the wells were subtracted before calculating the inhibition zones.

Minimum inhibitory concentration determination

The agar well diffusion method was used to test for the antimicrobial effect of the various concentrations of the extracts (50 to 400 mg/mL). Dilution of the extract concentration that had no visible growth was used as the MIC of the extract against the tested microorganisms. Negative control (30%) DMSO stood for negative control and then tests were performed in triplicates. This test was performed in order to compare the sensitivity of the microorganisms to different commercial antibiotics. The experiment was done in triplicates and inhibition zones were taken with a ruler. The diameters of the wells were subtracted before calculating the inhibition zones.

Statistical analysis

Statistically analysis of all obtained data was done using one-way ANOVA (analysis of variance) and the means were separated by Duncan's Multiple Range Test (SPSS 16.0 Version). Differences were examined significant at 0.05%

RESULTS

Qualitative phytochemical screening of mung bean seed revealed that Alkaloid, terpenoid, saponin, and steroid were present in the seed powder as well as in the seed powder extract. Anthraquinone, flavonoid, tannin, and phlobatanin were absent in mung bean samples subjected to phytochemical screening. All cardiac glycosides assayed for being positive in the seed powder, but Keller killion test was negative in the seed powder extract (Table 1). The Quantitative phytochemical component of unsprouted mung bean seed is presented on table (2).

Table 1: Qualitative phytochemical screening of mung bean seed.

Phytochemicals	Seed powder	Seed powder extract
Alkaloid	+	+
Anthraquinone	-	-
Terpernoid	+	+
Flavonoid	-	-
Tannin	-	-
Saponin	+	+
Phlobatanin	-	-
Steroid	+	+
Cardiac Glycosides		
Legal Test	+	+
Keller Killion Test	+	-
Salkowski Test	+	+
Lieberman Test	+	+

Key: + = present, - = absent

Table 2. Quantitative phytochemical component of unsprouted mung bean seed.

Extract source / Phytochemical content (mg/g)		
Phytochemicals (mg/g)	**Seed power**	**Seed powder extract**
Cardiac glycosides	17.48 ± 0.14^{c}	25.67 ± 0.03^{c}
Alkaloid	47.25 ± 0.01^{d}	27.73 ± 0.01^{d}
Terpernoid	13.75 ± 0.03^{b}	21.92 ± 0.03^{b}
Steroid	12.23 ± 0.01^{a}	8.05 ± 0.03^{a}
Saponin	ND	ND

Values are presented as Mean ± S.D (n=3). Means with the same superscript letters along the same column are not significantly different (P < 0.05).
Key: ND (Not determined).

The extract exhibited antimicrobial activity against some of the bacteria pathogens but two of the pathogens were resistant to the extract (Table 3). Also, the extract had no antimicrobial effect against all fungi isolates. The antimicrobial effect of the commercial antibiotics, when compared to that of the unsprouted mung bean extract, was higher and significantly different (P< 0.05) (Table 2). For bacteria, the assay revealed that commercial antibiotics (ofloxacin, and gentamycin exhibited antimicrobial effect against all the test bacteria except *Salmonella typhi* that was completely resistant to all commercial antibiotics used in this study. Antibiotics (cloxacillin, augumentin, ceftazidine, cefuroxin, chlorotetracyclin and erythromycin) had no antimicrobial effect on any of the test organisms (bacteria). The commercial antifungal compound, clotrimazole had an antifungal effect against all the test fungi (Table 4).

Table 3: Antimicrobial activities of mung bean extract and commercial antibiotics against common pathogens.

	Zones of inhibition (mm)							
Test Organisms	**MBE 50 mg**	**MBE 100 mg**	**MBE 200 mg**	**MBE 300 mg**	**MBE 400 mg**	**Ofloxacin (5 µg)**	**Gentamycin (10µg)**	**Clotrimazole (1 mg/ml)**
Klesiella pneumoniae ATCC 1883	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	19.00 ± 0.00^{e}	4.00 ± 0.00^{b}	ND
Enterobacter aerogenes ATCC 3048	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	9.00 ± 0.00^{b}	4.00 ± 0.00^{b}	ND
Salmonella typhi ATCC 6539	0.00 ± 0.00^{a}	2.03 ± 0.10^{b}	2.55 ± 0.10^{c}	2.73 ± 0.10^{c}	3.05 ± 0.77^{c}	0.00 ± 0.00^{a}	11.00 ± 0.00^{e}	ND
Pseudomonas aeruginosa ATCC 9027	2.03 ± 0.10^{b}	2.26 ± 0.12^{c}	2.50 ± 0.20^{b}	2.73 ± 0.10^{c}	3.10 ± 0.00^{c}	14.00 ± 0.00^{c}	11.00 ± 0.00^{d}	ND
Citrobacter freundii ATCC 8090	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	2.53 ± 0.10^{b}	2.85 ± 0.10^{b}	15.00 ± 0.00^{d}	5.00 ± 0.00^{c}	ND
Aspergillus fumigates	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	ND	ND	19.00 ± 0.66^{d}
Cryptococcus neoformans	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	ND	ND	14.00 ± 0.7^{d}
Candida albicans	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	ND	ND	0.9 ± 06^{c}

