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Biopreservative Potential of Lemon Grass (*Cymbopogon citratus*) Oil against Common Food Spoilage Fungi

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ABSTRACT: Food spoilage fungi are becoming a global challenge to food storage and food safety, hence tons of food are lost annually to the activities of these fungi while consumption of spoilt food are dangerous to human health. Therefore, the objective of this paper is to assess the antifungal activities of lemon grass oil (*Cymbopogon citratus*) against some food spoilage fungi using different standard techniques such as dilution plating technique, colonial and morphological characterization. Oil extraction was done using fractional distillation method and biocompounds evaluation with GC-MS, antifungal activity, the minimum inhibitory (MI) and fungicidal concentrations (MFC) were determine using standard methods. Results show that five fungi were identified as *Aspergillus niger*, *Penicillum* sp., *Aspergillus oryzae*, *Aspergillus flavus*and *Aspergillus terreus*. The GC-MS of the oil detectscitral, citronellol, neral, geranial, limonene and geranyl acetate, flavonoids, alcohol and terpenoids. The oil was cidal to 100 % of the fungi at stock concentration. The minimum inhibitory concentrations against *Aspergillus niger*, *Aspergillus oryzae* and *Aspergillus terreus* was 0.781 µL/ml, *Penicillum*sp. 0.391µL/ml, and *Aspergillus flavus*, 6.25µL/ml. The MFC for *Aspergillus niger*was 1.563µL/ml, 0.781µL/ml for *Penicillum*sp and *Aspergillus oryzae* 6.25µL/ml for *Aspergillus flavus* and 1.563 µL/ml for *Aspergillust erreus*. Lemon grass oil extracted was active against common food spoilage fungi examined and could be a potential biopreservative and antifungal agent.

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Spoilage occurs in food when there are changes in the organoleptic property of the food thereby rendering it unacceptable for ingestion by human and livestock. These changes could be attributed to many factors such as microbial presence and activities, food constituents, chemical reactions, packaging and storage conditions. Over one third of the global annual food produced is reportedly lost to spoilage from various aetiological agents (Garcha, 2018; Martín-Girela *et al.*, 2020; Bezirtzoglou, 2020). Growth and activity of microorganisms has been recognized as the major cause of food spoilage, chemical reactions, enzymatic activity and infestation by vermins are other known causes (Bezirtzoglou, 2020). Notable among microbial causes of spoilage are fungi and bacteria, fungi being the predominant on food with low moisture content and medium to low pH (Garcha, 2018)Fungal spoilage of food manifest in form of acidification, fermentation, oxidation, rancidity and putrefaction; that can create off odour and colour, powder and sliminess in food substances (Pitt *et al.*,2019). Fungi are reported to be the cause of about 5–10% of all cases of food spoilage and wastagein many African countries. Filamentous fungi are mostly

