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INVESTIGATION ON THE PHYSICOCHEMICAL AND PRESERVATIVE POTENTIAL OF ESSENTIAL OIL FROM *CUSSONIA BARTERI* SEEDS AGAINST POSTHARVEST SPOILAGE FUNGI ISOLATED FROM YAM (*DIOSCOREA ROTUNDATA*).

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ABSTRACT

The main objective of this study is to investigate the physicochemical and preservative potential of essential oil from *Cussonia barteri* seeds against postharvest spoilage fungi isolated from yam. Essential oils was extracted from the seeds using hexane extraction method and its antimicrobial activity was tested against postharvest spoilage fungi isolated from spoilt yam using disk diffusion method and its MIC and MBC calculated. The result from the physiochemical assessment of *Cussonia Barteri* (Jansa Seed) essential oil showed that the oil extracted was cream yellowish in colour with mild/subtle aroma and Smooth in texture and oil feeling. The pH is acidic (5.20), its electrical conductivity was 0.360Us/cm, concentration (0150mg/l), temperature 32.9^0c. three fungi strains (M*ucor spp,* than *penicillium spp* and *Aspergillus niger) were isolated from spoilt yam and f*rom the result, the *Cussonia Barteri* (Jansa Seed) essential oil showedinhibition zone of (20.00mm, 17.50mm, 0.00mm and 0.00mm) for M*ucor spp,in 500, 250., 125 and 62.50mg/L concentration respectively, Aspergillus niger* showedinhibition zone of (18.00mm, 12.50mm, 10.00mm and 0.00mm)*in 500, 250., 125 and 62.50mg/L concentration respectively,* while *penicillium spp* showed inhibition for only concentration of 500mg/l (15.00mm) and 250mg/l (10mm) but were resistance in all other concentrations. When compare with the commercial antibiotic (CFX), it showed inhibition forM*ucor spp,* (57mm) *Aspergillus niger (33mm)* and*penicillium spp*(57.50mm).The results of MIC and MBC of the *Cussonia Barteri* (Jansa Seed) oil suggested that it can be used to control and prevent postharvest spoilage fungiand food

poisoning diseases. the study have proved the seed to be potentially effective can be used as natural alternative preventives to control food poisoning diseases and preserve food stuff avoiding healthy hazards of chemically antimicrobial agent applications.

KEYWORDS: Antimicrobial, Food poisoning, preservative potentials, Extracts.

INTRODUCTION

Spices are indispensable components of cuisines used mainly for flavouring to improve palatability of food (Ghayur *et al.,* 2025). A spice is a dried seed, fruit, root, bark, flower, leave or any vegetative substance used in a very small quantity as food additive to colour, flavour or preserve food. Various oil extracted from spices are natural and contain aromatic phytochemicals and are widely used as a food flavour enhancer and natural preservatives to inhibit the microbial spoilage and improved the shelf life of food. (Denniff *et al.,* 2021). The major components of spices are tannins, resins, pigments, volatile, essential and fixed oils which contribute to flavouring (Cowan and Steel, 2025). Some well-known spices of commerce include red pepper, onions, sage, *Cussonia barteri* , nutmeg, clove, cinnamon, mustard, curry, turmeric, rosemary, onion, jansa and garlic (Abreu *et al*., 2022). Spices, because of many health-promoting phytochemicals they contain are known to fight cancer and many heart diseases. Apart from being used as food ingredients, spices are also used as components of many medicines, perfumery, cosmetics and natural colours. Many spices have been in use in many traditional medicines (Chrubasik *et al*., 2025).

Essential oils can be defined as naturally occurring complex mixtures of volatile lipophilic secondary metabolites that give plants and spices their characteristic essence and colours add (Ejimofor, 2022). Essential oils are complex mixtures, constituted by terpenoid hydrocarbons, oxygenated terpenes and sesquiterpenes. They originate from the plant secondary metabolism and are responsible for their characteristic aroma. The various applications of essential oils account for the great interest in their study. Such applications may be found in the cosmetic industry, as ingredients of fragrances, decorative cosmetic, fine fragrances and flavouring, in the food industry, as aromas and flavours, in the pharmaceutical industry, as active components of medicines and as antibacterials/antimicrobials, and in aromatherapy (Ahmed and Sharm, 2020).

C*ussonia barteri* is a small twisted savannah tree with thick corky bark. The leaves are borate with lateral nerves, the flowers are greenish white, the fruits are white and the wood is very soft and brittle. The seed of *Cussonia bateri* is used in soup and has a pleasant aroma and

sweet taste has been traditionally used to manage different conditions and in production of farm tools in Africa (Chang *et al*., 2025). In African traditional medicine, the uses of *Cussonia* and related species have been documented and reported to possess analgesic, anti-malarial, anti-inflammatory, anti-anaemic, anti-diarrhoea, wound healing, anti-poison, effects against mastitis, mental disorders, sexually transmitted diseases and as an anti-epileptic. Naturally, plant-derived molecules possess the ability to multi-target as they fight against predators like bacteria, fungi, viruses, insects, and herbivores, from their germination to maturity stages, and undergo challenging defense mechanisms for survival (Bartley and Jacobs, 2021). So, instead of developing new antibiotics, exploring potential antimicrobial agents from plant sources is convenient and cost-effective. The therapeutic effects of *Cussonia bateri* oil are due to its richness in several phytochemicals, nutritionally vital constituents, and fatty acids (Ejimofor *et al.,* 2023). *Cussonia bateri*oil has multiple bioactive molecules, including thymoquinone (TQ), *p*-cymene, α-thujene, carvacrols, β-pinene, limonenes, methyl linoleates, sabinenes, d-limonene, 4,5-epoxy-1-isopropyl-4-methyl-1-cyclohexene, and 4-terpineol (Banerjee *et al* 2019) which exerts many pharmacological activities, including antimicrobial, anticonvulsant, anticancer, anti-histaminic, anti-diabetic, anti-inflammatory, and antioxidant (Basu and Rastogi, 2023).

