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The Phytochemical and Antifungal Efficiency of Bean Leaf and Root against Some Pathogenic Fungi Isolated from Spoilt Vegetables Sold within Anambra Metropolies

Ejimofor Chiamaka Frances [a*], Nwakoby Nnamdi Enoch [b], Oledibe Odira Johnson [c], Afam-Ezeaku Chikaodili Eziamaka [c] and Mbaukwu Onyinye Ann [c]

[a] Department of Biological Sciences, Chukwuemeka Odumegwu Ojukwu University Uli, Anambra State, Nigeria.
[b] Department of Microbiology, Chukwuemeka Odumegwu Ojukwu University Uli, Anambra State, Nigeria.
[c] Department of Botany, Nnamdi Azikiwe University Awka, Anambra State, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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**Corresponding author: E-mail: cf.anyaegbu@coou.edu.ng;*

ABSTRACT

Investigated in vitro were the phytochemical and antifungal properties of bean leaf and root against certain pathogenic fungi isolated from spoiled vegetables marketed in Anambra metropolises. By combining 100g, 100ml of sterile distilled water, and ethanol with each plant part, two (2) distinct extract concentrations were produced from each plant component. The plants were subjected to phytochemical screening using several conventional techniques, which identified the presence of alkaloids, saponins, tannins, flavonoids, phytate, oxalate, and phenol in all of the plants but at various amounts. The effects of common antibiotics (Fluconazole 30 g/ml) were compared to those of plant extracts. According to a pathogenicity test, good vegetables rot was caused by Aspergillus niger, Fusarium solani, Penicillium sp, Rhizopus sp, and Mucor spp. The ethanol extract from beans root turned out to be more effective. The extraction solvent, extract concentration, and test pathogens all had an impact on the extract's effectiveness. When extract concentration rose, the amount of fungal growth inhibition also rose. All of the test fungi's mycelia development was effectively/highly inhibited by beans root, although water extracts exhibited a lower degree of inhibition. Farmers should utilise bean root ethanolic extracts instead of synthetic or commercial fungicides due to their fungitoxic potential against vegetable storage fungi.

Keywords: Bean root; Bean leaf; fungicides; phytochemicals.

1. INTRODUCTION

Due to the components that have therapeutic potential, medicinal plants have been utilised as treatments for human ailments for ages [1]. More than 80% of the world's population, according to the World Health Organization (WHO), relies on traditional medicine for their basic healthcare requirements in 2018. Traditional medicine plays a significant role in African civilizations, and regional and cultural differences influence the local medical systems [2]. Due to increased awareness of the effectiveness, safety, and quality control of ethnomedicine, herbs are becoming extremely popular in developing nations [3]. Secondary plant metabolites, or phytochemicals, have received a lot of attention recently as potential sources for pharmaceuticals.

As a result, it is expected that phytochemicals with strong anti-fungal action will be employed to treat fungus infections. This is due to the fact that, according to Nascimento et al. [4], the secret to chemotherapy's effectiveness is the ongoing development of new medications to address the problems presented by resistant strains of microorganisms. Many compounds found in several plants, including peptides, tannins, alkaloids, essential oils, phenols, and flavonoids, among others, have been shown in studies to be potential sources of antimicrobial synthesis [5].

In order to find new opportunities to create more strong medications against microbial diseases, researchers are increasingly focusing on ethnomedicine [6]. As a result, a number of medicinal plants have been screened for possible antibacterial activity [7]. Their high protein content and inexpensive price compared to meat and meat products, legumes, which are the seeds of the leguminosae family, include peas, beans, and pulses [8]. Legumes are important food items for people in tropical developing nations; a large number of species and varieties are consumed by the populace as a whole because they are affordable and a significant source of protein (20–40%), carbohydrate (50–60%), and other nutrients that are good for human health and wellbeing [9].

The legume plant known as beans (*Vigna unguiculata*) is recognised as a significant food crop in the third world. It is a great source of riboflavin, thiamine, folic acid, niacin and biotin [10]. It significantly contributes to the reduction of hunger and poverty in developing nations. Cowpea seeds are eaten as cooked seeds or in conjunction with foods like maize, rice, plantains, and more in various countries of West Africa, including Nigeria. They are also turned into paste to make a variety of traditional meals, including moimoi, steaming cowpeas, and akara, a fried cowpea paste [11]. The quality and quantity of food supply must be improved in a number of ways to keep up with the current yearly rate of population growth in Africa and to ensure that low-income groups have access to enough food to fulfil their protein needs.

1.1 Statement of Research Problem

Without any scientific backing, the usage of bean leaf and root extract has been based on trial and error in many tribes throughout Africa. Many components of plants have been utilised to cure various illnesses in various places, sometimes with varying degrees of success.

