Original Research Article

The phytochemical and antifungal efficiency of bean leaf and root against some pathogenic fungi isolated from spoilt vegetables sold within Anambra metropolies

ABSTRACT

The phytochemical and Antifungal efficiency of bean leaf and root against some pathogenic fungi isolated from spoilt vegetables sold within Anambra metropolies were investigated invitro. Two (2) different extract concentrations were obtained from each plant parts used by blending 100g, in 100ml of sterile distilled water and ethanol. Phytochemical screening of the plants was conducted using different standard methods; this revealed the presence of alkaloid, saponin, tannins, flavonoid, phytate, oxalate and phenol in all the plants but at different concentrations. Effect of standard antibiotics (Fluconazole 30µg/ml) comparative to the plant extracts was determined. Pathogenicity test revealed that Aspergillus niger, Fusarium solani, Penicillium sp, Rhizopus sp and Mucor spp induced rot in healthy vegetables. The beans root ethanol extract proved to be more potent. The efficacy of the extract varied with the solvent of extraction, extract concentration and the test pathogens. Inhibition of fungal growth increased with a corresponding increase in extract concentration. Beans root depicted an effective/high rate of inhibition on the mycelia growth of all the test fungi whereas, extract of water extracts showed a lower inhibition rate.. The fungitoxic potential of bean root ethanolic extracts on vegetable storage fungi recommends their use to farmers as alternative to commercial / synthetic fungicides.

Keywords : Bean root, Bean leaf, Fungicides

Introduction

Medicinal plants have been used for centuries as remedies for human diseases because they contain components of therapeutic value (Obafemi *et al.,* 2017). According to the World Health Organization (WHO) in 2018, more than 80% of the World's population relies on traditional medicine for their primary health care needs. Traditional medicine is an important part of African cultures and local medicinal systems vary among cultural groups and regions (Morebise and Fafunso, 2017). Herbs are now very popular in developing countries on account of improved knowledge about the safety, efficiency and quality assurance of ethno- medicine (Morebise et al., 2017). In recent years, secondary plant metabolites (phytochemicals) have been extensively investigated as a source of medicinal agents.

Thus it is anticipated that phytochemicals with good anti-fungal activity will be used for the treatment of fungal infections. This is because according to Nascimento et al., 2020 the success story of chemotherapy lies in the continuous search for new drugs to counter the challenges posed by resistant strains of micro-organisms. Studies indicate that in some plants, there are many substances such as peptides, tannins, alkaloids, essential oils, phenols and flavonoids among others which could serve as sources of antimicrobial production (Memudu et al., 2015).

Therefore, researchers are increasingly turning their attention to ethno-medicine, looking for new leads to develop more effective drugs against microbial infections (Mbanali et al., 2019) and this has led to the screening of several medicinal plants for potential antimicrobial activity (Maurya *et al*., 2018).

Legumes refer to the seeds of leguminosae including peas, beans and pulses, "it is considered as poor man meat" due to their high protein content and low cost compared to meat and meat products (Larone, 2015). Legumes are food stuffs of great significance to people in tropical developing countries, a large number of species and varieties of legumes are consumed by the teaming population, as they are

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UNDER PEER REVIEW

inexpensive and important source of protein (20-40%), carbohydrate (50-60%) along with other nutrients which have beneficial effects on human health and well being (Martinez *et al.,* 2010).

Beans (*Vigna unguiculata*) is a legume plant that is regarded as a prominent food crop in the 3rd world countries. It is an excellent source of thiamine, folic acid, niacin, riboflavin and biotin (Laestsh, 2019). It plays a great role in alleviating poverty and malnutrition in developing countries. In many parts of West Africa including Nigeria, cowpea seeds are consumed as boiled seeds or in combination with food such as maize, rice, plantain among others. They are also processed into paste for the preparation of various traditional foods, such as Akara (fried cowpea paste) and moimoi, steamed cowpea (Mandrell et al., 2016). As the population of Africa continues to increase at its present annual rate and the low income groups are to be provided with sufficient food to meet their protein requirement, efforts have to be made in several directions to improve the quality and quantity of food supplies.

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Statement of Research Problem

The use of bean leaf and root extract have been on the basis of trial and error in different communities in Africa without any scientific basis. Plant parts have been used in different locations for the treatment of different ailments which sometimes bring about conflicting results.