Values are presented as Mean ± S.D (n=3). Means with the same superscript letters along the same column are not significantly different (P<0.05)
Keys: ATCC: American Type Culture Collection; MBE: Mung bean seed extract, ND= not determined.

Table 4: Minimum inhibitory concentration (mg/ml) of unsprouted mung bean seed extract against test organisms.

Test Organism	Diameter of inhibition obtained for the Minimum inhibitory concentration (MIC)
Klesiella pneumoniae ATCC 1883	ND
Enterobacter aerogenes ATCC 3048	ND
Salmonella typhi ATCC 6539	2.03
Pseudomonas aeruginosa ATCC 9027	2.03
Citrobacter freundii ATCC 8090	2.53
Aspergillus fumigates	ND
Cryptococcus neoformans	ND
Candida albicans	ND

Values are presented as mean ± S.D (n=3).
Key: ATCC: American Type Culture Collection; ND: Not determined.

DISCUSSION

Mung bean is one of the most essential short-seasoned, summer-growing legumes widely grown throughout the tropic and subtropical regions. Almost all of the described components from plants that are active against microorganisms are aromatic or saturated organic compounds (Rand *et al.*, 2011). Bioactive compounds in plants are often extracted with ethanol (Ahmad *et al.*, 1998; Keerthiga and Anand, 2015; Lin *et al.*, 1999). Therefore, ethanol was used as a solvent to extract the bioactive compounds/antimicrobial agents in this study in order to prepare the basis for monitoring different microbial agents as a prelude for the future separation of antimicrobial compound(s).

The results of the quantitative analysis of phytochemicals revealed the presence of alkaloid, saponin, cardiac glycosides, steroids and terpenoids in the mung bean extract, but flavonoid, tannin, and phlobatanin, were present. These phytochemicals have been acknowledged to be active biologically and they usually act as a defense system in plants against microbial, insects and some herbivoral predation (Bonjar *et al.*, 2004; Tang *et al.*, 2014). This suggests that mung bean may be a good source of bioactive compounds which could be applicable in the treatment of disease infections d (Tang *et al.*, 2014). Taleb-Contini *et al*. (2003) in his experiment gave an insight into the antimicrobial identity of steroids from *Chromolaena* plant species against *Streptococcus sobrinus and Streptococcus mutans* strains. Also, Neumann *et al*. (2004) studied the antiviral identity of steroids. The anticarcinogenic (e.g., perilla alcohol), anti-ulcer, antimalarial (e.g., artemisinin), hepaticidal, antimicrobial or diuretic (e.g., glycyrrhizin) effects of terpenoids was been reported by (Dudareva *et al*., 2004). Theng and Korpenwar (2015) reported that *Pueraria tuberosa* tuber quantitatively revealed the presence of flavonoids, alkaloid, saponin, and terpenoids which subscribed to the medicinal value of the plant. Ververidis *et al.* (2015) also reported that flavonoids are directly or indirectly connected to the health of plant physiology and may change dramatically under biotic and abiotic stress (Christos *et al.*, 2016). Stimulation of plant defense systems, such as those comprising proteinase inhibitors, produces a response that protects the plant from these types of stresses (Kessler and Baldwin, 2002; Tang *et al.*, 2014) and as a result of this response, accumulation of secondary metabolites with various health benefits has been observed (Bowles 1990; Kessler and Baldwin, 2002). Lee *et al.* (2011) observed that saponin and flavonoid content of mung bean systemically contribute to its overall antioxidant activity.

Previous work of some scholars on sprouted mung bean seed gave some useful information its therapeutic value. The study carried out by Rand *et al*. (2011), revealed its antibacterial activities against *Helicobacter*. *pylori*. Hafidh *et al*. (2011) provided additional evidence that methanol extract of mung bean seed extract shows both antimicrobial and antifungal activities against multi-drug resistant bacteria including gram-positive organisms. More recently, Siti *et al.* (2013) reported a broad spectrum of activity of sprouted mung bean seed against all bacteria tested with the exception of *Klebsiella. pneumoniae* that showed negligible inhibitory activity.