Traditional healers frequently utilise plants in analogy to the illness being treated and in ways that are morphologically similar to it. For instance, conditions associated with menstrual issues and bleeding are treated using herbs that produce crimson juice [12]. So, it is necessary to determine the foundation for the claims made on the effectiveness of the plants utilised locally in ethnomedicine.

1.2 Aim of Study

To evaluate the phytochemical constituents and antifungal effect of ethanol and aqueous extracts of the bean leaf and root on various fungi isolated from spoiled food.

1.3 Objectives

To obtain the ethanol and aqueous extracts of bean leaf and root.

To determine qualitatively and quantitatively secondary metabolites present in the ethanol, and aqueous extracts of bean leaf and root.

To assess the antifungal activities of the aqueous, and ethanol, extracts of bean leaf and root.

1.4 Significance of the Study

As some microorganisms have gained resistance to many traditional medications, research is being done to create novel organic compounds from plants with antimicrobial capabilities for treating illnesses as a result of the rising resistance to antibiotics [13]. In order to treat infectious illnesses, a different strategy must be developed. Given the low cost, using local flora will be a good development.

The use of bean leaf and root extracts in the treatment of conditions including cancer, skin problems, ulcers, and more has been documented.

Thus, it is crucial to conduct scientific research on these plant components to determine their medicinal potential. The identification of this plant as a potent commercial medicinal plant depends on the determination of its chemical makeup as well as antibacterial activity against particular infections. Tests can indicate the smallest dosage needed for the treatment of diseases by determining how effective it is against a virus.

1.5 Scope of the Study

The research is restricted to the antifungal properties of ethanol and aqueous extracts of bean leaf and root on a variety of fungus isolated from contaminated food.

2. MATERIALS AND METHODS

2.1 Collection of Samples

Samples of spoilt vegetables and beans leaf and seed were collected from Eke Awka, in Anambra state Nigeria. These will besingly placed in sterile labelled polythene bags and transported to the lab.

2.2 Extraction of Plant

2.2.1 Ehanol and water extract

The obtained plant material (1000g) was air dried, ground up, and then mixed with water and ethanol. After standing for 48 hours, it was filtered. The filtrate was dried in a rotary evaporator at 55°C while being evaporated under decreased pressure. The dried extract was kept at -20°C in a labelled, sealed bottle. The extract's yield as a percentage was 0.11%.

2.2.2 Determination of extraction yield (% yield)

The yield (%, w/w) from all the dried extracts was calculated as:

$$\text{Yield } (\%) = (W1 * 100)/W2$$

where

W1 is the weight of the extract after lyophilization of solvent, and
W2 is the weight of the plant powder.

2.3 Qualitative Phytochemical Screening

In order to determine whether the chosen plant extracts contain saponins, tannins, alkaloids, flavonoids, triterpenoids, steroids, glycosides, anthraquinones, coumarin, saponins, or reducing sugars, a procedure based on those earlier reports by Banso and Adeyemo, 2016, will be used to perform phytochemical screening of the extract.

2.3.1 Test for tannins

Each powdered sample was individually cooked for five minutes in a water bath with 20 cc of distilled water, then immediately filtered. A few drops (2–3) of 10% ferric chloride were added to 1 ml of cold filtrate after it had been distilled to 5 ml with distilled water, and the mixture was then checked for precipitate development and colour changes. To establish the presence of tannins, the reaction mixture was examined for a brownish green or blue-black colouring.

2.3.2 Test for saponins

About 1g of each powdered sample was separately boiled with 10 ml of distilled water in a bottle bath for 10 mins. The mixture was filtered while hot and allowed to cool. The following tests were then carried out.

(a) Demonstration of frothing: 2.5 ml of filtrate was diluted to 10 ml with distilled water and shaken vigorously for 2 mins, formation of froth which is stable for some minutes indicate the presence of saponin in the filtrate.

(b) Demonstration of emulsifying properties: 2 drops of olive oil was added to the solution obtained from diluting 2.5 ml filtrate to 10 ml with distilled water (above), shaken vigorously for a few minutes, formation of a fairly stable emulsion indicated the presence of saponins.

2.3.3 Test for steroids

(a) About 0.2 g of each portion of the powdered sample was dissolved in 2 ml of chloroform.0.2 ml of concentrated H_2SO_4 was carefully added to form a layer. A reddish-brown colour at the interface between the layer indicates the deoxy-sugar characteristics of cadenolides which indicates the presence of steroid

(b) 2 ml of acetic anhydride was added to 0.5 g ethanolic extract of the sample with 2 ml of concentrated H_2SO_4. The colour change from violet to blue or green in some samples is an indication of the presence of steroids.

