Asian Journal of Research in Biology

Volume 8, Issue 1, Page 18-33, 2025; Article no.AJRIB.12609

The Preservation Potential of Utazi and Scent Leaf Extracts against Spoilage Microbes Isolated from Spoilt Water Yam

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.56557/ajrib/2025/v8i144

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/12609

Original Research Article

Received: 24/10/2024
Accepted: 27/12/2024
Published: 23/01/2025

ABSTRACT

Plant diseases represent a huge threat to global food security and agricultural sustainability. Water yam gotten from three different locations within Anambra State were allowed to rot before use. Four microorganisms (fungi) - *Aspergillus* spp, *Mucor* spp, *Fusarium* spp and *Penicillium* spp were isolated and identified from three rotten *Dioscorea alata* (water yam) varieties from three market sites in Anambra State, Nigeria, in West Africa. Pathogenicity test carried out using the microorganisms confirmed them to be the pathological agents of the rot. Antimicrobial activity test with aqueous extracts of Utazi leaf and Scent leaf showed that three pathogens, *Mucor* spp, *Fusarium* spp and *Penicillium* spp were substantially inhibited in the agar diffusion tests. The result obtained showed that Scent leaf had the best antimicrobial activity for the fungi isolates due to its

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Cite as: *Eziamaka, Afam-Ezeaku, Chikaodili, Eze, Hope Nkiruka, and Onyia, Precious Chidera. 2025. "The Preservation Potential of Utazi and Scent Leaf Extracts Against Spoilage Microbes Isolated from Spoilt Water Yam". Asian Journal of Research in Biology 8 (1):18-33. https://doi.org/10.56557/ajrib/2025/v8i144.*

higher zone of inhibition. Based on this result, utilizing plant extracts like utazi and scent leaf not only leverages their antimicrobial properties but also aligns with sustainable and eco-friendly preservation practices.

Keywords: Preservation-potential; utazi; scent-leaf, extracts, spoilage, microbes, water yam.

1. INTRODUCTION

"The food yam belongs to the genus *Dioscorea* in the family Dioscoreaceae and is a monocotyledon. It is one of the most rated and common food of the tropical world. The edible varieties of yam are important sources of carbohydrate staple for millions of people in the tropical and sub-tropical countries in West Africa, Caribbean, Northern and central parts of south-East Asia and some parts of China" (Okigbo et al., 2000).

Plant diseases represent a huge threat to global food security and agricultural sustainability. Microbes cause massive agricultural food loss and shortage by infecting and damaging food crops, leading to reduced crop yield, poor quality and economic losses (Lichterman, 2004; Ejimofor et al., 2023). Traditional approaches to managing phyto-pathogenic microbes include the use of resistant crop varieties, cultural practices and various chemicals. While the latter can manage microbial infestation, their frequent use negatively impacts the environment and raises concern regarding safety and its potential harm to human health, and this has led to more interest in sustainable alternatives using plant extracts (Achugbu et al., 2022).

The treatment and effective control of disease in water yam (*Dioscorea alata*) by the use of available medicinal plants in a locality will continue to play an important role in preventing food spoilage (Wukhe, 2000). These plants (utazi and scent leaf) are able to synthesize many chemical compounds for defence against microbes and insect infestation (Lichterman, 2004). "Herbaceous vegetables have unique benefits within the farmng systems and household gardens because they grow quickly. Many are disease resistant since they easily adapt to local and environmental conditions. They are counted as part of the most inexpensive sources of different kinds of nutrient, preservation and are culturally acceptable" (Onyekwere et al., 2021).

"Gongronema latifolium commonly called 'utazi' and 'arokeke' in the eastern and western Nigeria, is a tropical rainforest plant primarily used as spice and vegetable in traditional folk medicine" (Ugochukwu et al., 2003). The leaves are rich in various phytochemicals such as tannins useful in fighting microbes. The leaves of *G.latifolium* are rich in fats, proteins, vitamins, minerals and many essential amino acids collectively contributing to its high nutritional value.

Utazi (*Gongronema latifolium*): *"Gongronema latifolium* is a climbing perennial shrub capable of twining around vertical support, as well it can grow horizontally on the ground up to 5 metres long. The soft woody stem produces adventitious roots in contact with soil" (Osugwu et al., 2013). "The stem of the plant is hollow, soft and hairy in texture and contains white latex which is released on incision or injury. The stem base is hard and woody to provide firm support. The leaves are simple decussate and sometimes whorled green leaves with an entire margin and long petiole" (Osugwu et al., 2013). "The leaf blade is broadly ovate to almost circular with a deep cordate base and an acuminate apex" (Balogun & Obimma, 2016).

"The flowers of *G. latifolium* are small, fragrant, bisexual, star-shaped (actinomorphic) and pale yellow in colour with axilliary cymes types of inflorescence. The calyxlobes are rounded in shape with hairy apex. The corolla is long, tubular at the apex; the corona has five fleshy and cramy lobes with a brown base" (Osugwu et al., 2013).

"The fruits of *G. latifolium* is green initially and turns dark brown to black at maturity. It is a dehiscent seedpod called a follicle which is oblong-lanceolate. At maturity, the fruit splits open lengthwise releasing flat seeds which are attached to a white silky tuft which aids dispersal for pollination" (Balogun & Obimma, 2016).

The stem when grown from stem cuttings, matures in 12 months. It usually requires a hot climate of about 32 to 37.5 degree Celsius. Flowers are pollinated by insects due to its attractive colour and fragrance.