An antimicrobial is a substance that kills or inhibits the growth of microbes such as bacteria, fungi, protozoa or viruses. Antibiotics are those substances which are produced by microorganism that kills or prevents the growth of another micro-organism (Ernst, 2021). Several microorganisms derived antibiotics are currently in use to treat a variety of human disease, therefore the action must be taken to control the use of antibiotics, develop new drugs either synthetic or natural, for a long period of time, plant have a valuable source of natural products for maintaining human health. India has a rich tradition in use of medicinal plants to develop drugs (Bidwell, 2021). Antimicrobials are used in food for two main reasons: to control natural spoilage processes (food preservation), and to prevent or control growth of micro-organisms, including pathogenic micro-organisms (food safety). Natural antimicrobials are derived from animal, plant and microbial sources. There is considerable potential for utilization of natural antimicrobials in food, especially in fresh fruits and vegetables. Edible, medicinal and herbal plants and spices such as oregano, rosemary, thyme, sage, basil, turmeric, *Cussonia barteri* , garlic, nutmeg, clove, mace, savoury, and fennel, have been successfully used alone or in combination with other preservation methods. They exert direct or indirect effects to extend foodstuff shelf life or as antimicrobial agent against a variety of Gram positive and Gram-negative bacteria (Ahmed *et al.,* 2020).

MATERIAL AND METHOD

Sample Collection

Cussonia barteri **seed**sample were collected from AwkaAnambra state. The seed were dried for three days in the room without direct sunlight. After drying they were ground into powder using electronic grinder. The powdered sample were weighed using a digital weighing balance to determine the weight.

METHODS

Extraction of Black seed (*Nigella sativa)*

The*Cussonia barteri*oil was extracted using soxhlet apparatus with N hexane as the solvent. 300 ml of normal Hexane was poured into a round bottom flask and 100 g of ground sample were weighed and placed on a filter paper. The sample folded on the filter paper was then placed in the thimble and inserted into the centre of the extractor. The soxhlet heated at 60^0C. This was allowed to continue for 60 minutes. It was then removed from the tube, dried in the oven, cooled in the dessicators and weighed again. The solution left in the round bottom flask was a combination of oil and solvent. The solvent was removed through distillation and oil recovered, weighed and recorded.

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 121^0C 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 Ejimofor *et al*. (2023) 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 (90^0Capart) on solidified potato dextrose agar (PDA) and sabouard dextrose agar (SDA) plates. Two replicates for each sample were made. The plates were incubated a temperature of 28-30^0C 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 Oleidibe *et al.,* (2022). Fungal identification was done using morphological characteristics and comparing the findings with established keys as described by Firn (2024). 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.

Identification and Characterization of Isolates

The isolates were identified using cultural characteristics and morphology with reference to De Afam-Ezeaku *et al*. (2021).

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 sometime and then examined microscopically with the high power objective. Motile organisms were seen swimming around.

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 20^0C 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 20^0C 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 110^0C 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 121^0C for 10 min. It was filtered and dispensed into sterilized test tubes. Isolates were inoculated into the surface of the medium and incubated at 20^0C for 7 days. The occurrence of clearance in the medium column was taken as a positive result.

Pathogenicity test

The method of Oledibe *et al*. (2020) will be use for the pathogenicity test. Nine healthy cocoyam corms were properly washed with tap water and then rinsed with distilled water. Then the surfaces of the corms were dis-infected with 75% ethanol. The severity of rot seeks to measure the magnitude of the infection as well as the rate of the pathogenicity of the rot-causing fungi. This was determined by obtaining the rotted portions off the whole tubers and taking the final weight of the individual yam tuber. Un-inoculated controls were placed in clean polyethylene bags. The percentage severity of rot (Sr %) was calculated thus:

$$Sr\ \% = \frac{FW - w}{w} \times 100$$

Where,

FW = Final weight of infected yam tuber,

w = weight of rotted tuber portion.

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 (%) = dc- dt x 100

dc 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 (Giri *et al*., 2024), was used with slight modifications to determine minimal inhibitory concentration (MIC) values of plant extracts. Plant extracts were serially 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 Goyal and Kadnur (2024). After 72hrs 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 27^0C 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 × 3 / n = 3 × 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 ($p<0.05$).

RESULTS

PHYSIOCHEMICAL PROPERTIES OF *CUSSONIA BARTERI* (JANSA SEED) ESSENTIAL OIL

The result from the physiochemical assessment of *Cussonia Barteri* (Jansa Seed) essential oil showed that the oil extracted was cream yellowish in colour with mild/subtle aroma and Smooth in texture and oil feeling. The pH is acidic (5.20), its electrical conductivity was 0.360Us/cm, concentration (0150mg/l), temperature 32.9^0C.

Table 1: Physiochemical properties of *Cussonia barteri* (Jansa Seed) essential oil.

Test	Results
pH	5.2 (acidic)
Density	0.90g/cm^3
Conductivity	0360Us/cm
Concentration	0150Mg/L
Temperature	32.9^0C
Texture	Smooth in texture and oil feeling
Colour	Cream yellow
Aroma	Mild and subtle aroma(medicine)

Table 1 shows the physiochemical properties of *Cusonia barteri* essential oil indicating its natural natural preservative agents. This aligns with standard characteristics of high-quality essential oils. The slightly acidic pH, low density, minimal conductivity, and typical organoleptic properties (color, aroma, and texture) collectively indicate a stable, bioactive, and potentially valuable oil for pharmaceutical, cosmetic, and antimicrobial applications. These findings support further investigation into its chemical composition and biological activities.

ISOLATION OF SPOILAGE FUNGI

The total fungi count as well as the Colonial and Morphological features of the three fungi isolated from the yam samples are shown in tables 2 and 3. The fungi pathogens isolated includes *Mucor spp, Aspergillus niger and Penicillium digitatum.*

Table 2 Total fungal count of yam samples.