2.3.4 Test for alkaloids

About 1 g of each powdered sample was separately boiled with water and acidified with 5 ml of 1 % HCl on a steam bath. The solution obtained was filtered and 2 ml of the filtrate was treated with few drops of the following reagents separately in different test tubes and observed.

(a) Mayer's Test: Filtrates were treated with Mayer‟s reagent (potassium mercuric iodide). Formation of a creamy white precipitate indicated the presence of alkaloids in the extract.

(b) Wagner's Test: Filtrates were treated with Wagner‟s reagent (Iodine in potassium iodide). Formation of brown or reddish-brown precipitate was regarded as evidence for the presence of alkaloids in the extract.

(c) Dragendorff's Test: Filtrates were treated with dragendorff‟s reagent (solution of potassium bismuth iodide), Formation of orange-brown precipitate was regarded as evidence for the presence of alkaloids in the extract.

(d) Hager's Test: Filtrates were treated with Hager‟s reagent (saturated picric acid solution), Formation of yellow coloured precipitate was regarded as evidence for the presence of alkaloids in the extract.

2.3.5 Test for cardiac glycosides

a) About 5 ml of each extract was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underplayed with 1 ml of concentrated sulphuric acid. A brown ring at the interface indicated the deoxysugar characteristics of cardenolides. A violet ring may appear below the ring while in the acetic acid layer, a greenish ring may be formed.

b) About 10 ml of 50 % H_2SO_4 was added to 1 ml of the filtrate in separate test tubes and the mixtures heated for 15 mins followed by addition of 10 ml of Fehling‟s solution and boiled. A brick red precipitate indicated presence of glycosides.

2.3.6 Test for free anthraquinones

5 ml of chloroform was added to 0.5 g of the powdered dry seeds of each sample. The resulting mixture was shaken for 5 mins after which it was filtered. The filtrate was then shaken with equal volume of 10 % ammonia solution. The presence of a bright pink colour in the aqueous layer indicated the presence of free anthraquinones.

2.3.7 Test for combined anthraquinones

1 g of powdered sample of each sample was boiled with 2 ml of 10 % hydrochloric acid for 5 mins. The mixture was filtered while hot and filtrate was allowed to cool. The cooled filtrate was partitioned against equal volume of chloroform and the chloroform layer was transferred into a clean dry test tube using a clean pipette. Equal volume of 10 % ammonia solution was added into the chloroform layer, shaken and allowed to separate. The separated aqueous layer was observed for any colour change; delicate rose pink colour showed the presence of an anthraquinone.

2.3.8 Test for flavonoids

(a) 1 g of powdered sample of each sample was separately boiled in 20 ml of water and then filtered. 5 ml of dilute ammonia solution was added to a portion of the filtrate, followed by the addition of concentrated H_2SO_4. A yellow coloration was indicative of the presence of flavonoids.

(b) 1 g of the powdered dried seeds of each sample was boiled with 10 ml of distilled water for 5 minutes and filtered while hot. Few drops of 20 % sodium hydroxide solution were added to 1 ml of the cooled filtrate. A change to yellow colour which on addition of acid changed to colourless solution depicted the presence of flavonoids.

2.3.9 Test for terpenoids

5 ml of each extract was mixed in 2 ml of chloroform. 3 ml of concentrated H2SO4 was then added to form a layer. A reddish-brown precipitate colouration at the interface formed indicated the presence of terpenoids.

2.3.10 Test for phlobatannins

Deposition of a red precipitate when an aqueous extract of each plant sample was boiled with 1 % aqueous hydrochloric acid was taken as evidence for the phlobatannins.

2.3.11 Test for carotenoids

1 g of each sample was extracted with 10 ml of chloroform in a test tube with vigorous shaking. The resulting mixture was filtered and 85 % sulphuric acid was added. A blue colour at the interface showed the presence of carotenoids.

2.3.12 Phenolics

Each sample's powdered dried seeds weighed 0.5 g, and they were cooked in 10 ml of distilled water for 5 minutes before being filtered while still hot. A solution of ferric chloride in 1 ml was then added. The development of brown or blue-black colouring suggested the presence of phenol.

2.3.13 Check for reduced sugars

10 ml of distilled water were added to each sample, which weighed about 1 g, in the test tube. The mixture was boiled for 5 minutes. The combination was heated, filtered, and then allowed to cool. In a test tube, 5 ml of the Fehling's solution (A and B) mixture was added to 2 ml of the filtrate, and the resulting liquid was then heated for 2 minutes. Brick red precipitate at the test tube's bottom revealed the presence of reducing sugar.

