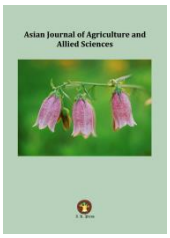

*Asian Journal of Agriculture and Allied Sciences*

*Volume 8, Issue 1, Page 99-113, 2025; Article no.AJAAS.12608*

# Preservative Potential of *Cussonia barteri* Seed Essential Oil against Fungal Spoilage in Yams (*Dioscorea rotundata)*

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***Authors' contributions***

*This work was carried out in collaboration among all authors. Author A-ECE designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors CMM and AAN managed the analyses of the study. Authors EHN and NAO managed the literature searches. All authors read and approved the final manuscript. All authors read and approved the final manuscript.*

***Article Information***

DOI: https://doi.org/10.56557/ajaas/2025/v8i156

**Open Peer Review History:**
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://prh.ikprress.org/review-history/12608

***Original Research Article***

***Received: 03/11/2024***
***Accepted: 07/01/2025***
***Published: 14/01/2025***

## ABSTRACT

This study investigated the preservative potential of essential oil extracted from *Cussonia barteri* seeds. The physicochemical properties of the oil, including pH, specific gravity, color, and aroma, were characterized and its antifungal activity against three fungal species (*Aspergillus* spp,

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***Cite as:*** *Eziamaka, Afam-Ezeaku, Chikaodili, Chukwuekee, Miracle Mmesomachukwu, Achugbu, Adaeze Nnedimma, Eze, Hope Nkiruka, and Nwakuche, Adaugo Ozioma. 2025. "Preservative Potential of Cussonia Barteri Seed Essential Oil Against Fungal Spoilage in Yams (Dioscorea Rotundata)". Asian Journal of Agriculture and Allied Sciences 8 (1):99-113. https://doi.org/10.56557/ajaas/2025/v8i156.*

*Penicillium* spp*, and Mucor* spp*)* associated with yam spoilage was evaluated, using *In vitro* assays. The results showed that the essential oil had a pH of 5.2, specific gravity of 0.85, and a mild, subtle aroma. The oil exhibited antifungal activity against the three fungal species, with the highest activity observed at a concentration of 500mg/ml. The minimum fungicidal concentration of the essential oil was also determined. The study concludes that the essential oil from *Cussonia barteri* seeds has the potential to serve as a natural preservative, thereby extending the shelf life of yams and reducing post-harvest losses. Further research is recommended to evaluate the oil's stability, toxicity, and regulatory compliance before commercial use. This study contributes to the development of natural preservative systems for sustainable food preservation methods.

*Keywords: Cussonia barteri; essential oil; antifungal activity; post-harvest preservation; sustainable food management; (Dioscorea rotundata); physiocochemical.*

## 1. INTRODUCTION

### 1.1 Background of the Study

*Cussonia barteri is a* flowering plant. It belongs to the family Araliaceae, which includes many species of flowering plants, and it produces small inconspicuous flowers typically arranged in clusters. *Cussonia bateri* is commonly known as the "Cabbage Tree". It is a dicotyledonous, medium sized deciduous tree. It grows up to 10-13m in height. The plant has digitate leaves (5-8 ovate-elliptic leaflets, with greenish white flowers contained in contained in clusters of narrow spikes up to 50cm long. The fruits of *Cussonia barteri* are fleshy and turn from purple to white upon maturation (Coulibaly et al., 2011). Seasonal variations affect the size of the plant especially in dry season where it undergoes complete defoliation. The defoliated tree looks like a 'cut off limb' in Mali and Volta, the leper's hands in Hausa', Northern Nigeria. The plant is also known as 'Ako-sigo' in Yoruba, Tuwongiwa in Hausa, Bolo koro in Senegal and kokobidua in Ghana. The seeds are also called 'Jansa seeds' (Cameroun),'Ugbaokwe'(Igbo),Tuwongiwa(Hausa),Bamarlahi(Fulani) and Shigo(Yoruba) in Nigeria (Nwokonkwo, 2013). The leaves of *Cussonia barteri* have been used in various African countries to treat a range of ailments. *Cussonia barteri h*as a lot of uses all over the world. It has medical uses. Traditional medicine uses various parts of the tree for treating ailments such as malaria, rheumatism, and digestive disorders. It also has culinary uses. The leaves are edible and can be cooked as vegetables or added to soups and stews. The leaves and branches are sometimes used as fodder for livestock. The wood of *Cussonia barteri* can be used in construction, furniture making, and carving due to its durability and workability. The wood is also used as firewood and charcoal. The bark fibers of *Cussonia barteri* are used for making ropes, baskets, and mats. In some parts of the world the tree is used in cultural and ritual practices. When planted it often serve as shade trees in agricultural fields and residential areas. The extensive root system of *Cussonia barteri* helps prevent soil erosion, making it useful for stabilizing slopes and riverbanks.