Scent leaf (*Ocimum gratissimum*): *"Ocimum gratissimum* commonly known as scent leaf or

African basil is a plant mainly found in the tropics and widely used as spice. It is a full developed flowering plant used naturally in the treatment of different diseases such as diarrhea due to its richness in antioxidants and phytochemicals" (Obioma *et al*., 2011). *Ocimum gratissimum* is an aromatic perennial herb, 1-3 metres tall; erect stem, much branched, woody at the base often with epidermis peeling in strips. The leaves are opposite, slender, blade is ovate, and sometimes glandular punctate.

"Inflorescence is arranged in terminal, simple or branched receme 5-30cm; rachis lax, softly pubescent; bracts sessile, ovate. Fruit consists of four, dry, one-seeded nutlet enclosed the persistent calyx (the lower lip closing the mouth of the fruiting calyx); nutlet is subglobose, 1.5 mm long, rugose brown; outer pericarp not becoming mucilaginous water" (Orwa et al., 2009). "Their flowering period starts after 136 days and continues until 195 days. Seed matures after 259 days. It occurs from sea level up to 1500m altitude in coastal scrub, along lake shores" (Orwa et al., 2009).

Statement of the problem: Farmers have long been using various chemicals to tackle post-harvest loss due to microbial attack. Many peasant farmers cannot afford to buy the various expensive chemicals used, coupled with the fact that those chemicals are not healthy and safe for both the natural environment and also for human consumption. Hence the introduction and use of medicinal plant extracts to help farmers prevent yam spoilage, increased yield, better yam quality and to ensure adequate food supply. This research is aimed at studying the preservation potential of utazi and scent leaf extracts against spoilage microbes isolated from spoilt water yam.

2. MATERIALS AND METHOD

2.1 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 (Ezeanwaji et al., 2014). The research is based on *in-vitro* antimicrobial activity of Utazi and Scent leave extracts on water yam spoilage microbes.

2.2 Materials Used

The materials used for the study included yam (gotten from three different locations within Anambra state), utazi leaf and scent powder (gotten from Eke Awka), 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.

2.3 Sample Preparation

The samples were ground into fine powder and stored in an air tight plastic container for extraction. Also, the water yam was allowed to rot before being taken to the laboratory.

2.4 Extraction

"Extraction was done with ethanol by cold maceration. 250g of powdered sample was weighed into a glass and extracting solvent (ethanol) was added until the plant residues were fully immersed. The vessel was closed with a tight-fitting glass cover and the contents in the vessel were shaken after every 4 h except at night, and left to stand for three days (72 h) but with subsequent agitation until this period was over. The contents of the flask were then strained through two clean pieces of cotton cloth placed on top of filter paper, both supported by a funnel, and the extracted solution (miscella) was collected in a flask with a tight-fitting cover. The maximum yield of ethanol extract was obtained by squeezing the marc (solid residue) in the top clean dry piece of cloth, while drippings of liquid extract were allowed to pass through the second clean dry piece of cloth, to the contents of the flask through Whatman's filter paper. The volume of the yield was noted. Both the ethanol and water extracts were transferred to a hot-air oven for drying at temperatures between 50 °C and 70 °C, and later transferred into a desiccator for further drying" (Ezeanwaji et al., 2014).

2.5 Phytochemical Screening

2.5.1 Preliminary phytochemical screening

"The extracts were subjected to preliminary chemical screening for their presence or absence of active phytochemical constituents" by the following methods according to (AOAC INTERNATIONAL, 2023).

2.5.2 Test for alkaloids

The extracts were treated with dilute (10%) hydrochloric acid and filtered. The filtrates were treated with various alkaloidal reagents.

a. Mayer's test: The extracts were with Mayer's reagent (Potassium mercuric iodide). Appearance of cream colour indicates the presence of alkaloids in chloroform, methanolic and aqueous extracts.
b. Wagner's test: The extracts were treated with the Wagner's reagent (Iodine solution) the appearance of brown colour precipitate indicates the presence of alkaloids in chloroform, methanolic and aqueous extracts.

2.6 Phenolics

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

2.6.1 Test for terpenoids

Two ml of chloroform was combined with 5 ml of each extract. Then, to create a layer, 3 milliliters of concentrated H2SO4 were added. Terpenoids were present because of the reddish-brown precipitate coloration that developed at the contact.

2.6.2 Test for Cardiac Glycosides

a. **Keller-Killani test:** When a pinch of the extracts was dissolved in the Glacial acetic acid and few drops of ferric chloride solution was added, followed by the addition of concentrated Sulphuric acid, formation of red ring at the junction of two liquids indicates the presence of glycosides in methanolic and aqueous extracts.

2.6.3 Test for flavonoids

a. The Shinoda test involved dissolving the extracts in alcohol, adding a piece of magnesium dropwise, then adding concentrated hydrochloric acid and heating the mixture. The presence of flavonoids in methanolic and aqueous extracts is indicated by their magenta appearance.

b. Ferric Chloride test: A few drops of neutral ferric chloride were added to the extracts. Methanolic and aqueous extracts showed a blackish red color.

2.6.4 Test for saponins

a. Foam test: The extracts were diluted to 20 ml with distilled water and shaken well in a graduated cylinder for 15 minutes. The formation of foam in the upper part of the test tube indicates the presence of saponins in each extract.

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

2.6.5 Test for steroids

a. Salkowski reaction: To 2 ml of extract, added 2ml chloroform and 2 ml conc. H_2SO_4. shook well. Chloroform layer showed red color and acid layer showed greenish yellow fluorescence.

b. Liebermann-Burchard test: The lack of green color showed that there were no steroids in any of the extracts after they were treated with strong sulfuric acid, a few drops of glacial acetic acid, and acetic anhydride.