Often traditional healers use plants according to their analogy and morphological similarities to the ailment being treated. For example, plants containing red juice are used to treat ailments connected to menstruation problems and bleedings (Lalieveld et al., 2016). There is therefore the need to ascertain the basis for the claims of the efficacy of the plants used locally in ethno-medicine.

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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.

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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.

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Significance of the study

The increasing resistance to antibiotics has resulted in the research to form new organic molecules from plants with antimicrobial properties for treating diseases since some micro-organisms have developed resistance to many orthodox drugs (Kozlowski, 2016). There is the need to find an alternative approach in the treatment of infectious diseases. Using local plants will be a welcome development as the cost will be minimal.

The bean leaf and root extracts have been reported in the treatment of various ailments such as ulcer, cancer, skin diseases e.t.c.

It is therefore important to scientifically investigate these plant parts to ascertain their therapeutic potentials.

Determination of their chemical composition as well as antimicrobial efficacy against specific pathogens is important in the recognition of this plant as a potent commercial medicinal plant. Tests can determine its efficacy against a pathogen and thus, establish the minimal dosage required for the treatment of ailments.

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Scope of the study

The study is limited to the phytochemical constituents and antifungal effect of ethanol and aqueous extracts of the bean leaf and root on various fungi isolated from spoiled food.

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MATERIALS AND METHODS

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.

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Extraction of plant

Ehanol and water extract

The plant material collected (1000g) was air dried, pulverized, and macerated with ehanol and water. It was allowed to stand for 48 h and then filtered. The filtrate was evaporated under reduced pressure and was dried using a rotary evaporator at 55°C. The dried extract was stored in a labeled sterile screw capped bottle at -20°C. The percentage yield of the extract was 0.11%.

Determination of extraction yield (% yield)

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

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 .

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QUALITATIVE PHYTOCHEMICAL SCREENING

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Phytochemical screening of the extract will be carried out by a procedure that was based on those earlier reports by Banso and Adeyemo, 2016 so as to detect the presence of saponins, tannins, alkaloids, flavonoids, triterpenoids, steroids, glycosides, anthraquinones, coumarin, saponins, reducing sugars in the selected plant extracts.

Test for tannins

1g of each powdered sample was separately boiled with 20 ml distilled water for five minutes in a water bath and was filtered while hot. 1 ml of cool filtrate was distilled to 5 ml with distilled water and a few drops (2-3) of 10 % ferric chloride was added and observed for any formation of precipitates and any colour change. The reaction mixture was observed for a brownish green or blue-black colouration for the confirmation of the presence of tannins.

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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.

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UNDER PEER REVIEW

(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.

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.

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.

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.

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.

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UNDER PEER REVIEW

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.

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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.

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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.

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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.

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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.

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Phenolics

0.5 g of the powdered dried seeds of each sample was boiled with 10 ml of distilled water for 5 mins and filtered while hot .Then 1ml of ferric chloride solution was added. Formation of blue-black or brown colouration indicated the presence of phenol.

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Test for reducing sugars

To about 1 g of each sample in the test tube was added 10 ml distilled water and the mixture boiled for 5 mins. The mixture was filtered while hot and the cooled; 5 ml of mixture of equal volume of Fehling‟s solution (A and B) was added to 2 ml of the filtrate in a test tube and the resultant mixture was boiled for 2 mins. Appearance of brick red precipitate at the bottom of the test tube indicated the presence of reducing sugar.

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UNDER PEER REVIEW

Determination of phenols

The method of Harborne (2018) was used for the assay. Two grams of the ground samples were defatted with 100 ml of diethyl ether using Soxhlet apparatus for 2 h. The fat free samples were boiled with 50 ml of ether for 14 min. Five millilitres of the extract was pipetted into a 50 ml flask after which 10 ml of distilled water was added. Two millilitres of $NH_4(OH)_2$ solution and 5 ml of concentrated ethyl alcohol were then added. The samples were then made up to mark and left to react for 30 min for colour development. The absorbance of the solutions was read using a visible spectrophotometer at 505 nm wavelength.