2.3.14 Measurement of phenols

The test was conducted using Harborne's [14] methodology. Using a Soxhlet device, two grammes of the ground materials were defatted for two hours with 100 cc of diethyl ether. For 14 minutes, 50 cc of ether was heated with the fat-free samples. Ten millilitres of distilled water were added after five millilitres of the extract were pipetted into a 50 millilitre flask. Next, 5 ml of concentrated ethyl alcohol and 2 ml of NH4(OH)2 solution were added. The samples were then prepared as directed and given 30 minutes to react in order to acquire their colour. At a wavelength of 505 nm, a visible spectrophotometer was used to measure the solutions' absorbance.

2.4 Fungal Isolation

2.4.1 Culture media

Two commercial media was used in this work. They were Potato Dextrose Agar (PDA), which is

a general purpose culture media, and Sabouraud Dextrose Agar (SDA), which is a modification of Dextrose Agar.

2.4.2 PDA media preparation

About 39g of the medium were suspended in one litre of distilled water, heated over a Bunsen flame while being stirred frequently, and allowed to boil for one minute to thoroughly dissolve the medium/contents. The solution was autoclaved for 15 minutes at a temperature of 1210 C and one atmosphere of pressure (15 PSI). Let to cool for ten minutes after withdrawing from the autoclave. To act as antibiotics, 500 mg of streptomycin sulphate was added to the molten solution.

2.4.3 SDA media preparation

About 65g of the medium were suspended and dissolved in 1 litre of distilled water by heating to boiling and stirring frequently. It was heated for one minute to dissolve the solution, and then sterilised for 15 minutes at 1210C in an autoclave. After that, while the solution was still molten, 500mg of the antibiotic streptomycin was added.

2.4.4 Isolation of fungi from samples

In this investigation, the Kuhnau, 2016, isolation approach was used. The surfaces were sterilised by dipping completely in a concentration of 40% hypochlorite solution for 60 seconds; the sterilised sections to be inoculated were then removed and rinsed with three changes of sterile distilled water. A small section of infected C. esculenta tissues containing the advancing margin of rot and adjoining healthy tissue was cut using a sterilised scalpel and cork borer. In a laminar airflow cabinet, the tuber pieces were dried by blotting with sterile filter paper. Four portions of each cut sample were individually inoculated (90° apart) on solidified potato dextrose agar (PDA) and sabouard dextrose agar (SDA) plates using sterile forceps. For each sample, two replicates were created. During 72 hours, the plates were kept in an incubator at a temperature of 28 to 30 °C. The rotting of the tubers was caused by fungi, which were seen.

2.4.5 Identification of fungi

To create a pure culture, isolated fungus were further sub-cultured. According to Kozlowski, identification was then completed using information on the colony's shape and microscopic traits [15]. According to Karimi et al. [16].'s description, morphological traits were used to identify the fungi, and the results were compared to existing keys. Each isolate was examined using a colony and a microscope, and their morphological characteristics were noted and documented. Based on growth patterns, mycelia colour, and microscopic investigations of vegetative and reproductive structures, morphological traits were explored. A little piece of mycelia was taken from the area between the colony's centre and edge using a sterile inoculating needle, and it was then put on a spotless microscopic slide with lactophenol in cotton blue. With the sterile needle and a cover slip that was put carefully and slightly pressed to remove air bubbles, the mycelia were evenly disseminated throughout the slide. The slide was heated by steam from some boiling water in order to better preserve the fungal formations on it. With sterile blotting paper, the cover slip's excess lactophenol was removed from the margins. Using the microscope's 10 and 40 objective lenses, the slide was examined. Isolates were identified using cultural morphology and characteristics according to De Hoog et al. (2020) and Jay (2012).

2.4.6 Culture-specific traits

To help with identification of the organisms, the growth pattern, colour, and size of colonies were noted during the incubation period.

2.4.7 Morphology of colonies

On a tidy microscopic slide, lactophenol (LP) was dropped upon it. A little amount of the isolate was added to the lactophenol (LP) drop and suspended. The suspension was covered with a spotless cover glass, then under a microscope was looked at.

2.5 A Spore's Stain

The heat-fixed slide containing the isolate's smear was placed over a steaming water bath, and blotting sheets were placed over the region of the smear without sticking out over the corners of the slide as part of the staining step for spore identification. The blotting paper was then steamed after being saturated with a 5.6% malachite green solution for 5mins. The slide was then carefully cleaned with tap water after being cooled to room temperature. After one minute of application, Safari was rinsed

completely yet quickly before being blotted dry. The slide was then inspected under a microscope.