2.6.6 Test for tannins

a. Lead acetate solution: The presence of tannins in methanolic and aqueous extracts was demonstrated by the formation of a white precipitate after the extracts were treated with a 10% lead acetate solution.

b. Ferric Chloride Solution: When the extracts were treated with ferric chloride solution, NaOH, and AgBr Solution appearance of green colour precipitate indicated the presence of tannins in methanolic and aqueous extracts.

2.7 Quantitative Phytochemical Screening

Steroids: One gram (1 g) of the extract will be macerated with 20 ml of ethanol. Two milliters (2 ml) of chromagen solution was added to 2 ml of the filtrate and allowed to stand for 30 minutes. Absorbance will be read at 550 nm. A standard was made following the same procedure at different concentrations using steroid hormone, a standard curve of absorbance vs concentration will be plotted and the concentration of steroid in the extract extrapolated from the standard curve.

Saponins: Ten milliliters of petroleum ether will be used to macerate the extracts (1 g each), which will then be decanted into a beaker. After adding an additional 10 milliliters of petroleum ether to the beaker, the filtrate was heated until it evaporated completely. Six milliliters of ethanol

were used to dissolve the leftovers. Two milliliters of the solution were then transferred into test tubes, and two milliliters of chromagen solution was added. After letting the combinations remain for half an hour, the absorbance at 550 nm will be measured. Using ursolic acid, a standard was created using the same process at various concentrations. The concentration of saponin in the extracts was calculated by extrapolating the standard curve of absorbance vs. concentration.

Alkaloids: An aliquot of (0.5 g) of the extract was dissolved in 96% ethanol and 20% H_2SO_4 and filtered, the filtrate (1 ml) was added to 5 ml of 60% tetraoxosulphate (VI) acid and allowed to stand for 3 hours after which reading was taken spectrophometrically at 565 nm wavelength. A standard was made following the same procedure at different concentrations using caffeine, a standard curve of absorbance vs concentration plotted and the concentration of alkaloids in the extracts extrapolated from the standard curve.

Quantitative test for terpenoids: Dried plant extract 100mg (wi) was taken and soaked in 9mL of ethanol for 24 hour (Indumathi et al., 2014). The extract after filtration, was extracted with 10mL of petroleum ether using separating funnel. The ether extract was separated in pre-weighed glass vials and waited for its complete drying (wf). Ether was evaporated and the yield (%) of total terpenoids contents was measured by the formula (wi-wf/wi×100).

Determination of Tannins by Titration: Twenty gram of sample was weighed in a conical flask and 100mls of n hexane or petroleum ether was added and covered for 24 hours. The sample was then filtered and allowed to stand for 15 minutes for the solvent to evaporate. It was then re-extracted by soaking 100mls of 1% acetic acid in ethanol for 4 hours. The sample was then filtered and the filtrate collected.

Twenty-five ml of ammonium hydroxide were added to the filtrate to precipitate the alkaloids. The alkaloid was heated with electric hot plate to remove some of ammonium hydroxide still in solution. The remaining volume was measured and 5ml of this was taken and 20ml of ethanol was added to it. It was titrated with 0.IM NaOH using 1ml of phenolphthalyne as indicator until a pink end point is reached. Tannin content was calculated in percentage ($C^1V^1 = C^2V^2$) molarity

Data

C_1 = Concentration of Tannic acid
C_2 = Concentration of Base
V_1 = Volume of Tannic acid
V_2 = SVolume of Base

$$Therefore\ C1\ = \frac{C_2V_2}{V_1}$$

$$\%\ of\ tannic\ acid\ content = \frac{C_1\ x\ 100}{Weight\ of\ sample\ analyzed}$$

Flavonoids: The extracts (1 g) each will be macerated with 20 ml of ethylacetate for 5 min and filtered. To (5 ml) filtrate was added 5 ml of dilute ammonium, shaken for 5 min, the upper layer will be collected and the absorbance read at 490 nm. A standard was made following the same procedure at different concentrations using quercetin as standard. A standard curve of absorbance vs concentration was plotted and the concentration of flavonoid in the extract extrapolated from the standard curve.

Phenol: Defatting of 2 g wood powder sample was carried out for 2 hours in 100 cm^3 of ether using a soxhlet apparatus. The defatted sample (0.50 g) was boiled for 15 minutes with 50 cm^3 of ether for the extraction of the phenolic components. Exactly 10 cm^3 of distilled water, 2 cm^3 of 0.1 N ammonium hydroxide solution, and 5 cm^3 of concentrated amyl alcohol were also added to 5 cm^3 of the extract and left to react for 30 minutes for colour development. The optical density was measured at 505 nm, and 0.20 g of tannic acid was dissolved in distilled water and diluted to 200 mL mark (1 mg/cm^3) in preparation for phenol standard curve. Varying concentrations (0.2–1.0 mg/cm^3) of the standard tannic acid solution were pipetted into five different test tubes to which 2 cm^3 of NH_3OH, 5 cm^3 of amyl alcohol, and 10 cm^3 of water were added. The solution was made up to 100 cm^3 volume and left to react for 30 minutes for colour development. The optical density was determined at 505 nm.

2.8 Microbial Analysis of Sample

Preparation of culture media: The major media used for the isolation and characterization of bacteria include: Nutrient agar (NA), MacConkey agar (MA), potato dextrose agar (PDA) and Peptone water. Appropriate grams of the agar were measured and poured into a conical flask, dispensed into the volume of water according to the manufacturer's instruction. The mixture was heated in the autoclave for 15 minutes at 121°C.