Locations	Mean total fungi count (x10^2cfu/g)
Location A	**137.00**
Location B	**107.00**
Location C	**144.00**
Mean Fungi count	**129.33**

***Values are mean scores of three (3) replicates**

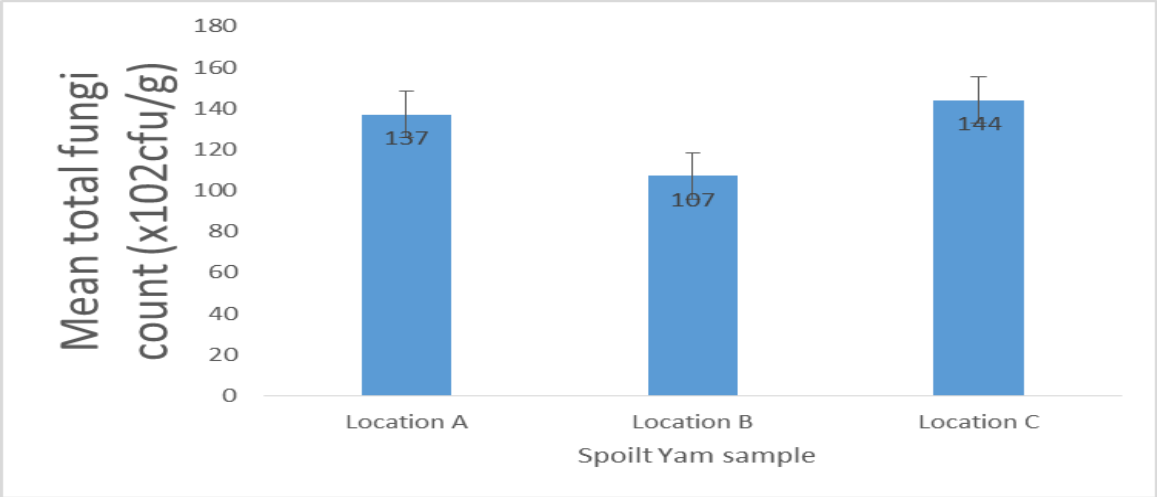

Figure 1: Anova representation of total fungal count of yam samples.

Table 3: Colonial and Morphological features of the fungi isolated.

Isolate	**Colonial Features**	**Morphological Features**	**Suspected Organism**
1	On SDA, colonies had rapid growth rate. However, colonies were flat and compact with yellow basal felt covered by a dense layer of black conidial heads with powdery texture. The colour on the reverse side was pale yellow. Colonies were incubated at 30 o C for 5 days	Septate hyphae with Conidiophores were hyaline or pale-brown to black, erect, simple, with foot cells basally, inflated at the apex forming globose vesicles, bearing conidial heads split into over 4 loose conidial columns with over 4 fragments apically composed of catenulate conidia	*Aspergillus sp*
2	Colonies are velvety and fast growing, with shades of green sometimes white.	Conidia are smooth and ellipsoidal. Conidiophores are smooth and short. Mycelia are arranged irregularly with branches of various length	*Penicillium sp*
3	On SDA, colonies were floccose (cottony in texture), pale greyish-brown. Growth rate was rapid, thus, colonies filled the entire petri-dish in	Sporangiophores were hyaline, erect, non-septate and branched sympodially and circinate. Sporangia were terminal, dark-brown, finely echinulate to smooth and spherical	*Mucor spp*

	3 days. Colour on the reverse side was yellow. Colonies were incubated at 30 o C for 5 days	(20- 80 µm in diameter). Sporangiospores were hyaline or pale-brown. Collumellae were ellipsoidal and 4.5-7 x3.5-5 µm in size. Chlamydospores were absent	

BIOCHEMICAL CHARACTERIZATION OF THE ISOLATED FUNGI

The biochemical characteristics of the isolated fungi were determined in terms Spore Formation, Motility and Biochemical Identification and the result shown in table 4.

Table 4: Biochemical Identification of isolated fungi.

S/N	Isolate	Carbohydrate assimilation	Spore formation	Amino acid assimilation	Motility	Hydrolysis	Lipase activity
1	*Aspergillus sp*	+	-	+	-	-	+
2	*Penicillium sp*	+	-	+	-	-	+
3	*Mucor spp*	+	+	-	-	-	-

PATHOGENICITY TEST

The pathogenicity test showed that all the test fungi (*Mucor spp,penicillium spp*and *Aspergillus niger*) were pathogenic, hence causes rot in healthy yam tubers after nine (9) days of inoculation. The most virulent among the test fungi was *Aspergillus* niger (68%) while the least virulent was *mucor spp*with rot incidence of 25% .

Table 5: Result of the Pathogencity Test.

Sample	*Aspergillus sp (mm)*	*Penicillium sp (mm)*	*Mucor spp(mm)*
Sample 1	9.80	0.00	13.00
Sample 2	42.10	8.70	0.00
Sample 3	9.00	56.00	25.00
Sample 4	50.00	9.00	8.70
Sample 5	68.00	0.00	0.00

Key

+: mildly pathogenic (>10/50mm in diameter).

++: very pathogenic (>/50mm in diameter).

- = not detected

ANTIMICROBIAL ACTIVITY OF EESSENTIAL OIL FROM *CUSSONIA BARTERI* (JENSA SEED) ON TEST ORGANISMS (MM)

The result of the antimicrobial efficacy of *Cussonia Barteri* (Jansa Seed) essential oil against M*ucor spp,penicillium spp*and *Aspergillus niger*are shown in table 6. From the result, the *Cussonia Barteri* (Jansa Seed) essential oil showedinhibition zone of (20.00mm, 17.50mm, 0.00mm and 0.00mm) for M*ucor spp,in 500, 250., 125 and 62.50mg/L concentration respectively, Aspergillus niger*showedinhibition zone of (18.00mm, 12.50mm, 10.00mm and 0.00mm)*in 500, 250., 125 and 62.50mg/L concentration respectively,* while *penicillium spp*showed inhibition for only concentration of 500mg/l (15.00mm) and 250mg/l (10mm) but were resistance in all other concentrations. When compare with the commercial antibiotic (CFX), it showed inhibition forM*ucor spp,* (57mm)*Aspergillus niger (33mm)* and*penicillium spp*(57.50mm).