Furthermore, when *Cussonia barteri* is planted in gardens and parks it serves ornamental purposes due to its attractive foliage and unique appearance.

The *Cussonia bateri* has been used over years in treatment of certain diseases and illnesses of various kinds. According to Van and Gericke (Van Wyk & Gericke, 2000), traditional remedies made from parts of *Cussonia* species have been used to alleviate digestive issues such as stomach pain and diarrhea. The leaves and other parts of the cabbage tree have been used in traditional medicine for their anti-inflammatory properties, which may be beneficial in treating rheumatism (Lawal, 2014; Tatsumi et al., 2001). It was also discovered in 2009 by Cheikhyoussef (Cheikhyoussef, 2009), some traditional medicinal practices in Africa have used parts of *Cussonia* species for treating malaria symptoms.

Yam (*Dioscorea rotundata)* cultivation dates back to ancient times, with evidence of its domestication found in Africa as early as 8000 BCE. Archaeological findings suggest that yams were cultivated in parts of West Africa, particularly in regions that are now Nigeria and Ghana. Yam cultivation spread from Africa to Asia and the Pacific through trade and migration. Different varieties of yams were cultivated in regions such as Southeast Asia, the Pacific Islands, and parts of India and China. According to Nweke and Enujeke (2006), yams have held significant cultural and ritual importance in many

societies. In Africa, yams are associated with fertility, prosperity, and cultural identity, often featuring prominently in ceremonies and festivals. In the modern era, yam (*Dioscorea rotundata)* cultivation has undergone significant advancements in breeding, cultivation techniques, and post-harvest handling to improve yield and quality. Research continues to explore the genetic diversity of yams and develop varieties resistant to pests and diseases (Lebot et al., 2009).

According to Oloyede and Obuotor, (2008), yams are rich in essential nutrients, including carbohydrates, fiber, vitamins (such as vitamin C and B6), and minerals (like potassium and manganese), all of which contribute to overall health and well-being.

Yams contain antioxidants such as vitamin C and beta-carotene, which help combat oxidative stress and reduce the risk of chronic diseases, including heart disease and cancer (Oboh et al., 2012).

Ogunbode and Olatunde (2013) said that ccompounds found in yams have been shown to possess anti-inflammatory properties, which may help reduce inflammation and alleviate symptoms associated with inflammatory conditions such as arthritis.

Also, fiber content in yams supports digestive health by promoting regular bowel movements, preventing constipation, and supporting a healthy gut microbiota Ogunbode and Olatunde (2013) said.

Yams are low in fat and calories but high in fiber, making them a filling and nutritious food choice that can aid in weight management and promote satiety (Adetuyi & Olayiwola, 2009). The potassium found in yams according to Mabekoje and Onilude (2003), plays a crucial role in regulating blood pressure and heart function, thus supporting cardiovascular health and reducing the risk of stroke and heart disease.

“The presence of vitamins and minerals in yams, such as vitamin B6 and manganese, contributes to brain health and cognitive function, supporting memory and concentration” (Osuagwu et al., 2010).

Also, yams have a low glycemic index, which means they cause a slower and steadier increase in blood sugar levels compared to high-glycemic foods, making them suitable for individuals with diabetes or those aiming to manage blood sugar (Gberikon et al., 2024).

In year (2000), Ishida et al. (2000), said that the vitamin C content in yams strengthens the immune system by supporting the production and function of white blood cells, which help protect the body against infections and illnesses. According to Uvere and Omjate (2008), yams contain minerals like manganese and copper, which are essential for bone formation and maintenance, thus supporting overall bone health and reducing the risk of osteoporosis.