In the present investigation, ethanol extracts of mung bean seed were screened for their antibacterial activities against six gram-negative enteric bacteria, and tree fungi selected on the basis of their clinical and pharmaceutical importance.

Results of the antimicrobial assay revealed unequal antimicrobial activities which might be due to a number of factors. Some scholars earlier discovered that the antimicrobial activities of biological extracts are variable, which may be dependent on the test organisms, media used in culturing test organisms, environmental nature, variation in physical and biochemical structure of the antimicrobial components of the biologically active agent, and the extraction solvent (Iwalokun *et al*., 2007; Ramesh and Manohar, 2010). The extract showed inhibitory activity against *Pseudomonas aeruginosa*, *Salmonella typhimurium* and *Citrobacter freundii*. However, *Klebsiella pneumoniae*, *Enterobacter aerogene* were resistant. *Candida albicans*, *Cryptococcus neoformans* and *Aspergillus fumigatus* were resistant to the antimicrobial effect of the extract.

The result of the antibacterial assay in this study is in agreement with the study carried out by Rand *et al*. (2011), Hafidh *et al*. 2011, Siti *et al*. (2013) but that of antifungal assay contrast the earlier report of Hafidh *et al* (2011). Also, Awala and Oyetayo (2015) reported that fungi were more susceptible than bacteria in their studies on *Trametes elegans* and their result is at variance with this study. The zone of inhibitions produced by ethanol extract is not close to that produced by the commercially available antibiotics used. This may be due to the fact that the extract of unsprouted mung bean seed used for antimicrobial assay in this study may not present much activities and more abundant of secondary metabolites since relevant biosynthetic enzymes are activated during the initial stages of germination. Thus, Tang *et al.* (2014) reported that germination may have improved the medicinal qualities of mung bean.

Results of the present study show unsprouted mung bean extract may be a good source of the antibacterial drug. Although reasonable numbers of natural or synthetic antimicrobials are available to control pathogenic microorganisms effectively, it is important to note the increasing global antimicrobial resistance which is becoming a public health problem. Therefore, more still need to be done on the search for new antimicrobial agents from various biological sources continuously. Since unsprouted mung bean seed extract (*Vigna radiata*) showed antimicrobial activity to some selected gram-negative organisms. This provides evidence that it may be effective and cheap antimicrobial source for both natural and pharmaceutical industries.

CONCLUSION

The present study was able to establish the antimicrobial potentials of Mung bean seed extract from mung bean samples obtained from Igasi Akoko, in Akoko North West Local Government Area of Ondo State, Nigeria. This reveals the therapeutic use of mung bean seeds as a natural alternative agent that could be used to complement antimicrobial therapy.

Conflict of interest: None.