Fungal isolation:

Culture Media

Two commercially available media will be used in this work. These were Potato Dextrose Agar (PDA), which is a general purpose culture media, and Sabouraud Dextrose Agar (SDA), which is a modification of Dextrose Agar

PDA media preparation

In one litre of distilled water, 39g of the medium was suspended, heated over a Bunsen flame with frequent agitation, and allowed to boil for one minute to completely dissolve the medium/contents. The solution was autoclaved at temperature of 121^0 C for 15 minutes, at a pressure of one (1) atmosphere (15 PSI).After removing from the autoclave, allowed to cool for 10 minutes. Five hundred (500 mg) streptomycin sulphate was added into the molten solution to serve as antibiotics.

SDA media preparation

In one litre of distilled water, 65g of the medium was suspended and dissolved by heating to boil, with frequent agitation. After heating for one minute and dissolving the solution, it was sterilized in an autoclave at 121^0C for 15 minutes. This was followed by the addition of 500mg streptomycin antibiotic while the solution was still in a molten state.

Isolation of Fungi from Samples

The isolation technique of Kuhnau, 2016 was adopted in this study. A small section of infected *C. esculenta* tissues containing the advancing margin of rot and adjoining healthy tissue were cut using sterilized scalpel and cork borer while the surfaces were sterilized by dipping completely in a concentration of 40% hypochlorite solution for 60 seconds; the sterilized sections to be inoculated were then removed and rinsed with three changes of sterile distilled water. The tuber pieces were made to dry by blotting with sterile filter paper in a laminar airflow cabinet. With the aid of a sterile forceps four pieces of each cut samples were separately inoculated (90° apart) on solidified potato dextrose agar (PDA) and sabouard dextrose agar (SDA) plates. Two replicates for each sample were made. The plates

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were incubated a temperature of 28-30 °C in an incubator for 72 hours. Fungi associated with the tubers spoilage were observed.

Identification of fungi

Isolated fungi were further sub-cultured to obtain a pure culture. Identification was then done based on colony characteristics, morphology and microscopic features according to Kozlowski (2018). Fungal identification was done using morphological characteristics and comparing the findings with established keys as described by Karimi et al., 2016. Each isolate was subjected to colony and microscopic examinations during which their morphological features were observed and recorded. Morphological features studied were based on growth patterns, color of mycelia and microscopic examinations of vegetative and reproductive structures. A sterile inoculating needle was used to get a small portion of mycelia from between the colony centre and the edge and placed on a clean microscopic slide containing lactophenol in cotton blue. The mycelia were spread well on the slide using the sterile needle and a cover slip gently placed with little pressure to eliminate air bubbles. The slide was placed above some boiling water to steam it for better staining of fungal structures. The excess lactophenol on the edges of the cover slip was wiped using sterile blotting paper. The slide was mounted on the microscope and observed with ×10 and ×40 objective lenses.

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Identification and Characterization of Isolates

The isolates were identified using cultural characteristics and morphology with reference to De Hoog *et al*. (2020) and Jay (2012).

Cultural Characteristics

The growth pattern, pigmentation and size of colonies were recorded at the incubation period to aid identification of the organisms.

Colony Morphology

A drop of lactophenol (LP) was placed on a clean microscopic slide. A small portion of the isolate was placed in the drop of lactophenol (LP) and suspended. A clean cover glass was placed over the suspension and observed microscopically.

Spore Staining

The staining procedure for identification of spore was carried out by placing heat-fixed slide (containing the smear of the isolate) over a steaming water bath and placing of blotting papers over the area of the smear without sticking out past the edges of the slide. The blotting paper was then saturated with 5.6% solution of malachite green and steamed for 5 min. Following this, the slide was cooled to room temperature and then rinsed thoroughly with tap water. Safari was then applied for one minute and rinsed briefly but thoroughly before blotting dry. After which the slide was examined microscopically.

Motility Test

Fungal motility was determined by transferring a small drop of live isolates to the centre of a slip of a depression slide using petroleum jelly or 2-3 drops of peptone water with growth of the organism replaced on a clean slide with wire loop. Then cover slip was placed over the slide, the slide was left for some time and then examined microscopically with the high power objective. Motile organisms were seen swimming around.

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UNDER PEER REVIEW

Biochemical Test

Carbohydrate Assimilation Test: Filtered and sterilized carbohydrates were added to the medium at concentration of 1% while the pH was adjusted to 5.4 by addition of NaOH or HCl. 2 ml of the media were dispensed into 10 ml test tube. The tubes were also inoculated with isolates and carbohydrates. All tubes were incubated at 20o C for 14 days. A change in the color of the medium of orange and yellow were taken as positive result. A change to pink or purple was considered negative result.