The presence of vitamins A and C in yams contributes to healthy skin by promoting collagen production, maintaining skin elasticity, and protecting against oxidative damage from UV radiation (Adetuyi & Olayiwola, 2009).

Beta-carotene, a precursor of vitamin A found in yams, supports eye health by reducing the risk of age-related macular degeneration and maintaining optimal vision (Erukainure & Oke, 2014).

Nweze and Okafor (2005) said that “yams are a rich source of carbohydrates, providing a sustained release of energy, making them an excellent choice for athletes and individuals with active lifestyles”. “Certain compounds found in yams, such as diosgenin, have been studied for their potential anti-cancer properties, including inhibiting the growth of cancer cells and inducing apoptosis (cell death) in tumor cells” (Ding et al., 2011).

**Objectives Of This Study:**

The objectives of the study include:

1. To assess the physicochemical properties of essential oil extracted from *Cussonia barteri* seeds, including composition, purity, and stability.
2. To investigate the antifungal activity of the essential oil against common spoilage microorganisms affecting yams, such as fungi.

## 2. LITERATURE REVIEW

### 2.1 *Cussonia barteri*

**Botany of *Cussonia barteri*:** *Cussonia barteri*, commonly known as the “cabbage tree”, is a distinctive tree species native to West Africa, particularly found in countries like Nigeria, Cameroon, and Ghana. It belongs to the family Araliaceae and is characterized by its large, rounded, cabbage-like leaves clustered at the top of a thick trunk. The tree can reach heights of up

to 15 meters and has a broad canopy. Its bark is smooth and greyish-brown, often with some irregularities (World Agroforestry Centre, 2009).

The leaves of *Cussonia barter*i are an important part of traditional medicine in Africa, used for their medicinal properties in treating various ailments. Additionally, the tree provides habitat and food for a variety of wildlife. It prefers moist, well-drained soils and can tolerate both full sun and partial shade. Due to habitat loss and overexploitation for medicinal use, *Cussonia barteri* is facing threats in some areas and conservation efforts are needed to protect this species (World Agroforestry Centre, 2009).

It is a small tree, glabrous except the inflorescence, with the ends of the branches very thick. Leaves digitate of 6 oblong-obovate leaflets cuneate at the base, narrowly and acutely acuminate or caudate at the apex, entire, minutely reticulate, 7-8 1/2 inches long. Petioles terete-sulcate, 15 inches long, pithy. Spikes about 6 1/2 inches long, with numerous sessile flowers spirally arranged. Rachis 1/7- 1/6 inches thick, pubescent; bracteoles ovate-lanceolate, glabrescent. Fruit subglobose, 1/5- 1/4 in. long, glabrate, white; calyx with sinuous margin; epigynous disk shortly conical, continuous with style, which is bilobed at the spreading apex.

**Fruits:** The fruits of *Cussonia barteri* are small, round, and typically green when unripe, turning purplish-black as they mature. They contain small seeds within, dispersed by birds and other animals. The fruits are not typically consumed by humans but play a role in the tree's reproductive cycle and as a food source for wildly (Coulibaly et al., 2011).

Cotyledons: The cotyledons of Cussonia barteri, which are the embryonic leaves within the seed, are typically lanceolate or elliptic in shape. They serve as the initial energy and nutrient source for the seedling as it germinates and establishes itself (World Agroforestry Centre, 2009).

**Roots:** Cussonia barteri has a well-developed root system, with a taproot that extends deep into the soil to provide stability and access to water and nutrients. The roots also have lateral branches that spread outwards, helping to anchor the tree and absorb resources from the surrounding soil. This root system is crucial for the tree's survival and growth in various environmental conditions (Coulibaly et al., 2011).

**Flowers:** The flowers of *Cussonia barteri* are small, greenish-white, and arranged in terminal clusters. They are typically bisexual, containing both male and female reproductive organs, and are pollinated by insects such as bees and butterflies. The flowers play a crucial role in the tree's reproductive cycle, leading to the formation of fruits and seeds (World Agroforestry Centre, 2009).