2.5.1 Motility test

Fungal motility was measured by replacing the growth of the organism on a clean slide with a wire loop after putting a small drop of live isolates to the centre of a slip on a depression slide using petroleum jelly or 2-3 drops of peptone water. The slide was covered with a cover slip, left alone for a while, and then inspected under a high power microscope. Swimming about were movable creatures.

Biochemical Test Carbohydrate Assimilation Test: The pH of the medium was raised to 5.4 by adding NaOH or HCl, and filtered and sterilised carbohydrates were added at a concentration of 1%. A 10 ml test tube was filled with 2 ml of the medium. Moreover, isolates and carbs were injected through the tubes.

All tubes underwent a 14-day incubation period at 20° C. A shift in the medium's orange and yellow tint was viewed favourably. Pink or purple changes were seen as undesirable outcomes.

The medium preparation and prescription for the amino-acid absorption test were the same as those for the carbohydrate assimilation test. The isolate and control tubes for each fungus and amino acid were inoculated into 10 mm test tubes containing 2 ml of the medium. Moreover, tubes were kept at 20° C for 14 days. A move to pink or purple was regarded as a favourable outcome, but a change to orange was viewed as a failure.

2.5.2 Hydrolysis test

After the addition of 0.05 mg milk and 1.2 mg agar, the base medium was comparable to that of the test for amino acid absorption. The medium was put into a petri dish after an autoclave at 110o C for 30 minutes. At 20°C for 14 days, isolates were incubated after being inoculation in the plate's centre. It was deemed successful when a clean zone appeared around the fungus colony.

2.5.3 Test for lipase activity

The medium, which contains 1.0% agar, 0.3% yeast extract, and 0.5% peptin, was autoclaved at 121° C for 10 minutes. It was filtered and then put into test tubes that had been sterilised. Incubation was place at 20° C for 7 days after the isolates were injected onto the medium's surface. Clearance occurring in the middle column was viewed as a successful outcome.

2.5.4 Filter paper technique for assessing the sensitivity of antifungals

Whatman No. 1 filter paper was used to create and sterilise 6mm filter paper discs. The discs were put aseptically onto the agar plates containing the test microorganisms after being inserted into the various concentrations of the extracts using ethanol-dipped and flamed forceps. Each plate included three discs total—three for each of the different spice intensities. 48 hours were spent incubating the inoculation plates at room temperature. By measuring the zones of inhibition—the area around each disc that is clear—in millimetres, the antifungal activity was assessed. At the conclusion of the incubation time, the diameter of the radial growth of the fungus was determined, and the fungitoxicity level of the powders and extracts was calculated using the following formula:

$$Percentage\ growth\ inhibition\ (\%) = \frac{dc - dt}{dc} x \frac{100}{1}$$

Where

dc = average diameter of fungal colony in control treatment

dt = average diameter of fungal colony with powder or extract.

2.5.5 Determination of minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC)

With a few minor adjustments, the microplate approach that was previously reported [17] was utilised to figure out the minimum inhibitory concentrations (MIC) of plant extracts. Plant extracts were serially diluted, with dilutions ranging from 1/2 to 1/100 of the original amount. Each extract dilution was combined with 100 mL of fungal spore suspension (2 106 spores mL-1 in fresh PDB) in each well. The microplates were incubated at 27 °C for 2-3 days while being checked every day. Three copies of each experiment were performed. A microplate reader set to 595 nm was used to spectrophotometrically read the MIC values. By comparing the growth in control wells and the

extract blank, which consisted of uninoculated plates, MIC values were determined. The lowest concentration of plant extract that resulted in growth inhibition of more than 90% at 48 h as compared to the control was referred to as the MIC of the extracts.

Jackson determined the in vitro fungicidal activity (MFC) and characterised it [18]. 20 L were subcultured onto PDA plates after 72 hours from each well that had no discernible growth (growth inhibition of > 98%), from the last positive well (growth comparable to the growth control well), and from the growth control (extract-free medium). The plates were incubated at 27 °C until the growth control subculture showed signs of growth. The lowest extract concentration that did not result in any fungus growth on the solid medium was considered to be the minimum fungicidal concentration.

2.6 Statistical Analysis

For each treatment, each outcome was repeated twice or three times using triplicates (n = 2 3 / n = 3 3), and the data were presented as a mean standard deviation. Using Minitab® Version 16 for Windows (Minitab Inc., USA), a one-way analysis of variance (ANOVA) was carried out, followed by a posthoc Tukey's test for means separation (p 0.05).

3. RESULTS

3.1 Qualitative Phytochemicals

The result of the qualitative phytochemical composition of the ethanolic extracts of the plants are shown in Table 1.