Sterilization of materials: All glassware used for the research including Conical flask, Petri dishes, test tubes were washed with detergent and rinse with clean water and were assemble in the autoclave with the more fragile equipment wrap in aluminium foil and were sterilized at 121°C for 15 minutes.

2.9 Procedure

Total bacterial count: "One gram of each sample was homogenized using vortex mixer (VM-300, Taiwan) with 9 ml sterile peptone water to obtain first dilution, and 1ml from the first test tube was pipette into the second test tube already containing 9ml of peptone water, this continued following the same procedure till the last dilution (i.e the last test tube).using the pour plate method 1ml each of each sample unit from the test tubes was pipetted into the sterile Petri dishes containing already prepared Nutrient agar media. The plates were incubated at 37°C for 24hr. After incubation the representative colonies on the plates was subcultured on fresh nutrients agar to obtain pure cultures of the isolates. The pure cultures were then transferred into nutrient agar slants for biochemical identification" (Abbott et al., 2008).

Total fungi count: "The serially diluted sample was used in the total fungi analysis. Using the streak method 1ml each of each sample unit from the test tubes was collected with wire loop and streaked into the already prepared PDA agar Petri dishes. The plates were incubated at 37°C for the 72hr. After incubation the representative colonies on the plates were subcultured on fresh PDA agar to obtain pure cultures of the isolates. The pure cultures were then used for biochemical identification" (Abbott et al., 2008).

The number of colonies were counted on all the agar and calculated using the formula below;

$$CfU\ (ml) = \frac{N}{VxD}$$

Where

Cfu = Colony forming unit
N = Mean number of colonies
V = Volume of innoculum
D = Dilution factor.

2.10 Identification and Characterization of Bacterial Isolates

Purification of isolates: Single colonies of bacteria were randomly selected from different media plates based on their morphologies These bacterial cultures were subsequently isolated in pure forms by subculturing on nutrient agar plates incubated for 24hrs and used for microscopic characterization and biochemical analysis.

2.10.1 Identification of microorganisms

(a) Morphological identification: The isolated bacteria were identified on the basis of motility and Gram-staining.

Gram staining: "The pure bacterial isolates were stained according to Gram's techniques. A thin smear was prepared on clean glass slide, air dried, and heat fixed by placing the slide gently over the flame of the spirit lamp. The smear was stained with crystal violet for 1 minute, and then rinsed with tap water. The smear was then covered with Lugol's iodine for 60 seconds and washed off under gentle running tap water. The slide was then decolourized using 70% ethanol after which it was washed under tap water and then counterstained with safranin for 30 seconds. It was again rinsed with tap water and the slide blotted dry with a piece of filter paper. The stained cells were examined with the oil immersion objective lens of the light microscope. The gram positive organism is characterized by a purple colour while a gram negative organism takes on a pink colour as well as the shape of the cells were also examined" (Ezeanwaji et al., 2014).

Motility test: "The stabbing technique was used to carry out this test. Test-tubes containing sterilized Sim Agar were prepared. Sterilized inoculating needle was used to pick up isolates from their pure cultures. Each test-tube was stabbed with the needle rubbed with each isolate in the middle. The test-tubes were then incubated at 37^0C for 24hours. After which the tubes were observed for the motility of the isolates. A motile isolate usually grows away from the point where the medium was stabbed" (Wukhe, 2000).

Urease Test: "This test was used to demonstrate the ability of the isolates to produce the enzyme urease which splits urea forming ammonia. The test is usually used to differentiate organisms like proteus from other non-urease positive organisms. A loop full of the isolates was used to inoculate a tube of urea-agar. The tubes were incubated at 37^oC. A change in colour from yellow to red confirmed the presence of urease" (Wukhe, 2000).

Catalase Test: This test was used to demonstrate which of the isolates produced the enzyme catalase that releases oxygen from hydrogen peroxide. A loopful of the pure colony was transferred into a plain, clean glass slide. The sample was then mixed with a drop of 3% v/v hydrogen peroxide. The reaction was observed immediately. Gas production indicated by the production of gas bubbles confirmed the presence of catalase.

Indole Test: "This test was used to determine which of the isolates had the ability to split indole from tryptophan present in buffered peptone water. The test is usually used as an aid in the differentiation of Gram negative, *Bacilli* especially those of the *Enterobacteriaceae* (Baker & Breen, 1976). Tubes of peptone water were inoculated with young cultures of the isolates. The tubes were incubated at 37°C or 48hrs. About 4 drops of Kovac reagent were added into 1ml of each of the culture tubes. Positive test was indicated by a red colour that occurs immediately at upper part of the test tube" (Ezeanwaji et al., 2014).

Citrate Utilization Test: "This test was used to identify which of the isolates utilized citrate as the sole source of carbon for metabolism. The- test is usually used as an aid in the differentiation of organisms in the *Enterobacteriaceae* and most other genera. The medium used for this test was the Simon's citrate agar. Slant tubes of Simon's citrate agar were inoculated with young cultures of the isolates. The inoculation was done by stabbing the medium on the tubes using sterile straight inoculating wire containing the culture. The tubes were then incubated at 37°C for about 24hours. Change in colour from green to blue after about 24hours of incubation indicated positive result" (Ezeanwaji et al., 2014).

Coagulase Test:

1. A very homogenous suspension of the inoculum was mixed on a drop of normal saline in a grease free slide.
2. Loopful undiluted rabbit plasma was added to the suspension and mixed thoroughly for 5 seconds.
3. A control was set up in the same manner without blood plasma.
4. Coagulase positive staphylococci showed clumping or agglutination within 5-15 seconds while negative suspension showed no clumping.