**Taxonomic Hierarchy of *Cussonia bateri*:**

Kingdom : Plantae
Sub kingdom : Stretophyta
Class : Equisteopsida
Sub-Class : Magnoliidae
Order : Apiales
Family : Araliaceae
Genus : *Cussonia*
Species : *Cussonia barteri*
(World Agroforestry Centre, 2009)

## 2.2 Ethnomedicinal and Economic Importance of *Cussonia barteri*

*Cussonia barteri* as a plant has a lot of economic importance. Some of them are:

Bark decoction of *Cussonia barteri* is used to treat malaria in Nigeria. In Ghana, Malawi and Zimbabwe, extracts are used to treat mental health related issues. Its leaves are used in a decoction to treat rheumatism and oedema. A water decoction of bark extracts is used as a topical treatment of gonorrhea and a root decoction is drunk for the treatment of diarrhea.

**Plate 1. *Cussonia bateri* (Jansa seeds)**

**Plate 2. Yam (*Discorea rotundata)***

On the other hand, *Cussonia bateri* has ethnomedicinal and pharmacological applications. Leaves, stem bark and seed of *C. barteri* have been shown to be rich in saponins, flavonoids, phenols, sugars and alkaloids. Some of these constituents have been isolated and elucidated from *C. barteri*. Several compounds isolated from plant include triterpenes, saponins, polyenyne and quinic esters. Phytochemical constituents are also partly responsible for biological activities o*f C. barteri*. Extracts and components isolated from the plant have demonstrated neuropharmacological, anti-larvicidal, anti-microbial, anti-inflammatory and antioxidant activities. Overall, the insights provided by this review reinforce the potential of *C. barteri* for drug development and create the need for further scientific probe of constituents of the plant with the aim of developing novel drug candidates.

## 2.3 Botany and Description of *Dioscorea rotundata* (Yam)

**Yams:** Yams botanically called *Dioscorea* (family *Dioscorea*ceae) are grown for their edible tubers. Yams are native to warmer regions of both hemispheres, and several species are cultivated as staple food crops in the tropics. In certain tropical cultures, notably in West Africa and New Guinea, the yam is the primary agricultural commodity and the focal point of elaborate rituals. Yams are consumed as cooked starchy vegetables. They are often boiled and then mashed into a sticky paste or dough, but they may also be fried, roasted, or baked in the manner of potatoes or sweet potatoes, which are unrelated. Botanically Yams (*Dioscorea rotundata*) can be described using the physiological features such as these:

**Leaves:** Yam leaves are large, heart-shaped, and often have prominent veins. They can vary in color from green to dark green, depending on the variety. The leaves grow alternately along the stem and may have a slightly rough texture.

**Bark:** The bark of the yam plant is typically smooth when young but becomes rough and fissured as the plant matures. It may range in color from grayish-brown to dark brown, with irregular patterns and ridges.

**Stem:** The stem of the yam plant is a climbing vine that can grow several meters in length. It is cylindrical and may have a slightly ridged or grooved texture. The stem is usually green when young, turning brown or grayish-brown as it matures.

**Cotyledon:** The cotyledon, or seed leaf, of yam is usually oval-shaped and fleshy. It is the first leaf-like structure that emerges from the seed during germination. Cotyledons contain stored nutrients that provide energy for the seedling as it begins to grow.

**Fruits:** Yams produce fruits that are typically small, round, and berry-like. The fruits may vary in color, ranging from green to yellow or orange, depending on the variety. Each fruit contains several seeds, which are dispersed when the fruit ripens and splits open.

**Taxonomic Hierarchy of Yam (*Dioscorea rotundata)*:**

Kingdom : Plantae
Sub Kingdom : Magnoliopsida
Class : Liliopsida
Order : *Dioscorea*les
Family : *Dioscorea*ceae
Genus : *Dioscorea*
Species : *Dioscorea rotundata*

**Diseases and Pest of Yam (*Dioscorea rotundata)*:** Yam as a plant has diseases and pests affecting its growth, development and propagation. These factors hinder its development and retards their growth. Some of these diseases and pests are:

i. **Anthracnose (*Colletotrichum gloeosporioides)*:** According to Akinyele et al. (2019) this fungal disease causes dark lesions on yam leaves, stems, and tubers, leading to reduced yield and quality.
ii. **Yam mosaic virus (YMV)**: YMV causes mosaic symptoms on yam leaves, stunting, and reduced tuber size, ultimately affecting yield.
iii. **Yam anthracnose virus (YamAV):** According to Mignoina and Thottappilly (2000), yam anthracnose virus causes necrotic lesions on yam leaves, reducing photosynthesis and tuber yield.
iv. **Yam beetle (*Heteroligus* spp.*)*:** The yam beetle damages yam leaves and stems by feeding on them, leading to defoliation and reduced photosynthesis (Agbeniyi, 2007).
v. **Yam nematode (*Scutellonema bradys)*: a**ccording to (Dossou-Aminon et al., 2018) this nematode infects yam roots, causing root lesions and deformities, which can lead to reduced nutrient uptake and stunted growth.
vi. **Yam tuber moth *(Glyphodes* spp.*)*:** The yam tuber moth larvae feed on yam tubers, causing tunneling and rotting, leading to post-harvest losses (Dossou-Aminon et al., 2018; Birhanu et al., 2014).

## 3. MATERIALS AND METHODS

**Study Area:** This work was conducted at Alpha laboratory Awka, Anambra State. Anambra State is located in the south-eastern part of Nigeria and situated between latitudes 6° 13' and 16' N and longitude 7° 4' and 7° 41' E and Altitude 160.8m respectively (Ezenwaji et al.,, 2014; CLSI, 2008). The research is based on physiocochemical and preservative potential of essential oil from (*Cussonia barteri)* seeds on post harvest losses of yam (*Dioscorea rotundata).*

**Materials Used:** The materials used for the study included white yam (gotten from three different locations within Anambra state), Jansa seed powder (gotten from Eke Awka market), Whitman's filter paper No 42, beakers, volumetric flasks, measuring cylinder, spatula, inoculating loop, bunsen burner, aluminum foil, cottonwool, scapel, microscope, sterile polythene bags, masking tape, petri dishes, blotting paper, sodium hypochlorite, test tubes and rack, micro pipette, funnels, slide, cover slip.

**Samples Preparation:** The samples were ground into fine powder and stored in an air tight plastic container for extraction. Also, the white yam was allowed to rotten before taking to the laboratory.

**Extraction of Jansa seed (*Cussonia barteri)*:** 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 $60^0$C. 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 (Imanirampa & Alele, 2016).

**Fungal isolation:** 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" (AOAC International, 2023).

**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 $121^0$C for 15 minutes. This was

followed by the addition of 500mg streptomycin antibiotic while the solution was still in a molten state" (Nwachukwu and Osuji, 2018).

**Isolation of Fungi from Samples:** The isolation technique of Onuh *et al*. (2015) 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 potatoes 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°C in an incubator for 72 hours. Fungi associated with the tuber's 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 Marthur and Kongsdal, (2003). Fungal identification was done using morphological characteristics and comparing the findings with established keys as described by Nwachukwu and Osuji (2008). 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 Hoog et al. (2020).

**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" (AOAC International, 2023).

**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" (AOAC International, 2023).

**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.

**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 $20^0$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" (AOAC International, 2023).

**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 $20^0$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" (AOAC International, 2023).

**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 $110^0$C for 30 min, the medium was poured into petri dish. Isolates were inoculated at the centre of the plate and incubated at $20^0$C for 14 days. The appearance of a clear zone around the fungal colony was taken as a positive result" (AOAC International, 2023).

**Lipase Activity Test:** The medium of 0.5% peptin, 0.3% yeast extract and 1.0% agar were autoclaved at $121^0$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 $20^0$C for 7 days. The occurrence of clearance in the medium column was taken as a positive result.

**Pathogenicity test:** The method of Ogbo and Agu, (2015) 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 of 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:

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

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 antifungal 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:

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

Where
dc = average diameter of fungal colony in control treatment
dt = average diameter of fungal colony with powder or extract (AOAC International, 2023).

**Determination of minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC):** A microplate method, 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 ($2 \times 10^6$ 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 as described by Espinel-Ingroff et al. (Espinel-Ingroff et al., 2002). After 72 h of incubation, 20 µL was subcultured from each well and 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.