Table 6: Sensitivity of Essential oil from *Cussonia Barteri* (Jensa Seed) on Test Organisms. (mm)

Test isolates	**CFX**	**500 mg/ml**	**250 mg/l**	**125 mg/l**	**62.5 mg/l**
M*ucor spp*	55.00	15.00	15.00	0.00	0.00
M*ucor spp*	60.00	25.00	20.00	0.00	0.00
Mean	**57.50**	**20.00**	**17.50**	**0.00**	**0.00**
Aspergillus niger	37.00	18.00	12.00	10.00	0.00
Aspergillus niger	33.00	18.00	14.00	10.00	0.00
Mean	**33.00**	**18.00**	**13.00**	**10.00**	**0.00**
penicillium spp	60.00	15.00	0.00	0.00	0.00
penicillium spp	54.00	15.00	20.00	0.00	0.00
Mean	**57.00**	**15.00**	**10.00**	**0.00**	**0.00**

Table 6 illustrates how responsive ***Cussonia Barteri*** is to microorganisms in terms of inhibition or destruction

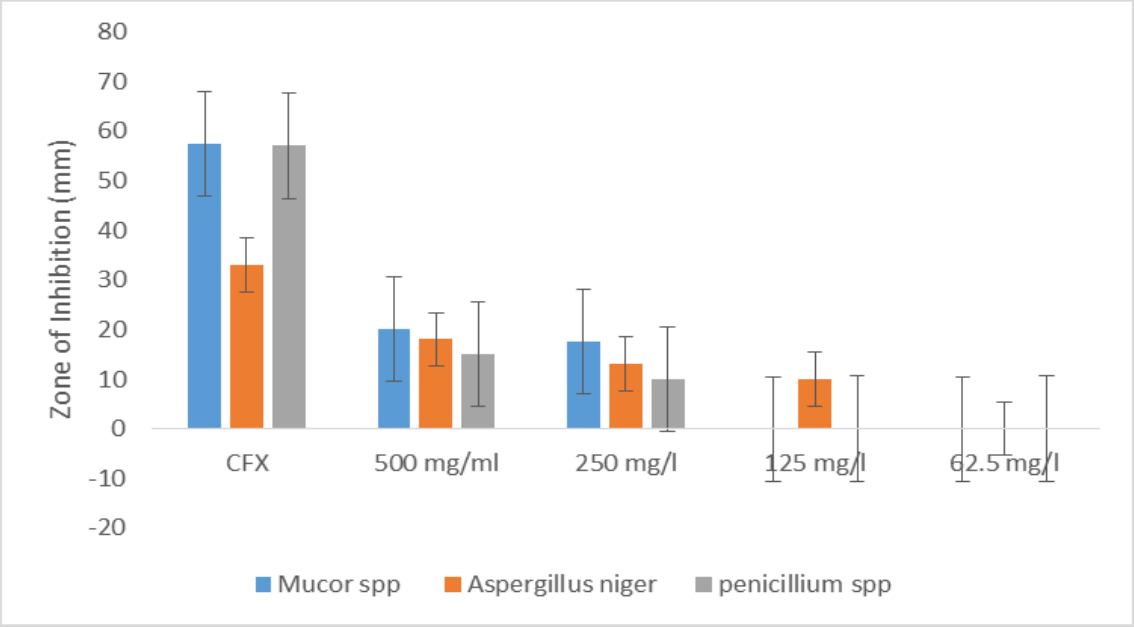

Figure 2: Anova representation of sensitivity of essential oil from *Cussonia Barteri* (Jensa Seed) on Test Organisms. (mm)

Table 7: Minimum Inhibitory Concentration (MIC) of Plant Extracts on Test isolates (ƛ=340 nm)

Jensa Seed Extract	Test Organisms	500 mg/ml	250 mg/ml	125 mg/ml	62.5 mg/ml
Mean	*Mucor spp*	**0.0315**	**0.054**	**0.047**	**0.048**
Mean	*Aspergillus niger*	**0.0490**	**0.058**	**0.0219**	**0.060**
Mean	*penicillium spp*	**0.0495**	**0.067**	**0.0215**	**0.059**

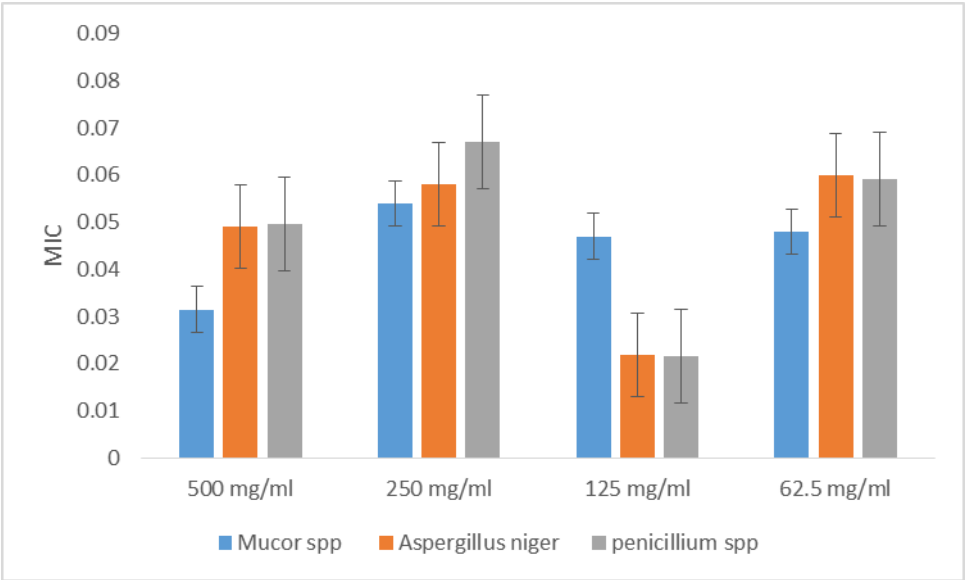

Figure 3: Anova representation of sensitivity of Minimum Inhibitory Concentration (MIC) of Plant Extracts on Test isolates.