3.2 Isolation of Spoilage Fungi

The fungi pathogens that were constantly isolated from the sammples includes *Aspergillus niger, Fusarium solani, Rhizopus stolonifer and Penicillium digitatum.* The frequency of occurrence varied with different fungi associated with the rotten vegetable cormels. The most frequently occurred were *Aspergillus niger* (Table 3).

3.3 Minimum Inhibitory Concentration (Mg/mL)

The Minimum inhibitory concentration (MIC) of all the extract against the isolated fungal were presented in Table 6. The result showed that mucor spp have the highest MIC value for all the extract while penicillium spp have the lowest MIC value.

Table 1. Qualitative phytochemical composition

Phytochemicals	Beans root ethanol extract	Beans root water extract	Beans leaf ethanol extract	Beans leaf water extract
Saponin	++	+++	+++	-
Flavonoid	-	+++	++	+
Alkaloid	++	-	-	-
Tannin	-	+	+	-
Steroids	+	-	-	+
Terpeniods	+	+++	+++	-
Glycosides	++	++	+	-
Phenol	-	-	-	+

Key: *+++ = Present in high concentration*
++ = Present in moderate concentration
+++ = Slightly or sparingly present
- = Absent.

Table 2. Mean fungi count

Sample	Mean total fungi count (cfu/g)
1	2.50×10^4
2	2.80×10^4
3	2.00×10^4
4	2.45×10^4
5	1.90×10^4

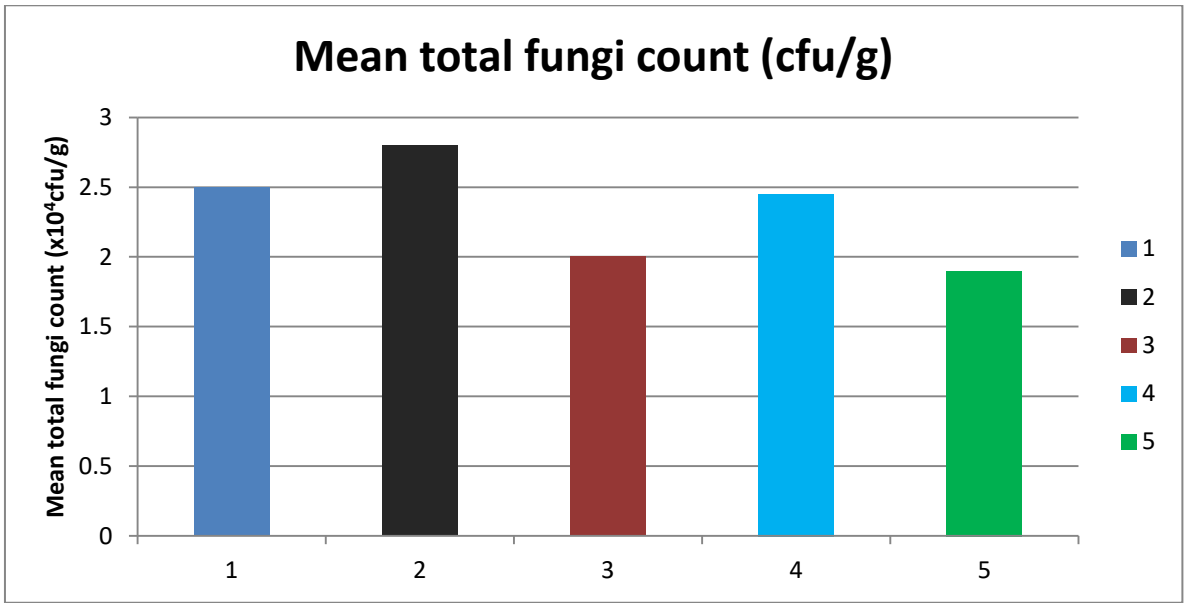

Table 3. Morphological Characteristics of Fungal Isolates

S/N	Colour of spores	Reverse of the agar	Aerial hypae	Abundance	Growth	Pigmentation
1	Black	Light green	Powdery, spores embedded	Abundant	Fast	No
2	Black	Light green	Powdery, spores embedded	Abundant	Fast	No
3	Blue – green	Cream	Powdery, spores embedded	Abundant	Fast	No
4	White	Cream	Fluffy, raised a little	Abundant	Fast	No