Amino-acid Assimilation Test:

Medium preparation and indication were as described for the carbohydrate assimilation test. 10 mm test tubes containing 2 ml of the media were inoculated with the isolate and control tubes for each fungus and amino acid. Also tubes were incubated at 20o C for 14 days. A change to pink or purple was considered positive result while a change in color of the medium to orange was taken as negative result.

Hydrolysis Test:

The basal medium was similar to that of amino acid assimilation test with addition of 0.05 mg milk and 1.2 mg agar. After autoclaving at 110o C for 30 min, the medium was poured into petri dish. Isolates were inoculated at the centre of the plate and incubated at 20oC for 14 days. The appearance of a clear zone around the fungal colony was taken as a positive result.

Lipase Activity Test:

The medium of 0.5% peptin, 0.3% yeast extract and 1.0% agar were autoclaved at 121o C for 10 min. It was filtered and dispensed into sterilized test tubes. Isolates were inoculated into the surface of the medium and incubated at 20o C for 7 days. The occurrence of clearance in the medium column was taken as a positive result.

Antifungal sensitivity testing using filter paper method

Filter paper discs of 6mm were prepared from Whatman No.1 filter paper and sterilized. Using ethanol dipped and flamed forceps, the discs were inserted into the various concentrations of the extracts and placed aseptically over the agar plates seeded with the test microorganisms. A total of three discs were placed on each plate, with three for the various spice concentrations. The inoculated plates were incubated at room temperature for 48 hours. The antifungalal activity was evaluated by measuring the zones of inhibition, which is the clear zone around the various discs in millimetres.

The diameter of the radial growth of the fungus 0 was measured at the end of the incubation period and then used to determine the fungitoxicity level of the powders and extracts using the formula:

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

Where

dc = average diameter of fungal colony in control treatment

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

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

A microplate method, as previously described (Kar, 2017), was used with slight modifications to determine minimal inhibitory concentration (MIC) values of plant extracts. Plant extracts were serially

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UNDER PEER REVIEW

diluted, ranging from 1/2 up to a 1/100 dilution from the crude extract. In each well, 100 µL of each extract dilution was mixed with 100µL of the fungal spore suspension (2×10^6 spores mL-1 in fresh PDB). The microplates were incubated for 2-3 d at 27 °C with daily monitoring. All experiments were done in triplicate. The MIC readings were performed spectrophotometrically with a microplate reader at 595 nm. MICs values were calculated by comparing growth in control wells and the extract blank, which consisted of uninoculated plates. The MIC of the extracts was defined as the lowest concentration of plant extract that caused growth inhibition of more than 90% at 48 h, as compared to the control.
The in vitro fungicidal activity (MFC) was determined described by Jackson (2016). After 72 h of incubation, 20 µL was subcultured from each well that showed no visible growth (growth inhibition of over 98%), from the last positive well (growth similar to that for the growth control well), and from the growth control (extract-free medium) onto PDA plates. The plates were incubated at 27 °C until growth was seen in the growth control subculture. The minimum fungicidal concentration was regarded as the lowest extract concentration that did not yield any fungal growth on the solid medium used.

Statistical analysis
All results were replicated two or three times with triplicates ($n = 2 \times 3$ / $n = 3 \times 3$) for each treatment and the data were expressed as a mean ± standard deviation. One-way analysis of variance (ANOVA) was performed using Minitab® Version 16 for Windows (Minitab Inc., USA) followed posthoc Tukey's test for means separation ($p<0.05$).

Result

QUALITATIVE PHYTOCHEMICALS
The result of the qualitative phytochemical composition of the ethanolic extracts of the plants are shown in table 1.

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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.

UNDER PEER REVIEW

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).

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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}