Table 8: Minimum Fungicidal Concentration (MBC) of Plants Extract on Test Organisms

Fungi Isolates	MFC of Extract (mg/ml)
M*ucor spp*	500.00
Aspergillus niger	500.00
penicillium spp	500.00

The results presented indicate that the Minimum Fungicidal Concentration (MFC) of the plant extract against all tested fungal isolates—Mucor spp., Aspergillus niger, and Penicillium spp.—was 500 mg/ml. This uniform MFC value across the three fungal genera suggests that a relatively high concentration of the extract is required to achieve fungicidal (complete killing) activity.

DISCUSSION

The present investigation revealed the potency of *Cussonia Barteri* (Jansa Seed in inhibition of M*ucor spp, penicillium spp* and *Aspergillus niger*which are among the spoilage fungi associated with spoiled yam. The result obtained from this study showed that *Cussonia Barteri* (Jansa Seed) has a better inhibition zone for *Muccor spp* than *penicillium spp*and *Aspergillus niger*. This result is also similar to the report of Jenssen *et al*., 2025, whose study showed *Cussonia Barteri* (Jansa Seed) to be of higher antifungal efficacy when tested on M*ucor spp,* isolated from spoilt yam tubers. The high zone of inhibition obtained in this study for M*ucor spp, penicillium spp*and *Aspergillus niger* could be attributed to the solvent used in the extraction (hexane). Ejimofor *et al*. (2025) found that the Hexane extract of major spices such as ginger and garlic has the moderate antibacterial activity (24mm) since the control CFX has shown the highest inhibition 57.5MM mm, therefore, it is essential to compare the capability of the extract with the best standard of inhibition activity.

As shown in Table 1 and also the statistical representation in Figure 1; The physicochemical and organoleptic characteristics presented in the table provide important insight into the quality, stability, and potential applications of Cussonia barteri essential oil. These parameters are widely used in essential oil characterization to determine purity, identity, and functional properties in pharmaceutical and industrial applications. The pH value of 5.2 indicates that the essential oil is slightly acidic. Although essential oils are generally hydrophobic and do not have a true pH in the strict aqueous sense, measured acidity often reflects the presence of oxygenated compounds such as phenols, aldehydes, or carboxylic derivatives. Slight acidity in essential oils has been associated with antimicrobial activity, as acidic environments can

inhibit microbial growth and enhance preservation potential (Yadav, 2022) . This suggests that C. barteri oil may possess bioactive properties relevant to medicinal or preservative uses. The density of 0.90 g/cm³ is consistent with typical essential oils, which are generally less dense than water (density <1.0 g/cm³). This characteristic confirms the oil's volatile and lipophilic nature, as most essential oils float on water due to their lower density (Dhifi *et al.,* 2016) . The observed density supports the classification of the extract as a true essential oil and indicates minimal contamination with heavier non-volatile compounds. Electrical conductivity (360 µS/cm) is relatively low, which is expected since essential oils are poor conductors due to the absence of free ions. However, the measurable conductivity may suggest the presence of trace polar constituents or residual moisture from the extraction process. Conductivity in essential oils is rarely high because they are composed mainly of non-polar terpenes and related compounds (Leite *et al.,* 2020) . The reported concentration (150 mg/L) reflects the amount of dissolved oil or active constituents in the tested medium. This relatively low concentration aligns with the fact that essential oils are potent substances, often effective at low doses due to their complex mixture of bioactive compounds such as monoterpenes and sesquiterpenes (Dhifi *et al.,* 2016). This further supports its potential pharmacological relevance. Temperature (32.9°C) at the time of analysis indicates ambient tropical conditions, which may influence volatility and viscosity. Essential oils are known to be temperature-sensitive; increased temperature can enhance evaporation and alter physicochemical measurements such as density and viscosity (Yadav, 2022). Thus, this value is important for reproducibility and standardization. The texture described as smooth and oily is characteristic of essential oils, confirming their lipophilic and non-viscous nature. This property is important for applications in cosmetics, aromatherapy, and topical formulations, where spreadability and skin absorption are key considerations. The cream-yellow coloration of the oil is also typical, as most essential oils range from colorless to pale yellow depending on their chemical composition. Pigmentation may be influenced by the presence of certain compounds or slight oxidation during extraction and storage (Boughendjioua & Djeddi, 2017). The mild and subtle medicinal aroma suggests the presence of volatile aromatic compounds such as terpenoids and phenolics, which are responsible for the characteristic scent of essential oils. Aroma is not only an organoleptic property but also an indicator of chemical composition and potential therapeutic value, as many aromatic compounds exhibit antimicrobial, anti-inflammatory, and antioxidant activities (Dhifi *et al*., 2016).

Also as seen in table 2, The total fungal count of yam samples across the three locations shows noticeable spatial variation, with values ranging from 107.00 × 10^2 cfu/g to 144.00 ×

10^2 cfu/g and an overall mean of 129.33 × 10^2 cfu/g. This indicates a moderate to high level of fungal contamination in the sampled yam, which is significant from both food safety and postharvest quality perspectives. Location C recorded the highest fungal load (144.00 × 10^2 cfu/g), followed by Location A (137.00 × 10^2 cfu/g), while Location B had the lowest count (107.00 × 10^2 cfu/g). The higher fungal counts observed in Locations A and C may be attributed to factors such as poor storage conditions, higher moisture content, and increased environmental exposure, all of which are known to favor fungal proliferation. Fungal growth in yam is strongly influenced by humidity, temperature, and handling practices during storage and transportation, which can accelerate spoilage and microbial colonization.

The mean fungal count (129.33 × 10^2 cfu/g) suggests that the yam samples are generally susceptible to fungal contamination, which is consistent with previous studies reporting that yam and its products are prone to microbial spoilage during storage. For instance, studies in Nigeria have documented fungal contamination in yam products, often associated with storage fungi such as Aspergillus, Penicillium, and Fusarium, which are capable of producing harmful mycotoxins . These fungi not only reduce the shelf life of yam but also pose serious health risks due to toxin production, including aflatoxins and fumonisins.