Table 4. Fungi identification

S/N	Description	Probable identity
1	They are normally powdery black, with tall conidiophores emerging from long, wide, thick-walled, occasionally branching foot cells. Conidia are big, globose, irregularly roughed, and have radiating heads.	*Aspergillus niger*
2	Colonies are whitish to olivaceous-buff in colour, with a fragrant odour and tall and short sporangiophores that may be distinguished in the dark. Sporangia with ellipsoidal, pyriform, or subgloblose features are blackish. Absence of chlamydospores.	*Mucor sp.*
3	Colonies are rapidly expanding, with sparse to abundant, floccose aerial mycelium that becomes felted and is either white or peach in colour with a hint of violet. Aromatic scent that is distinctive and suggests lilae.	*Fusarium sp.*
4	Colonies are loosely synematous, rapidly developing conidiophores in new isolates that give the colony a zonate look. Colonies are light green, inverted colourless, yellow-brown conidiophores that are typically smooth walled, pencilli that are 2-3 stages branching, and conidia that are subglobose to ellipsoidal smooth-walled. The odour is fragrant, fruity, and reminiscent of apples.	*Penicillium sp.*

Table 5. Antifungal activities of the extracts

Fungi isolate	Extract	Zone of inhibition(mm)	Result
Aspergillus spp	Beans root ethanol extract	12.33± 0.28	S
	Beans root water extract	12.00± 0.00	S
	Beans leaf ethanol extract	10.16± 0.28	1
	Beans leaf water extract	8.65± 1.32	R
	Fluconazole	12.00± 0.00	S
Mucor spp	Beans root ethanol extract	25.00±0 .00	S
	Beans root water extract	15.00± 0.00	S
	Beans leaf ethanol extract	12.00± 0.00	S
	Beans leaf water extract	10.00± 0.00	I
	Fluconazole	32.27± 1.36	S
Fusarium spp	Beans root ethanol extract	17.33± 0.28	S
	Beans root water extract	10.00± 0.00	S
	Beans leaf ethanol extract	7.16± 0.28	R
	Beans leaf water extract	5.00± 1.32	R
	Fluconazole	18.00± 0.00	S
Penicillium spp	Beans root ethanol extract	0.00±0 .00	R
	Beans root water extract	0.00± 0.00	R
	Beans leaf ethanol extract	0.00± 0.00	R
	Beans leaf water extract	0.00± 0.00	R
	Fluconazole	32.27± 1.36	S

N/B: R = Resistant, I = Intermediate, S = Susceptible;

Table 6. Minimum inhibitory concentration (Mg/mL)

Microorganisms	Beans root ethanol extract	Beans root water extract	Beans leaf ethanol extract	Beans leaf water extract	Fluconazole
Aspergillus sp.	5.30	0.30	7.30	0.30	0.30
Penicillium sp.	0.50	0.30	2.90	0.30	0.30
Fusarium sp.	0.30	0.50	0.50	0.50	1.00
Rhizopus sp.	5.30	0.30	0.30	0.30	0.30
Mucor spp.	10.20	10.60	12.50	10.60	0.30

4. DISCUSSION

The result of this study revealed the presence of 5 different fungal species from spoilt vegetable obtained from eke Awka Anambra state (*Aspergillus niger, Penicillium citrinum, Fusarium solani, Rhizopus stolonifer* and *Mucor piriformis*). Using the pathogenicity test, it was determined that these fungi were the spoilage's main culprits. Due to the threat posed by post-harvest vegetable spoiling brought on by various fungal species, particularly in developing nations like Nigeria, as stated by Igbozuluike (2015). When there is *Aspergillus* spp. And *Rhizopus* spp. as vegetable spoilage agent in this research was in conformity with the findings of Jenkins, [19] among the spoilt vegetable vended in some selected markets in Lagos. Abang and Shittu [20] who isolated and reported *Aspergillus niger* as the most dominant mycological flora that was associated with spoilage of vegetable. This finding was also in conformity with that of Adipala et al. [21] and Chukwu et al. (2018) who isolated *A. niger* and *R. stolonifer* from vegetable in Nigeria.

The finding was also in conformity with the findings of Alcarraz et al. [22] on the isolation of fungal pathogens from vegetable stored and sold in the market. Furthermore, Ali et al. [23] stated that *A. niger* was the cause of post-harvest spoilage in sweet orange and acid lime at field. Agrios [24] reported that *A. niger*, *Alternaria* species, *Botryodiplodia theobromae* and *Colletotrichum gloeosporioides* were isolated from the spoilt vegetable. However, the value obtained for the prevalence of *A. niger* which caused a disease called black mold on certain fruits and produced potent mycotoxins called ochratoxins that can be harmful to human beings and animals, was higher than the one reported by Mailafia et al. [25] who reported the highest occurrence of 38%.