Oxidase Test: This was carried out to identify bacterial species that produced the cytochromeoxidase enzyme.

A piece of filer paper was placed in a clean Petri dish and 2-3 drops of fresh or nascent oxidase reagent was added. A colony of test organism was collected using a glass rod and smeared on the filter paper and observed. Blue-purple color within few a seconds showed a positive test.

Identification and Characterization of Fungal Isolates: The isolates were identified using cultural characteristics and morphology.

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. Sclean 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" (Ezeanwaji et al., 2014).

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" (Ezeanwaji et al., 2014).

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 20^0C 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 of isolated fungi: "Pathogenicity or decay test was carried out in order to know if the isolated fungi were really responsible for the spoilage of citrus and banana fruits. Healthy fruits were surface sterilized with ethanol. Cylindrical plug tissues were cut out from the fruits using a sterilized 2mm sized cork borer. Agar plate containing a week-old fungal culture were aseptically placed in these holes, then covered and sealed off by means of petroleum jelly. The procedure was repeated separately across each of the fungal isolates. The inoculated samples and the control were placed in sterile polythene bags and incubated in an oven for 5days. The point of inoculation of each type of fungus was examined and recorded. The diameter of the rotten portion of the watermelon fruits was measured. The fungi were later re–isolated from the inoculated fruits and compared with the initial isolates" (Wukhe, 2000).

Antibacterial activity: "The 100µl of adjusted bacterial suspension was pipetted using a micropipette and applied on the surface of Mueller Hinton agar and was swabbed at 60^o rotation to uniformly distribute bacteria throughout media surface using a cotton swab. The swabbed Mueller Hinton agar stood for 15 min to provide time for the attachment of bacteria on the media. After that, the sterilised cork borer of 6 mm diameter was perforated with the swabbed media to create 6 mm diameter wells. At the time of punching media for different test bacteria, the cork borer was sterilised by immersing in alcohol and burning with Bunsen burner flames (Umer et al., 2013). The positive control disc (gentamicin) was positioned on the media surface, and 50 µl extracts at concentrations of 400, 200, and 100 mg/ml were added to the wells that had been produced. To aid in the diffusion of extracts or fractions in the media, the Petri dishes were refrigerated at 4°C for two hours after all of the wells had been filled and the positive control had been put on top of them. Petri dishes were then incubated in the incubator (BioTechnics India) for 24 hours at 37°C. Following a 24-hour incubation period, the diameter of the inhibitory zone was measured in millimeters using a ruler and noted. Three duplicates of the experiment were conducted (Wukhe, 2000).

Antifungal activity: The 100 µl adjusted fungi suspension was pipetted using a micropipette and applied on the surface of sabouraud dextrose agar and swabbed at 60^o rotation to uniformly distribute yeast throughout the media surface using a cotton swab. The swabbed sabouraud dextrose agar stood for 15 min to provide time for the attachment of fungi on the media. After that, the sterilised 6 mm diameter cork borer was used to perforate the swabbed media to create a 6 mm diameter of wells. The concentration of extracts for the experiment was determined based on a previous study on the plant. The 50 µl extracts at 400, 200, and 100 mg/ml, as well as the negative and positive controls, were added to the wells that had been made. To promote the spread of extracts or fractions in the media, the inoculated Petri dishes were refrigerated at 4°C for two hours. Following that, Petri dishes were incubated for 24 hours at 37°C. Following a 24-hour incubation period, the diameter of the inhibitory zone was measured in millimeters using a ruler and noted. Three duplicates of the experiment were conducted.

Determination of minimum inhibitory concentration for pathogenic bacteria: "Minimum inhibitory concentration is the minimum concentration of extracts or fractions which have inhibited the growth of microorganisms. The minimum inhibitory concentrations were determined using the broth microdilution technique for extracts or solvent fractions as their inhibition zones equal to or greater than 7 mm in agar well diffusion techniques. The serial double dilution technique was employed for extracts in broth filled wells. The serial twofold dilution was carried out by adding 100 µl of extracts or fractions to the first well and completely mixing them five times with a micropipette. Then, using a fresh micropipette tip, 100 µl of the mixture was transferred to the second well and thoroughly mixed as before. Using a fresh micropipette tip, 100 µl of the second well mixture was pipetted and moved to the third well, where it was thoroughly mixed as before. In order to have an equal volume of fluid in each well, the procedure was repeated until the tenth well and the 100 µl mixture of the tenth well were pipetted and discarded" (Clinical Laboratory Standards Institute, 2008).

"A prior investigation on the plant was used to determine the twofold serially diluted quantities of extracts for the experiment. 200, 100, 50, 25, 12.5, 6.25, 3.125, 1.5625, 0.78125, and 0.3906 mg/ml were the serially diluted concentrations employed in the experiment" (Abew et al., 2014). The growth and sterility controls were the 11th and 20th wells, respectively, filled with 100 µl of broth. To achieve a final concentration of 5 × 105 CFU/ml bacteria in each well, the 10 µl diluted bacterial suspension (10% of 100 µl well volume) was pipetted to the wells from the eleventh to the first wells. However, 10 µl broth was pipetted to the 12th well.

Lastly, microtitre plates were parafilm-sealed and incubated for 24 hours at 37°C (Clinical Laboratory Standards Institute, 2008). From the 12th to the first well, 0.01% resazurin sodium salt indicator was added to the incubated microtitre plate wells, which were then incubated for two hours at 37°C. "Color changes from the salt reaction with actively growing microorganisms are crucial for figuring out the minimum inhibitory concentration (MIC) of extracts or fractions. Inhibiting the growth of microorganisms results in a blue or purple color, whereas actively proliferating cells that reduced resazurin sodium salt to resorufin show a pink or colorless alteration. Three duplicates of the experiment were conducted" (Wukhe, 2000).