Although the fungal counts reported in this study (10^2 cfu/g range) are lower than some values reported in processed yam products (which can reach 10^3 cfu/g or higher), they still indicate contamination and potential quality deterioration. Even relatively low fungal loads can increase over time under favorable storage conditions, leading to rapid spoilage and possible toxin accumulation. The variation observed among the locations highlights the influence of environmental and handling conditions on microbial load. Location B's comparatively lower fungal count suggests better storage or handling practices, which could serve as a model for reducing contamination in other areas. Improved postharvest handling, including proper drying, hygienic storage, and moisture control, is essential to minimize fungal growth and ensure food safety.

Table 4 illustrated the biochemical characterization of the fungal isolates—Aspergillus sp., Penicillium sp., and Mucor spp.—reveals distinct metabolic and physiological traits that are critical for their identification and functional differentiation. These tests, including carbohydrate assimilation, spore formation, amino acid utilization, motility, hydrolytic activity, and lipase production, are widely employed in mycological studies to complement morphological identification and provide insights into fungal ecology and enzymatic potential. All three isolates demonstrated positive carbohydrate assimilation, indicating their ability to utilize sugars as primary carbon and energy sources. This is a fundamental

metabolic trait in filamentous fungi, as carbohydrates serve as essential substrates for growth and secondary metabolite production. Previous studies have similarly reported that *Aspergillus* and *Penicillium* species efficiently metabolize a wide range of sugars, contributing to their ecological versatility and prevalence in food spoilage and soil environments. Spore formation results showed variability among the isolates. Mucor spp. tested positive, while Aspergillus sp. and Penicillium sp. were negative in this dataset. Although all these fungi are generally known to produce spores, differences in observed spore formation may be attributed to culture conditions, incubation time, or methodological limitations. Mucor species are characterized by rapid formation of sporangiospores, which enhances their dispersal and colonization efficiency in moist environments . In contrast, delayed or absent sporulation in Aspergillus and Penicillium under specific laboratory conditions has been previously documented.

Amino acid assimilation was positive for Aspergillus sp. and Penicillium sp., but negative for Mucor spp.. This suggests that the former possess more versatile nitrogen metabolism, enabling them to utilize amino acids as nitrogen sources for growth and biosynthesis. This metabolic flexibility contributes to their adaptability in nutrient-variable environments. Similar findings have been reported, where Aspergillus and Penicillium species showed strong amino acid assimilation capabilities compared to other fungal genera. Motility was negative across all isolates, which is consistent with the general characteristic of filamentous fungi. Unlike some bacteria or lower eukaryotes, these fungi are non-motile and rely on spore dispersal mechanisms such as air currents or water movement for propagation. Hydrolysis activity was absent in all isolates, indicating a lack of detectable extracellular enzyme activity for substrate breakdown under the test conditions. This result suggests that the isolates may either not produce certain hydrolytic enzymes (e.g., amylases or proteases) or that the experimental conditions were not optimal for enzyme expression. However, fungal hydrolytic activity is often substrate-specific and inducible, meaning that different media compositions could yield different results. Lipase activity showed a clear distinction among the isolates. Aspergillus sp. and Penicillium sp. were positive, while Mucor spp. was negative. Lipase production is an important biochemical trait, as these enzymes catalyze the hydrolysis of lipids into fatty acids and glycerol, with significant industrial applications. Numerous studies have identified Aspergillus species as prolific producers of lipases, often exhibiting high enzymatic activity under optimized conditions . Similarly, Penicillium species are recognized for their lipolytic capabilities, contributing to their role in biodegradation and biotechnology .

The absence of lipase activity in Mucor spp. in this study contrasts with some reports but may reflect strain-specific variability or environmental influences.

The results of the pathogenicity test in Table 5 revealed clear differences in the susceptibility of the tested fungi—*Aspergillus spp., Penicillium spp*., and *Mucor spp*.—to the various samples, as indicated by the diameter of zones of inhibition (mm). These variations suggest differences in antifungal potency among the samples and differential sensitivity of the fungal isolates. Across all samples, Aspergillus spp. exhibited the highest overall susceptibility. Notably, Sample 5 recorded the largest inhibition zone (68.00 mm), followed by Sample 4 (50.00 mm) and Sample 2 (42.10 mm). This indicates a strong antifungal effect of these samples against Aspergillus spp., which may be attributed to the presence of bioactive phytochemicals such as terpenoids, phenolics, and flavonoids known for their antifungal activity (Bakkali *et al.,* 2008). The relatively lower inhibition observed in Samples 1 (9.80 mm) and 3 (9.00 mm) suggests weaker antifungal efficacy or lower concentrations of active compounds in those samples. In contrast, Penicillium spp. showed selective susceptibility. The highest inhibition was observed in Sample 3 (56.00 mm), indicating a strong antifungal potential of this sample against Penicillium. Moderate activity was observed in Samples 4 (9.00 mm) and 2 (8.70 mm), while Samples 1 and 5 showed no inhibitory effect (0.00 mm). This selective response may reflect intrinsic resistance mechanisms in Penicillium species, such as cell wall composition and enzymatic detoxification systems, which can limit the efficacy of certain antifungal agents (Hyldgaard *et al.,* 2012). For Mucor spp., Sample 3 again demonstrated the highest inhibition (25.00 mm), followed by Sample 1 (13.00 mm) and Sample 4 (8.70 mm). Interestingly, Samples 2 and 5 showed no activity against Mucor spp. The moderate sensitivity of *Mucor spp*. compared to *Aspergillus spp*. may be due to structural and physiological differences, including faster growth rates and spore resistance, which often reduce antifungal susceptibility (Pitt & Hocking, 2009). Overall, Sample 3 appears to exhibit broad-spectrum antifungal activity, as it inhibited all three fungal isolates significantly, particularly Penicillium spp. and Mucor spp. On the other hand, Sample 5 demonstrated highly selective activity, showing strong inhibition only against Aspergillus spp. but no effect on the other fungi. This suggests that the antifungal compounds present in Sample 5 may be more specific in their mode of action. The observed differences in antifungal activity across samples could be linked to variations in chemical composition, concentration of active constituents, or extraction methods. Essential oils and plant extracts are known to exhibit variable antimicrobial effects depending on their phytochemical profiles, which influence

their ability to disrupt fungal cell membranes, interfere with enzyme activity, or inhibit spore germination (Burt, 2004).