The isolation of more than one pathogenic organisms from a particular cormel confirms the possibility of multiple infections whose cumulative effect may cause rapid rottening of root and tuber crops this agrees with the reports of Adebiyi et al. 2015 on yam. Most often, natural openings and wounds caused by harvesting, transporting, handling, and marketing allow fungus to enter vegetable cormels. Ejimofor et al. [25] has out that infections originating from diseased foliage, roots, or mother tubers/cormels may already be present in root and tuber crops at the time of harvest.

This study found that bean root and leaf extracts contained fungitoxic compounds because they were able to stop the test fungi from growing. This finding is consistent with earlier reports of numerous studies that focused on different fungi [25], so the plant extracts used may have a place in the defence of mechanically damaged vegetable corms and cormels against rot fungi. The effectiveness of the extracts varied depending on the plant material, concentration, extraction solvent, and individual test fungus, though.

As ethanol extracts were more effective than aqueous extracts, the water used in the extraction procedure presumably was unable to completely dissolve the active ingredients found in plants, which are present in ethanol extracts. According to reports by Ejimofor et al. 2022 and Oledibe *et al.* 2022 on garlic, the higher yield from the ethanol extract was due to the fact that ethanol is an organic solvent and will dissolve organic compounds better than other solvents, freeing the active compounds (phytochemicals) needed for antifungal activity. The variation in the fungitoxic between the extraction medium might also be as a result of the variable sensitivity of each of the test isolates to varying concentrations of the extracts, this also agrees with the findings of some workers (Amadioha, 2020), The antifungal properties of these plant extracts are likely due to the presence of phytochemicals, which are anti-microbial agents [26], that are inhibitory to the growth of these pathogens. The presence of bioactive substances has been reported to confer resistance to plants against bacterial, fungi, and pest [27], which explains the demonstration of antifungal activity by the plant extracts used in this study. The plants' phytochemical screening revealed that all of the phytochemicals tested were positive (Alkaloid. Flavonoid, Phytate, Saponin, Tannins, Oxalate and Phenols). All of these phytochemicals have been shown to have medicinal and pharmacological potential by the reports of several researchers [28].

Minimal inhibitory concentrations (MIC) for ethanolic extracts of bean root and leaf have been reported. The three samples that were examined all shown fungistatic and fungicidal action. The MIC values were in the range of 0.30 and 7.0 g mL-1. The uziza extract's lowest fungicidal concentration showed to have the strongest fungicidal activity against all five isolated fungi, as shown by the low value (Table 5).

Our findings are in line with those of Ejimofor et al. [26], who hypothesised that each materialsolvent system exhibits a unique behaviour depending on the chemical properties of the solvent, the extraction technique employed, and various structural and compositional aspects of the natural products. Variations in the degree of activity have been found to be caused by changes in the polarity of the solvents used to dissolve the active plant components.

This result is consistent with the findings of Banso et al. [29], who also noted that antimicrobial drugs at higher doses exhibited more growth inhibition. Moreover, the antibacterial properties of plant extracts may result from the synergistic effects of multiple minor chemicals found in plants rather than the action of a single active molecule (Davicino et al. 2017). These findings suggest that the type of plant employed and the extract's properties determine the proper extract concentration to exhibit a certain effect [30-38].

This highlights the need of doing research to isolate, identify, and characterise the biomolecules in order to determine the compound(s) responsible for the inhibitory effect. To identify the chemical composition of the bioactive substances responsible for the reported antifungal action, more research is required. Natural fungicides generated from plants may provide novel, alternative active substances, particularly those with antifungal activity.

5. CONCLUSION

This study has shown the potentials of bean root and leaf in the control of vegetables in storage, with bean root ethanol extract demonstrating the most fungitoxic activity. This study also showed

that ethanol extracts demonstrated a higher antifungal activity over aqueous extract, indicating that ethanol extract of bean root and leaf could be an alternative or complementary to synthetic chemicals in controlling vegetable spoilage, where bean root are not available, beaver leaf extract could be used instead. The use of plant extracts in controlling rot-causing organisms and pests, however, could reduce over reliance on one source of agricultural chemicals to the farmers, which is reported to predicate long-term harmful consequences on environment, Man, and wildlife, as well as reduce production costs. This study's results have gone a long way in providing a better alternative to the over dependence on synthetic fungicides.

6. RECOMMENDATIONS

Future problems with regard to the plants employed include additional pharmacological assessment, toxicological studies, and potential therapeutic antifungal isolation from these plants; as a result, it is advised that further research be done on the chemical make-up of the active components of the plants.

Further research involving invivo assay would be necessary to investigate the fungistatic effects of these botanicals on the fungal inducing rot of vegetable corms and cormels that are not included among the test fungi in this research work. Additionally, further investigations can combine the plant extracts for possible synergistic effects.

To extend the shelf life of vegetables after harvest, it is crucial to design effective storage facilities.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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