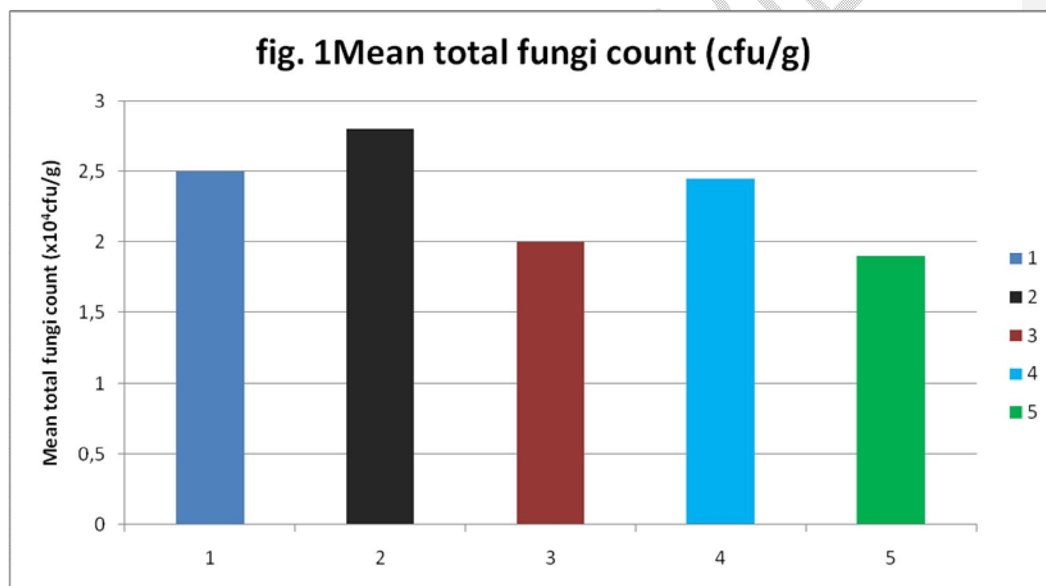

Table 3 : Morphological Characteristics of Fungal Isolates

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 –	Cream	Powdery,	Abundant	Fast	No

	green		spores embedded			
4	White	Cream	Fluffy, raised a little	Abundant	Fast	No

Table 4 : Identification of Fungi

S/N	Description	Probable identity
1	They are typically powdery black, Conidiophores arising from long, broad, thick-walled, sometimes branched foot cell, it has tall conidiophores. Conidia are large with radiating heads, mostly globose and irregularly roughed.	*Aspergillus niger*
2	Colonies are whitish to olivaceous-buff, odour aromatic; in the dark differentiated into tall and short sporangiophores. Sporangia blackish with ellipsoidal, pyriform or subgloblose. Chlamydospores absent.	*Mucor sp.*
3	Colonies are fast growing, aerial mycelium sparse to abundant and floccose, becoming felted, white or peach, but with a violet tinge. Characteristic aromatic odour suggesting lilae.	*Fusarium sp.*
4	Colonies are fast growing conidiophores in fresh isolate typically loosely synematous, giving the colony a zonate appearance. Colonies are light green, reversed colourless, yellow-brown conidiophores usually smooth walled, pencilli 2-3 staged branched with numerous usually oppressed mutulae, conidia sub-globose to ellipsoidal smooth-walled, odour aromatic, fruity and suggesting 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

PEER REVIEW

	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;*

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.

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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

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*). These fungal species were confirmed to be causative agents of the spoilage through the pathogenicity test. In view of the threat poses by post-harvest spoilage of vegetables induced by several fungal species especially in the developing countries like Nigeria as reported by Igbozuluike (2015). The presence of *Aspergillus* spp. and *Rhizopus* spp. as vegetable spoilage agent in this research was in conformity with the findings of Jenkins, (2020) among the spoilt vegetable vended in some selected markets in Lagos. Abang and Shittu (2015) who isolated and reported *Aspergillus niger* as the most dominant mycological flora that was associated with spoilage of vegetable. This finding was also

Comment [M87]: result of this study revealed the presence of 5 different fungal species from spoilt vegetable obtained from ekes

UNDER PEER REVIEW

in conformity with that of Adipala *et al* (2017) 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., 2017 on the isolation of fungal pathogens from vegetable stored and sold in the market. Furthermore, Ali *et al* (2015) stated that *A. niger* was the cause of post-harvest spoilage in sweet orange and acid lime at field. Agrios (2015) 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* (2018) 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. In most cases fungi gain entrance into vegetable cormels though natural opening and wounds created during harvesting, transportation, handling and marketing. However, Ejimofor et al., 2021 noted that root and tuber crops at time of harvest may already be infested by pathogens derived from disease foliage, roots or mother tubers/cormels.

This study revealed that fungitoxic compounds were present in bean root and leaf extracts since they were able to inhibit the growth of the test fungi, this result is in consonance with the earlier reports of several researches but on different fungal organisms (Ejimofor et al., 2021), hence the plant extracts used have the potential application in the protection of mechanically injured vegetable corms/cormels against rot fungi. However, the efficacy of the extracts differed with the plant material, concentration, solvent of extraction and with each test fungus.