The results presented in table 6 and figure 2 respectively indicated the Minimum Fungicidal Concentration (MFC) of the plant extract against all tested fungal isolates—Mucor spp., Aspergillus niger, and Penicillium spp.—was 500 mg/ml. This uniform MFC value across the three fungal genera suggests that a relatively high concentration of the extract is required to achieve fungicidal (complete killing) activity. The finding implies that while the extract may possess antifungal properties, its fungicidal potency is relatively low, since effective killing occurs only at the highest concentration tested. This observation aligns with the general understanding that many plant-derived extracts exhibit fungistatic activity at lower concentrations and fungicidal activity only at higher doses. According to Cowan M.M. (1999), plant extracts often require high concentrations to exert lethal effects due to the complex mixture of bioactive compounds, which may act synergistically but are less potent than purified antifungal drugs. The susceptibility pattern observed—where all organisms required the same high concentration—suggests broad-spectrum but weak fungicidal activity. However, it is noteworthy that *Aspergillus niger* and *Penicillium spp.* are known for their robust cell wall structures and spore-forming abilities, which contribute to increased resistance against antimicrobial agents. Studies by Gupta R. and Sharma A. (2012) indicate that fungal species with thick cell walls and efficient detoxification mechanisms often require higher concentrations of antifungal agents for complete inhibition or killing. The sensitivity profile of the essential oil extracted from Jensa seed against the tested fungal isolates—*Mucor spp, Aspergillus niger*, and *Penicillium spp*—reveals a concentration-dependent antifungal activity, with clear variability in susceptibility among the organisms. For Mucor spp, the essential oil exhibited very high inhibitory activity at 500 mg/ml (mean inhibition zone: 57.50 mm), which even exceeded the activity of the standard antifungal agent (CFX, 55.00 mm). This suggests strong fungicidal potential at high concentrations. However, a sharp decline in activity was observed as the concentration decreased, with mean inhibition zones dropping to 20.00 mm at 250 mg/ml and 17.50 mm at 125 mg/ml. No inhibition was recorded at 62.5 mg/ml and lower concentrations, indicating a relatively high minimum inhibitory threshold for Mucor spp. This pattern aligns with previous findings that members of Mucor are moderately resistant to plant-derived antifungal agents due to their robust cell wall composition and rapid growth rate. In the case of Aspergillus niger, the essential oil demonstrated moderate but consistent antifungal activity across a broader concentration range. The highest mean inhibition zone (33.00 mm) at 500 mg/ml was lower than that

observed for Mucor spp, indicating comparatively reduced susceptibility. However, unlike Mucor spp, A. niger maintained measurable sensitivity even at lower concentrations (10.00 mm at 62.5 mg/ml), suggesting that it is more responsive to sub-lethal doses of the essential oil. This observation is consistent with literature reports that A. niger is generally susceptible to essential oils rich in terpenoids and phenolic compounds, which disrupt membrane integrity and enzymatic systems. For Penicillium spp, the essential oil showed the highest activity at 500 mg/ml (mean: 57.00 mm), comparable to the control (60.00 mm), indicating strong antifungal efficacy. However, its sensitivity declined more rapidly than that of A. niger, with mean inhibition zones reducing to 15.00 mm and 10.00 mm at 250 mg/ml and 125 mg/ml, respectively, and complete resistance observed at 62.5 mg/ml. This suggests that while Penicillium spp is highly susceptible at high concentrations, it lacks sustained sensitivity at lower doses. Similar trends have been reported in studies where Penicillium species exhibit high initial susceptibility but quickly adapt to reduced concentrations of phytochemicals.

The essential oil from Jensa seed demonstrated strong antifungal activity at higher concentrations, particularly against Mucor spp and Penicillium spp, while Aspergillus niger showed more stable sensitivity across concentrations. The observed antifungal effects can be attributed to the presence of bioactive constituents such as monoterpenes, sesquiterpenes, and phenolic compounds, which are known to interfere with fungal cell membranes, inhibit spore germination, and disrupt metabolic pathways. The concentration-dependent decline in antifungal activity observed in this study is consistent with the general pharmacodynamic behavior of plant essential oils, where efficacy is closely tied to dosage. These findings support the potential application of Jensa seed essential oil as a natural antifungal agent, particularly in postharvest preservation systems where higher concentrations can be effectively utilized.

The high MFC value in table 8 may be attributed to the phytochemical composition of the plant extract. Compounds such as alkaloids, flavonoids, tannins, and terpenoids are known to disrupt fungal cell membranes and metabolic processes, but their effectiveness depends on concentration and bioavailability. As reported by Sofowora A. (2008), crude plant extracts typically exhibit lower antimicrobial efficacy compared to isolated active compounds due to dilution of active constituents. Another important consideration is the comparison between MFC and Minimum Inhibitory Concentration (MIC). In many studies, when the MFC is significantly higher than the MIC, it suggests that the extract is primarily fungistatic rather than fungicidal. This could indicate that the extract inhibits fungal growth at lower

concentrations but requires much higher levels to achieve complete eradication. This pattern has been widely reported in antifungal studies involving plant extracts (Hammer *et al.,* 1999). From an application perspective, the high MFC value may limit the practical use of the extract as a standalone antifungal agent, particularly in clinical or food preservation settings where lower effective doses are preferred for safety, cost, and sensory reasons. However, the extract could still be valuable in combination therapy, where it may enhance the efficacy of conventional antifungal agents or serve as a natural preservative when used at optimized concentrations.