Determination of minimum inhibitory concentration for pathogenic fungi: From the first to the tenth wells, extracts in broth-filled wells were subjected to the serial twofold dilution procedure. The serial twofold dilution was carried out by adding 100 µl of extracts or fractions to the first well and completely mixing them five times with a micropipette. Then, using a fresh micropipette tip, 100 µl of the mixture was transferred to the second well and thoroughly mixed as before. Using a fresh micropipette tip, 100 µl of the second well mixture was pipetted, moved to the third well, and mixed completely as before.

In order to get an equivalent volume of fluid in each well, the procedure was repeated until the tenth well and its 100 µl mixture were pipetted and disposed of (European Committee on Antimicrobial Susceptibility Testing [EUCAST], 2003). A prior investigation on the plant was used to determine the twofold serially diluted quantities of extracts for the experiment. The experiment employed the following serial double dilution concentrations: 200, 100, 50, 25, 12.5, 6.25, 3.125, 1.5625, 0.78125, and 0.3906 mg/ml. The growth and sterility controls were the 11th and 20th wells, respectively, filled with 100 µl of broth.

"To minimize contamination on sterility control and the final concentration of yeast suspension (2.5 × 104 CFU/ml) in each well, 10 µl of diluted yeast suspension (10% of 100 µl broth volume) was pipetted to wells from the eleventh to the first wells; nevertheless, 10 µl of broth was pipetted to the 12th well. From the 12th to the first well, 0.01% resazurin sodium salt indicator was added to the incubated microtitre plate wells, which were then incubated for two hours at 37°C. When the blue or purple resazurin color turned pink or colorless, the minimum inhibitory concentration (MIC) of the extracts and fractions was established" (Wiegand et al., 2008). Three duplicates of the experiment were conducted.

Determination of minimum bactericidal concentration (MBC): Subculturing 10 µl of microtitre plate well that was more than or equal to the lowest minimum inhibitory concentration on Mueller Hinton agar allowed for the determination of the minimum bactericidal concentration, which was then incubated for 24 hours. The Petri dish was evaluated for growth after 24 hours of incubation, and the lowest concentration of extracts or fractions that showed no growth was determined to be the minimum bactericidal concentration (Akinduti et al., 2019).

Three duplicates of the experiment were conducted.

Determination of minimum fungicidal concentration (MFC): "Subculturing 10µl of micro-titre plate well that was larger than or equal to the lowest minimum inhibitory concentration on the sabouraud dextrose agar allowed for the determination of the minimum fungicidal concentration, which was then incubated for 24 hours. The Petri dish was examined for growth after a 24-hour incubation period, and the lowest concentration of extracts or fractions that showed no growth was determined to be the minimum fungicidal concentration" (Akinduti et al., 2019). Three duplicates of the experiment were conducted.

2.11 Data Analysis

The Statistical Package for Social Science (SPSS) version 20 was used to enter the data into an Excel spreadsheet for statistical analysis. Statistical analysis and inference were conducted using descriptive statistics, one-way ANOVA, Tukey's post hoc test, and linear regression R 2 (Coefficient of determination). The group mean of inhibitory zone diameter was calculated using descriptive statistics as mean ± SEM. To find the significant difference between group means, a one-way ANOVA was used. Tukey's post hoc test, on the other hand, used one-way ANOVA to ascertain whether there was a significant difference between the means of each group. To ascertain the concentration dependency of extracts and fractions on antibacterial activity against test microorganisms, the linear regression R 2 was computed. Differences were deemed statistically significant when the p-value was less than 0.05.

3. RESULTS

Table 1. Extraction yield (%)

Utazi	Scent Leaf
9.80%	12.70%

Table 2. Qualitative phytochemical

Phytochemicals	Utazi Ethanol extract	Scent leaf ethanol extract
Saponin	++	+++
Flavonoid	+++	+
Alkaloid	+++	+++
Tannin	-	+
Steroids	-	-
Terpeniods	+++	+
Glycosides	+++	++
Carbohydrates	+++	+
Protein	-	-
Anthrocynin	_	-
Phenol	++	+++
Oil And Resin	-	-
Reducing Sugar	-	-

Key

+++ = Present in high concentration
++= Present in moderate concentration
+ = slightly or sparingly present
- = Absent

Table 3. Quantitative Phytochemical Composition (Mg/100g) of the utazi and Scent leaf

Parameter	Utazi Leaf	Scent Leaf
Alkaloid	3.67	6.20
Flavonoid	11.10	3.55
Saponin	24.77	6.45
Terpenoid	1.23	1.47
Steroid	0.16	1.90
Tannins	18.54	11.80
Phenol	91.70	7.51

Results are expressed in Means ± SD (n = 3)