REFERENCES

1. Adegoke AA, Iberi PA, Akinpelu DA, Aiyegoro OA, Mboto CI Studies on phytochemical screening and antimicrobial potentials of Phyllanthus amarus against multiple antibiotic resistant bacteria. Int. J. Appl. Res. Nat. Prod, 2010; 3(3): 6–12.
2. Altaf, M.M., Khan, M.S.A. and Ahmad, I., Diversity of Bioactive Compounds and Their Therapeutic Potential. In *New Look to Phytomedicine*, 2019; 15-34.
3. Anjana MK, Gangarao B, Hari priya T, Venna NM, Reeshma SK A phytopharmacological review on *Vigna* species. Intl. J. of Adv. in Pharm. Sci., 2011; 2(1): 2231-0541.
4. Awala SI, Oyetayo VO The Phytochemical and Antimicrobial Properties of the Extracts Obtained from *Trametes elegans* Collected from Osengere in Ibadan, Nigeria. Jor. J. of Biol. Sci., 2015; 8(4): 289 – 299. ISSN 1995-6673.
5. Baudoin JP, Vanderborght KPM, Mwang'ombe AW Common beans In: Crop Production in the Tropical Africa. Eds Romain, H. R.). Macmillian Press, USA, 2001; 354-359.
6. Bonjar GHS, Nik AK, Aghighi S Antibacterial and antifungal survey in plants used in indigenous herbal-medicine of south east regions of Iran. J. Biol. Sci., 2004; 4: 405-412.
7. Bowles DJ Defense-related proteins in higher plants. Annual Rev. Biochem, 1990; 59(1): 873–907.
8. Boxi M, Rajesh Y, Raja KV, Praveen B, Mangamma K Extraction, phytochemical screening and in-vitro evaluation of anti-oxidant properties of *Commicarpus chinesis* (aqueous leaf extract). Int. J. Pharm. Biosci., 2010; 1: 537–547.
9. Christos K, Sri S, Clemens van de W, Richard GF, VisserC, Gerard van der L, Yuling B Responses to combined abiotic and biotic stress in tomato are governed by stress intensity and resistance mechanism. J. Expl. Bot., 2016; 67(17): 5119-5132.
10. Dudareva N, Pichersky E, Gershenzon J Biochemistry of plant volatiles. Plant Physiol, 2004; 135: 18931902.
11. El-Olemy MM, Al-Muhtadi FJ, Afifi AFA Experimental Phytochemistry: A Laboratory Manual. King Saud Univ. Press, Saudi Arabia, 1994.
12. Fuller DQ Contrasting pattern in crop domestication and domestication rates: Recent Archeological insights. The Wld. Annals of Bot, 2007; 100(5): 903-924.
13. Gautam R, Saklani A, Jachak SM Indian medicinal plants as a source of antimycobacterial agents. J. of Ethnopharmacol., 2007; 110: 200-234.
14. Guriqbal S, Sekhon HS, Gurdip SJS, Brar TSB, Shanmugasundram S Effect of plant diversity on the growth and yield of mungbean [*Vigna radiata* (L.) Wilczek] genotypes under different environment in India and Taiwan. Intl. J. of Agric. Research, 2011; 6(7): 573-583.
15. Hafidh RR, Abdulamir AS, Law S, Fatimah A, Faridah A, Fatemeh J, Zamberi S Novel in-vitro antimicrobial activity of *Vigna radiata* (L.) R. Wilczek against highly resistant bacterial and fungal pathogens. J. of Medicinal Plant Res., 2011; 5(16): 3606-3618.
16. Hamad AM, Fields ML Evaluation of the protein quality and available lysine in germinated and fermented cereals. J. of Food Sci., 1979; 44: 456.
17. Harborne JB Phytochemical methods. A guide to modern techniques of plant analysis, third ed. Springer Pvt Ltd, New Delhi, 2005.
18. Iwalokun BA, Usen UA, Otunba AA, Olukoya DK Comparative phtyochemical evaluation, antimicrobial and antioxidant properties of