Ethanol extracts were more effective than aqeous extract, this suggests that water used in the extraction process was probably not able to dissolve all the principles compounds present in the plants, which are contained in the ethanol extract. The ethanol extract gave higher yield in all the plants, this agrees with the reports of Ejimofor et al., 2022; Oledibe et al., 2022) on garlic who attributed this to the fact that ethanol is an organic solvent and will dissolve organic compounds better, hence liberate the active compounds (phytochemical) required for antifungal activity. The difference in the fungitoxic between the extraction medium can also be as a result of the different susceptibility of each of the test isolates to different concentrations of the extracts, this also agrees with the findings of some workers (Amadioha, 2020),

The presence of bioactive substance have been reported to confer resistance to plants against bacterial, fungi and pest (Oledibe et al., 2022), this therefore explains the demonstration of antifungal activity by the plant extracts used in this study, hence the antifungal properties of these plant extracts is probably due to the presence of phytochemicals which are anti microbial agents (Ejimofor, 2022), that are inhibitory to the growth of these pathogens. Phytochemical screening of the plants showed positive for all the phytochemicals tested (Alkaloid. Flavonoid, Phytate, Saponin, Tannins, Oxalate and Phenols). Medicinal and pharmacological potential of all these phytochemicals was proved by the report of several workers (Anyaegbu *et al*., 2019).

Minimal inhibitory concentration (MIC) were established for ethanolic extracts of bean root and leaf. From the three samples evaluated, they all showed fungistatic activity fungicidal activity. The MIC values ranged from 0.30 to 7.0 µg mL-1.The minimum fungicidal concentration of the uziza extract proved to posses the highest fungicidal action against all the five fungi isolated as indicated by the low value (Table 5).

Comment [M88]: avoid abbreviation
Comment [M89]: organism
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Comment [M93]: and solvent
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UNDER PEER REVIEW

Our results are in accordance with Ejimofor et al. (2022), who suggested the that chemical characteristics of the solvent, the method used during the extraction process and diverse structural and compositional aspects of the natural products result in each materialsolvent system showing distinct behaviour. Differences in polarity among various solvents have been reported to account for the differences in solubility of active plant active properties, hence variations in the degree of activity.

This finding is in agreement with the report of Banso et al. (2019), who also observed that higher concentrations of antimicrobial substances showed more growth inhibition. In addition, the antimicrobial activity of plant extracts might not be due to the action of a single active compound, but the synergistic effect of several compounds that are in minor proportion in a plant (Davicino et al., 2017). These data indicate that the appropriate extract concentration to show a specific effect depends on the plant used and the nature of the extract. This emphasizes the need to know the compound/s responsible for the inhibitory activity through studies to purify, identify and characterize the biomolecules. Further studies are needed to determine the chemical identity of the bioactive compounds responsible for the observed antifungal activity. Natural plant-derived fungicides may be a source of new alternative active compounds, in particular with antifungal activity.

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CONCLUSION

This study have revealed the potentials of bean root and leaf in the control of vegetable in storage, with bean root ethanol extract exhibiting the most fungitoxic activity, this study also depicted that ethanol extracts demonstrated a higher antifungal activity over aqeous extract, indicating that ethanol extract of bean root and leaf could be an alternative or complimentary to synthetic chemicals in controlling vegetable spoilage, where bean root are not available, bean leaf can also be used as a second option because they exhibit a moderate fungitoxic activity on the test organisms.

However, the result of this study has gone a long way in providing better alternative to the over dependence on synthetic fungicides, the use of plant extracts in controlling rot causing organisms and pests could reduce over reliance on one source of agricultural chemicals to the farmers, that are reported to predicate long term harmful consequences on environment, Man and wildlife, as well as reduce production cost.

UNDER PEER REVIEW

Recommendations

With respect to the plants used, further pharmacological evaluation, toxicological studies and possible isolation of the therapeutic antifungal from these plants are the future challenges, hence it is

recommended that further investigations should be done on the chemical nature of the active principles of the plants.

Also further investigations can combine the plant extracts for possible synergistic effect, further research involving invivo assay would be needed 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.

Finally, very essential is the need to devise good storage facilities to prolong the shelf life of vegetable after harvest.

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