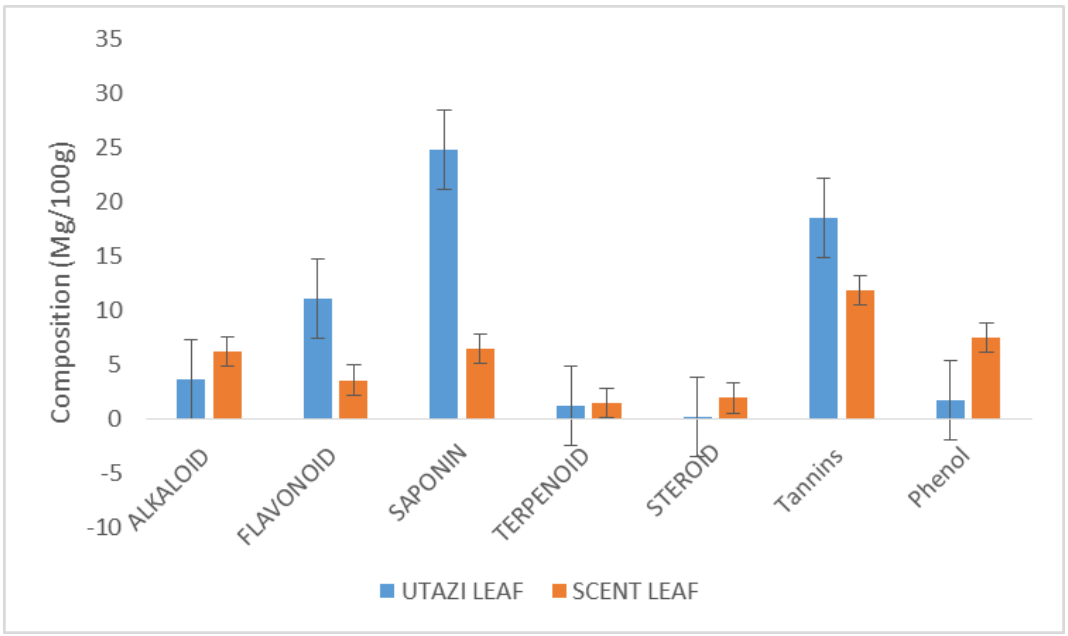

Fig. 1. Bar graph showing composition of different organic nitrogen-containing bases

3.1 Isolation of Spoilage Fungi

Table 4. Mean fungi count isolated from water yam samples

Sample location	Mean total fungi count (cfu/ml)
Eke Awka 1	2.50×10^4
Eke Awka 2	2.80×10^4
Amenyi market	2.00×10^4
Ifite market	2.45×10^4
Mean	**2.40×10^4**

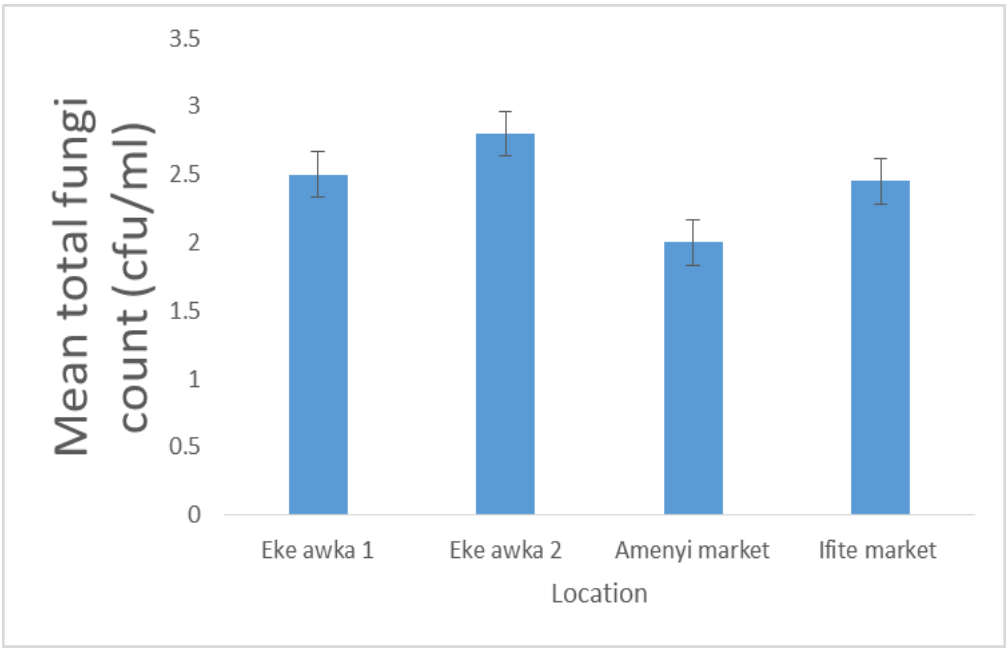

Fig. 2. Mean total fungi count in different regions

Table 5. Morphological characteristics of fungal isolates

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

Table 6. Identification of fungi

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

Table 7. Pathogenicity of fungal isolates on healthy water yam

Test isolates	Inoculated sample diameter after 4 days(mm)	Texture of the inoculated spoiled area	Spoiled diameter of control after 4 days (mm)	Texture of control after 4 days
Fusarium sp.	4	Rot	3*	Turgid
Aspergillus spp	4	Soft	3*	Turgid
Penicillium spp	4	Soft	3*	Turgid
Mucor sp.	4	Rot	3*	Turgid

3 = no spoilage*

Table 8. Antifungal activities of the extracts

Fungi Isolate	Extract	Zone of Inhibition(Mm)	Result
Aspergillus spp	Utazi ethanol extract	12.33± 0.28	R
	Scent leaf ethanol extract	10.16± 0.28	R
	Fluconazole	12.00± 0.00	S
Mucor spp			
Mucor spp	Utazi ethanol extract	16.00± 0.00	S
	Scent leaf ethanol extract	22.00± 0.00	S
	Fluconazole	32.27± 1.36	S
Fusarium spp	Utazi ethanol extract	17.33± 0.28	S
	Scent leaf ethanol extract	20.00± 0.00	S
	Fluconazole	18.00± 0.00	S
Penicillium spp	Utazi ethanol extract	16.00±0 .00	S
	Scent leaf ethanol extract	30.70± 0.00	S
	Fluconazole	32.27± 1.36	S