Pleurotus ostreatus. Afr. J. Biotechnol, 2007; 6: 1732-1739.
19. Jayaraman P, Mathivanan K, Sivaprakasam E, Palani R, Rajesh V Analysis of antimicrobial activity and phytochemical potential of *Cassia absus* Linn., *C. auriculata*, 2014.
20. Kayode JA, Oyetayo VO Effect of Fermentation on the Microbial, Proximate and Mineral Composition of Mung Bean (*Vigna radiata*). J. of Applied Life Sci. Int., 2016; 5(4): 1-12, ISSN: 2394-1103.
21. Keerthiga M, Anand SP Bioactive Compound Evaluation of Ethanol Extract from Geodorum densiflorum (Lam.) Schltr. by GC-MS analysis. Intl. J. of Pharmacol., 2015; 5(6): 2277-3312.
22. Kessler A, Baldwin IT Plant responses to insect herbivory: the emerging molecular analysis. Annu Rev Plant Biol, 2002; 53: 299–328.
23. Lee JH, Jeon JK, Kim SG, Kim SH, Chun T, Imm JY. Comparative analyses of total phenols, flavonoids, saponins and antioxidant activity in yellow soy beans and mung beans. Intl. J. of Food Sci. Technol., 2011; 46: 2513–2519.
24. Lin J, Opoku AR, Geheeb-Keller, M, Hutchings AD, Terblanche SE, Jager AK, Staden J Preliminary screening of some traditional zulu medicinal plants for anti-inflammatory and antimicrobial activities. J. of Ethnopharmacol., 1999; 68: 267-274.
25. Linn. and *C. fistula* Linn. Indian J. of Med. and Healthcare, 3(1): 2278-2966.
26. Martín-Cabrejas, M.Á. ed., *Legumes: Nutritional Quality, Processing and Potential Health Benefits* (Vol. 8). Royal Society of Chemistry, 2019.
27. Mitchell AM, Strobel GA, Hess WM, Vargas PN, Ezra D *Muscodor crispans*, a novel endophyte from *Anans ananassoides* in the Bolivian Amazon. Fungal Divers, 2008; 31: 37-43.
28. Neumann UP, Berg T, Baha M, Puhl G, Guckelbeger O, Langreh JM, Neuhaus P Long-term outcome of liver transplant for hepatitis C: A 10 year follow-up. Transplant, 2004; 77: 226-231.
29. Obadoni BO, Ochuko PO (2001). Phytochemical studies and efficacy of the extracts of some haemostatic plants in Edo and Delta States of Nigeria. Global J. Pure Appl. Sci., 8: 203-208.
30. Obianime AW, Uche FI The phytochemical screening and effects of methanolic extract of Phyllanthus amarus leaf on the biochemical parameters of male guinea pigs. J. Appl. Sci. Environ. Manage., 2008; 12(4): 73–77.
31. Ramesh C, Manohar GP Antimicrobial Properties, Antioxidant Activity and Bioactive Compounds from Six Wild Edible Mushrooms of Western Ghats of Karnataka, India. Pharmacognosy Res., 2010; 2: 107-112.
32. Rand RH, Ahmed SA, Law Se V, Fatimah A, Faridah A, Fatemeh J, Zamberi S Inhibition of Growth of Highly Resistant Bacterial and Fungal Pathogensby a Natural Product. The Open Microbiol. J., 2011' 96: 1061874-2858.
33. Schinor EC, Salvador MJ, Ito IZ, Dias DA Evaluation of the antimicrobial activity of crude extracts and isolated constituents from *Chresta scapigera*. Braz. J. Microbiol., 2007; 38: 145-149.
34. Shahid-Ud-Duaula AFM, Anwarul BM Phytochemical screening, plant growth inhibition, and antimicrobial activity studies of Xylocarpus granatum. Malaysian. J. Pharm. Sci., 2009; 7(1): 9.
35. Siti NC, Zuhaida MZ, Zulkhairi A, Maimunah M, Emida M, Azlin SR In vitro Antimicrobial Activity of *Vigna radiata*(L) Wilzeck Extracts against Gram Negative Enteric Bacteria. Wld. Appl. Sci J., 2013; 21(10): 1490-1494.
36. Sperandio FF, Huang YY, Hamblin MR Antimicrobial Photodynamic Therapy to Kill Gram-negative Bacteria. Recent Pat Anti infect Drug Discov, 2013; 8: 108–120.
37. Stadler M, Keller NP Paradigm shifts in fungal secondary metabolite research. Mycol. Res., 2008; 112: 127-130.
38. Taleb-Contini SH, Salvador MJ, Watanabe E, Ito IY, Oliveira DCR Antimicrobial activity of flavonoids and steroids isolated from two *Chromolaena* species. Braz. J. Pharm. Sci., 2003; 39: 403-408.
39. Tang DY, Dong H, Ren L, Congfen H Review of phytochemistry, metabolite changes, and medicinal uses of the common food mung bean and its sprouts (*Vigna radiata*).Central J. Chem., 2014; 8: 1-4.
40. Tateda K, Ishii Y Multiple-drug resistant bacteria: How do they come to exist, spread, and express their pathogenicity? Nippon Naika Gakkai Zasshi, 2003; 92: 2097-2103.
41. Theng KB, Korpenwar AN Quantitative Estimation of Some Phytochemical and Determination of Metalic Elements from *Pueraria tuberosa* (Roxb. ex Willd) DC. Tuber. Int. J of Sci. and Res., 2015; 4(2): 434-438.
42. Trease GE, Evans MC Pharmacognosy, fourteenth ed. Elsevier, New Delhi, India, 2005.
43. Venkidasamy, B., Selvaraj, D., Nile, A.S., Ramalingam, S., Kai, G. and Nile, S.H., Indian pulses: A review on nutritional, functional and biochemical properties with future perspectives. *Trends in Food Science & Technology*, 2019.
44. Ververidis Fi, Trantas EA, Mattheos AG, Koffas Peng Xu When plants produce not enough or at all: metabolic engineering of flavonoids in microbial hosts. Front. in Plant Sci., 2015; 6: 7.
45. Walshaw SC Converting to rice: Urbanization, islamization and on pembe, 700-1500 AD. J. of Wld. Archeol, 2010; 42: 137-154.
46. Wilczek LR Brief introduction of mung bean (*Vigna radiata*) extract, green mung bean extract, powder (*Phaseolus aureus*), Roxb *Vigna radiata*, MD idea extract, 2008.
47. Wink M Evolution of secondary metabolites in legumes (Fabaceae). South Afr. J. of Bot., 2013;

89: 164–175.