### 3.4 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 ($p<0.05$).

## 4. RESULTS AND DISCUSSION

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

| TEST | RESULTS |
|---|---|
| pH | 5.2 (acidic) |
| Conductivity | 0360Us/cm |
| Specific gravity | 0.85 |
| Temperature | 32.9c |
| Texture | Smooth in texture and oil feeling |
| Colour | Cream yellow |
| Aroma | Mild and subtle aroma(medicine) |

**Table 2. Total fungal count of yam samples**

| Locations | Mean total fungi count ($x10^2$cfu/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*

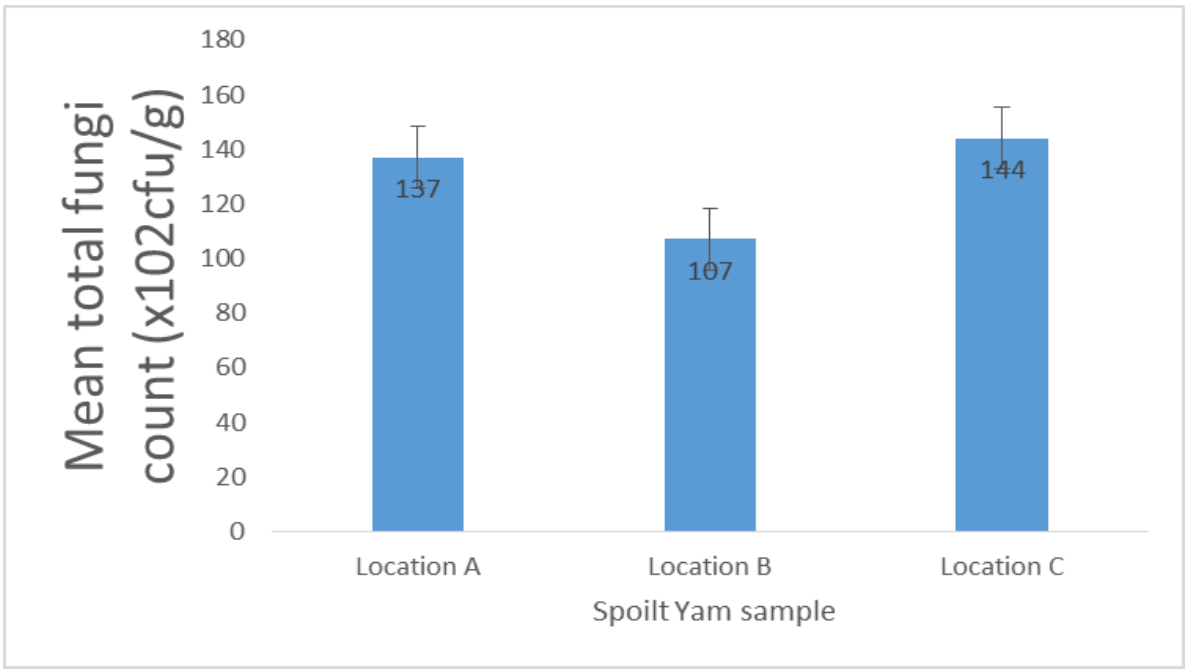


**Plate 3. Mean Total Fungi Count**

**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 3 days. Colour on the reverse side was yellow. Colonies were incubated at 30 o C for 5 days | Sporangiophores were hyaline, erect, non-septate and branched sympodially and circinate. Sporangia were terminal, dark-brown, finely echinulate to smooth and spherical (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 | *Mucor* spp |

**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** | **+** | **+** | **-** | **-** | **-** | **-** |

**Table 5. Result of the Pathogen city 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*

**Table 6. Sensitivity of Essential oil from Jansa 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 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** | M*ucor* 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** |

**Table 8. Minimum Fungicidal Concentration (MFC) 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 physiochemical properties are important because they allow the characterization and identification of an essential oil. They make it possible to decide their future utilisation. The pH obtained in this study was 5.2(acidic), this result is higher than that of *Ocimum americanum* essential oil which was reported to have a lower acidic pH of 3.39. The pH plays a key role in chemical and biochemical reactions and can influence the stabilizing properties of an essential oil. Specific gravity is the ratio of the density of respective substance to the density of water at 4°C" (Bamgboye & Adejumo, 2010). "Specific gravity values of oils are less than 1 for most of the oils except few containing oxygenated aromatic compounds. This agrees with the result of the present study, showing essential oil from Jansa seed to be 0.85. the result shows that the essential oil from Jansa seed is cream yellow and with a mild aroma. This is not to correlated with previous studies done on essential oil of *Zanthoxylum armatumis* which was extracted from N-hexane which showed a brownish yellow color (Al-Rehaily et al., 2003). Though the sample was extracted with same solvent, but the difference in their color might be due to difference in the plant where essential oil was obtained.