N/B: CLSI specification and standard zone of inhibition ranges:
Less than or equal to 15mm = microbes are resistant to the extract
16 - 20 = Microbes are intermediate to the extract
21 and above = Microbes are susceptible to the extract.
R = Resistant,
I = Intermediate,
S = Susceptible;

Table 9. Minimum inhibitory concentration (Mg/mL)

Microorganisms	Utazi	Scent leaf	Fluconazole
Aspergillus sp	>100	>100	10
Penicillium sp	50	25	1.00
Fusarium sp	0.50	0.50	1.00
Mucor spp	100	12.50	12.50

4. DISCUSSION

"The fungi isolated from water yam which was gotten from different market locations (Eke-Awka, Amenyi and Ifite market) of Anambra state were *Aspergillus* spp*, Mucor* spp*, Fusarium* spp *and Penicillium* spp. This result agrees with the previous findings showing *Aspergillus flavus, Aspergillus niger, Aspergillus tamari, Botryo-diplodia theobromae, Cladosporium herbarum, Fusarion oxysporum, Fusarium solani, Penicillium chryso-genom, Penicillium oxalicum, Rhizopus nodosus, Rhizoctonia* spp*. and Trichoderma virideon* as pathogenic fungi for yam tuber" (Okigbo & Emeka, 2010). This isolated fungus causes rot and post-harvest decay in water yam. *Fusarium* spp are known to cause dry rot in yam tubers (Amusa & Baiyewa, 1999), *Mucor* spp are known to be among the fungi that cause soft rot in water yam. *Aspegillus* spp and *Penicillium* spp have also been shown to be associated with soft rot (Amusa et al., 2003).

The water yam from Eke-Awka market had a higher microbial growth than that of Ifite market and Ameanyi market. This may be as a result of Eke-Awka being a larger market than Ifite market and Ameanyi market, which leads to higher volume of water yam being sold at Eke-Awka market than other markets, this higher volume can lead to more handling thus increasing contamination in the yam. The fungal organisms causing spoilage in the water yam must have been present right from the field, and after harvest transferred to storage houses and market places.

The result from this study also shows that both Utazi and Scent leaf extracts possess significant phytochemical compounds present in their ethanolic extracts, such as alkaloids, flavonoids, saponins, steroid, terpenoid, phenol and tannins, which is responsible for their biological activities. The study revealed that the extracts from both leaves have antimicrobial activity against microorganisms, which is in line with the previous research that has been done on both leaves by Okoi and Afuo (2009).

Exract's effectiveness against fungal isolated from the water yam which are common spoilage

agents in water yams, shows its potential as a natural antifungal agent. The observed antimicrobial effects of Scent leaf extract could be due to its essential oils and other phytochemicals, which have been reported to disrupt microbial cell membranes and interfere with their metabolic processes (Aneke et al., 2019; Njoku et al., 2011; Weigand et al., 2008).

Similarly, Utazi ethanolic extract demonstrated an antimicrobial activity, against the fungi isolated from the water yam. The extract was particularly effective against *Fusarium* spp*, Mucor* spp *and Penicillium* spp*,* showing substantial inhibition zones in the agar diffusion tests. This suggests its potential use as a natural preservative in preventing bacterial spoilage of water yams.

The comparative analysis between the two extracts showed that while both were effective, Scent leaf extract generally exhibited higher antimicrobial activities than Utazi leaf extract, due to its higher zones of inhibition against the fungi isolates and also, it's lower minimum inhibition concentration (mic). This could be due to Scent leaf ethanolic extract which showed a higher extraction percentage (12.70%) than that of Utazi ethanolic extract (9.80%).

The higher concentration of strong bioactive compounds like phenol, saponins and alkaloids in scent leaf ethanolic extract can also have influence in its antimicrobial activity. It has been reported that these bioactive compounds are very potent in antimicrobial activities. This showed that the scent leaf extract was more potent than utazi leaf extract in preserving the water yam, due to its higher antimicrobial activities.

However, both extracts showed inhibition on the growth of fungi isolated from the water yam to a certain extent, but *Aspergillus* spp were resistant to the antifungal activities of both extracts. This showed that though the leaf extract can preserve the water yam from spoilage to a certain extent, but it could not totally inhibit the growth of fungi that causes water yam to spoil, thus further studies and research is still needed to enhance the antifungal effectiveness of both leaf extracts so to increase the preservation potential of the extracts on water yam.

5. CONCLUSION

This research shows that Utazi and Scent leaf ethanolic extracts have the potential to serve as a preservative against spoilage microbes in water yams. The antifungal activities of these extracts suggest they could be effectively utilized to extend the shelf life of water yams, thereby reducing post-harvest losses. Scent leaf ethanol extract in particular, showed a higher potency and effectiveness against a broad range of spoilage microorganisms, indicating its superior preservation activity than utazi ethanol extract. However, the combined use of both extracts may enhance its synergistic antifungal effectiveness thus making it to be able to preserve the water yam for a longer period of time. 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. Moreover, this study shows the importance of natural preservatives as alternatives to synthetic chemicals, which are often associated with health risks and environmental concerns. Utilizing plant extracts like Utazi and Scent leaf not only leverages their antimicrobial properties but also aligns with sustainable and eco-friendly preservation practices.

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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Peer-review history:
The peer review history for this paper can be accessed here:
https://prh.ikprress.org/review-history/12609