The results of MIC and MBC as demonstrated in Table 7,figure 3and table 8 respectively also suggested that it can be used to control and prevent food borne microorganisms and food poisoning diseases. Fungal strains included in this study were chosen for their importance in food spoilage and food poisoning. *Mucor spp* considered as the one of the most common source of food borne disease while M*ucor spp,penicillium spp*and *Aspergillus niger* produce toxins and other metabolites that induce human gastroenteritis diseases. *Cussonia Barteri* (Jansa Seed) extract suppressing microbial growth of all tested fungal strains which appear to be potentially effective against the three fungal strains tested in this study. These results are in accordance with those of Kumar *et al*., 2020. A great variation in MIC of *Cussonia Barteri* (Jansa Seed) extract demonstrated in several investigation may be due to considerable variation in their method of extraction, constituents as well as bacterial strains used. On the other hand, a higher concentration of *Cussonia Barteri* (Jansa Seed) extract reached to 250 mg/l may be required to be effective against food spoilage fungi and these results were coincident with that previously reported by Lans *et al*. (2021). The Minimum Inhibitory Concentration (MIC) results of Jensa seed extract against Mucor spp, Aspergillus niger, and Penicillium spp demonstrate notable antifungal activity across the tested concentration range (500–62.5 mg/ml), as evidenced by variations in absorbance at 340 nm. Since lower absorbance values correspond to reduced fungal growth, the data indicate that the extract possesses inhibitory effects against all tested isolates, though with organism-specific responses.

For Mucor spp, the lowest absorbance value (0.0315) was recorded at 500 mg/ml, indicating maximum inhibition at the highest concentration. Although a slight increase in absorbance was observed with decreasing concentration, inhibition remained evident even at 62.5 mg/ml. This suggests that Mucor spp is moderately susceptible to the extract and that the bioactive constituents retain efficacy across a broad concentration range. The persistence of antifungal activity at lower concentrations may be attributed to the presence of stable phytochemicals

such as terpenoids and phenolic compounds, which are known to disrupt fungal cell membranes and metabolic processes (Bakkali et al., 2008).

In contrast, Aspergillus niger exhibited a non-linear response to increasing extract concentration, with the lowest absorbance (0.0219) observed at 125 mg/ml rather than at 500 mg/ml. A similar trend was observed for Penicillium spp, where the minimum absorbance (0.0215) also occurred at 125 mg/ml. This pattern suggests that the antifungal activity of Jensa seed extract may be optimized at intermediate concentrations. Such non-linear dose-response relationships have been reported in plant-derived antimicrobials and may result from synergistic interactions among phytochemical constituents at specific concentrations, or from antagonistic effects and compound saturation at higher doses (Cowan, 1999).

Comparatively, Penicillium spp and Aspergillus niger were more susceptible to the extract than Mucor spp, as indicated by their lower MIC values. This differential susceptibility may be linked to variations in fungal cell wall composition, permeability, and enzymatic defense mechanisms. Filamentous fungi such as Aspergillus and Penicillium may be more vulnerable to phytochemicals that interfere with ergosterol synthesis or compromise membrane integrity, while Mucor spp may possess structural or physiological traits that confer relatively higher resistance (Tajkarimi *et al.,* 2010).

The observed antifungal activity supports the potential application of Jensa seed extract as a natural preservative agent, particularly in the control of postharvest spoilage fungi. The effectiveness of the extract at relatively low concentrations (notably 125 mg/ml) is of practical significance, as it suggests cost-effectiveness and reduced risk of toxicity in real-world applications. Furthermore, the broad-spectrum activity demonstrated in this study aligns with previous reports on the antimicrobial properties of plant essential oils, which have been widely recognized for their roles in food preservation and plant disease management (Bakkali et al., 2008). The findings indicate that Jensa seed extract exhibits promising antifungal properties, with optimal inhibitory activity occurring at intermediate concentrations for certain organisms. These results highlight the importance of identifying appropriate dosage levels in the development of plant-based antifungal agents and provide a scientific basis for further investigation into the extract's mode of action and potential applications in postharvest preservation systems.

Several researchers investigated the efficiency of plant extracts and their effective compounds as antimicrobial agents to control growth of food borne and spoilage bacteria. Some researchers have suggested that antimicrobial components of the plant extracts (terpenoid, alkaloid and phenolic compounds) interact with enzymes and proteins of the microbial cell

membrane causing its disruption to disperse a flux of protons towards cell exterior which induces cell death or may inhibit enzymes necessary for aminoacids biosynthesis (Mabberley, 2024). Other researchers attributed the inhibitory effect of these plant extracts to hydrophobicity characters of these plants extracts which enable them to react with protein of microbial cell membrane and mitochondria disturbing their structures and changing their permeability (Masuda *et al*. 2024: Wilasrusmee *et al*., 2024). The present study suggested that plant extracts which proved to be potentially effective can be used as natural preservatives to control food poisoning diseases and preserve food avoiding application of health hazards of chemical preservatives.

CONCLUSION

Food spoilage is often caused by the growth of many pathogenic bacterial strains. Prevention of food spoilage in food industry and food stuff is mainly based on the application of chemical preservatives. The adverse effects of these chemical preservatives on human health increases the demand to search for potentially effective, healthy safer and natural food preservative. The result from this study have confirmed that *Cussonia Barteri* (Jansa Seed) derived antimicrobials have promising probability to be used as preservative in food. The *Cussonia Barteri* (Jansa Seed) extracts which proved to be potentially effective can be used as natural alternative preventives to control food poisoning diseases and preserve food stuff avoiding healthy hazards of chemically antimicrobial agent applications. Although the result obtained from this study showed that *Cussonia Barteri* (Jansa Seed) has a better inhibition zone for M*ucor spp,* than *penicillium spp*and *Aspergillus niger*, but the work suggests the possible exploration of these plants as sources of natural product for future use in the management of multi-drug resistant pathogens such as M*ucor spp,penicillium spp*and *Aspergillus niger*that cause wide range of postharvest losses in yam and other tubers.

RECOMMENDATIONS

- The study proved that *Cussonia Barteri* (Jansa Seed)could be used to decrease the possibility of food poisoning and spoilage, to increase the food safety and shelf-life of products, and to treat some infectious diseases but In the future, as the combinations of several spices were proven to possess higher inhibitory effects on specific bacteria than those of individual spices, the interactions of more spices should be studied and evaluated to inhibit different microorganisms in different food products.

- Finally, the mechanisms of antimicrobial action of spices remain to be clarified in order to make the best use of spices. Furthermore, the potential toxicity of spices on humans should be evaluated.

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