The result also shows that the essential oil from Jansa seed has a mild subtle aroma which is different from the essential oil of *Zanthoxylum armatumis* which have an unpleasant odor, it also has a conductivity of 0360Us/cm, a smooth

and oil feeling and it's temperature is measured to be 32.9°C.

The result of this study revealed the presence of three different fungal species from yam samples obtained from Ekeawka market, Anambra state (*Aspergillus* spp*, Penicillium* spp*,* and *Mucor* spp). These fungal species were confirmed to be causative agents of the spoilage through the pathogenicity test. This conforms with the previous findings showing that, *Mucor* spp is known to be among the fungi that causes soft rot in water yam. *Aspegillus* spp and *Penicillium* spp has also been shown to be associated with soft rot (Larone, 2011).

The isolation of more than one pathogenic organism 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 Anukwuorji et al. (2024) on yam.

Essential oil from plant has shown its ability to inhibit the growth of the microbes from earlier reports of several researches (Kalemba & Kunicka, 2003), This study revealed that fungitoxic compounds were present in the essential oil from *Coussonia barteri* seed, this agrees with previous studies showing the antimicrobial activities of *Cussonia barteri* (De Villiers et al., 2010).

“It has also been shown in several reports that the bioactive components present in essential oils might attach to the surface of the cell, and thereafter penetrate to the phospholipid bilayer of the cell membrane. The structural integrity of cell membrane is disturbed by their accumulation, which can detrimentally influence the cell metabolism causing cell death” ((Bajpai et al., 2013; Djenane et al., 2012). “Generally, essential oils characterized by a high level of phenolic compounds, such as carvacrol, eugenol, and thymol, which have important antimicrobial activities” (Lv et al., 2011). “These compounds are responsible for the disruption of the cytoplasmic membrane, the driving force of protons, electron flow, active transport, and also coagulation of cell contents” (Kalemba & Kunicka, 2003; Lambert et al., 2001). “An important feature of essential oils is their hydrophobicity, which allows them to partition into lipids of the cell membrane of microrganisms disrupting the structure, and making it more permeable” (De Villiers et al., 2010). This can then cause leakage of ions and other cellular molecules, hence this explains the ability of essential oil of C*ossonia bateri* used in this study to inhibit fungi rot in water yam. However, the efficacy of the essential oil differed with the different concentration, and with each test fungus.

From the result the essential oil of Jansa seed exhibited its antifungal sensitivity activities on *Mucor* spp at the concentration of 500mg/ml and 250mg/l, while *Aspergillus* spp at the concentration of 500mg/l, 250mg/l and 125mg/l, *Penicillium* spp at concentration of 500mg/ml and 250mg/l.

Minimal inhibitory concentration (MIC) was of the essential oil was tested at different concentrations of 500mg/ml - 65mg/l on the test organisms. The minimum fungicidal concentration of 500mg/ml of the essential oil proved to possess the highest fungicidal action against all the three fungi isolated.

This finding is in agreement with the report of Banso et al. (2016), who also observed that higher concentrations of antimicrobial substances showed more growth inhibition. In addition, the antimicrobial activity of essential oil 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., 2013). The data obtained indicate that the essential oil gotten from Jansa seed has the ability to prevent post-harvest decay in Water yam due to its ability to inhibit fungal growth. The physiochemical result obtained from the oil shows the purity and stability of the essential oil. Further research is still needed to know other applications of the essential oil, determine its preservative potential for use in other root and tuber crops. Additionally, the oil's stability, toxicity, and regulatory compliance need to be further evaluated before its commercial use as a preservative.

## 5. CONCLUSION

This research shows the physicochemical and preservative potential of essential oil from *Cussonia barteri* seeds as a natural and sustainable solution to mitigate post-harvest losses of yam (*Dioscorea rotundata*), making it an alternative to synthetic preservatives. The antifungal activities of essential oil suggest they could be effectively utilized to extend the shelf life of water yams, thereby reduce. This study provides the opportunity for the development of

natural preservative systems, which could revolutionize the preservation of water yams and other perishable crops, thereby promoting healthier and more sustainable food preservation methods.

## DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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