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concerned with spoilage of food. Members of the general *Aspergillus*, *Penicillium*, *Mucor*, *Rhizopus*, *Fusarium* are well documented as spoilage agents (Criado *et al.*, 2015; Pitt *et al*., 2019). Such food items become unfit for consumption. Fungal growth in food can generate toxic metabolites into the food. Such metabolites; often regarded as mycotoxins are variously pathogenic (Mallmann *et al.,* 2018). Moreover, when such food items were consumed, it may result in food poisoning of various degrees and ultimately, death. The overall impact of food spoilage is enormous and cannot be overemphasized. Food spoilage also leads to loss of essential nutrients, wastages of food, economic loss to farmers, famine and starvation, depreciation in the total national income. Food preservation is any activity that is aimed at increasing food shelf life in quality and quantity. The idea of preserving food preservation started very early in human history; drying, smoking, salting and fermenting were the commonly engaged methods. Technological breakthrough led to the use of modern formulations for food preservation while the recent surge in discouragement of the use of chemical preservatives led to the development of techniques that are based on extracted biological compounds which were used for food preservation. Any substance, biochemical, natural or synthetic substances engaged in extending food shelf life are known as preservatives (Riddervold and Astri, 2015). Such include salt, smoke, sugar, organic acids and essential oil from plants. On the other hand, synthetic preservatives include vinegar, sodium benzoate, sodium meta-bisulphite among others (Abakarov *et al.,* 2021).Chemical preservatives have detrimental effects on food consumers and the environment. Their use has been reported toxic to consumers and associated with development of recalcitrant substances in the environments (Prakash *et al.,* 2015; Chaudhari *et al.,* 2019). To mitigate the menace of fungal spoilage in foods, alternative measures to synthetic pesticides have been sought using natural agents such as essential oils that cause no environmental pollution, side effects and are safe for human use (Bhatt and Kale, 2019). The use of plant as antifungal in treating human infections has been well documented (Hay *et al.,* 2019). Many living organisms especially plants are natural reservoir of biological molecules with antimicrobial activity (Odds, 2018). The whole plant or any of its parts are resident to many biological metabolites that could be used to prevent fungal growth. Large array of active metabolites against fungal growth and activity have been reported in plants, these metabolites are of great importance to humans in combating issues of microbial activities. They could be engaged directly or prepared into formulations for commercial and local uses (Arif *et al.,* 2015). Lemon grass (*Cymbopogon Flexuosus*) and *(Cymbopogon Citraus)* is a commonly found grass with a strong aromatic smell (Nyamath and Karthikeyan, 2018), it is found in Asia, India and many parts of Africa (Srivastava *et al.,* 2013; Mukarram *et al.,* 2021; Mukarram *et al.,* 2022). It has essential oil known to possess antimicrobial characteristics (Ewanishi *et al* 2012; Rangari, 2013; De Silva *et al*., 2017; Chinen-chun *et al*., 2018). Lemongrass is widely known and used; the oil from the plant is rich in citral, a substance used by the perfumery, cosmetics and pharmaceutical industries (May *et al.,* 2008). Studies such from authors such as Pandey *et al.* (2003); Jaramillo-Colorado *et al.* (2020); Lima *et al.* (2020); Lee *et al.* (2020) have demonstrated intense antibacterial, antifungal, antiviral, antiprotozoal, antinematidal and larvicidalactivities of the lemon grass oil. The menace of food spoilage poses challenging threat to food production and human health in developing countries like Nigeria. To ensure food availability and safety for all, food spoilage must be eliminated or reduce to the barest minimum, hence, the objective of this paper is to assess the antifungal activities of lemon grass (*Cymbopogoncitratus*) oil against some food spoilage fungi.

MATERIALS AND METHODS

Isolation of Fungi from spoilt food items: Fungal isolation was done following the method used by Adedayo *et al*. (2023); Kawata *et al*. (2023). One gram each of the spoilt orange was weighed into 10 ml of sterile distilled water. Serial dilution was performed and 1 ml of the aliquot was inoculated on to the surface of already solidified PDA plates using spread plate method. The plates were incubated at 28^{0} C for 72 hours. Sub culturing of visible distinct colonies were done repeatedly to obtain pure culture of isolates. Isolates were maintained on agar slants.

Macroscopic and Microscopic Characterization and Identification of Fungi: The obtained fungal colonies were characterized macroscopically based on their colonial appearance on plate. Microscopic characterization was done on wet mount of isolates and after staining with cotton blue in lactophenol. The slide was examined under the microscope at ×10 and × 40 magnifications. The morphological characteristics and appearance were properly documented and compare with literature on mycological taxonomy for identification (Adedayo *et al*., 2023; Kawata *et al*., 2023).

Collection and Preparation of Lemon Grass Leaf: Lemon grass leaf was collected at Oluponna Iwo in Osun State. They were shred into long thin pieces using scapel. Oil extraction was done using steam

distillation method. The extraction was done using 1000 g of the lemon leaf in 5 liter round- bottomed flask to which was added 200 ml of distilled water. The distillation was run for four hours using a Clevenger apparatus (AOAC, 1990). Based on the differences in density of water and oil, the oil floated while the water was removed using a separating funnel. The extracted oil was dried using anhydrous sodium sulfate (Mohamed *et al.,* 2014).

*Characterization of the Lemon Grass Oil using GC-MS:*The method described and used by Syarif *et al.* (2023) with little modifications was followed. The lemon grass oil was characterized using a Shimadzu Gas Chromatograph (Model GC.MS-QP2010 Ultra) connected to a non-polar Rtx-MS capillary column 30 m by 0.25 mm and 0.25µl thick using a mass spectrometer detector while helium was used as a mobile phase (carrier gas). The set up was attached with a computer with special software to record and analyze the data. Retention indices (RI), using n-alkanes were used as the basis. The percent of each compound was based on the peak area divided by the total area of component peaks. The temperature range was from 50-300°C, with a temperature program rate of 10°C/min, starting at three minutes and finishing at thirty minutes. The pressure applied in this experiment was 100kpa with a total flow of 50ml/min and 1.69ml/min of column flow. The injection, ion source and the interface temperatures were 300°C, 200°C and 250°C respectively.

Assessment of the Antifungal Activity of Lemon Grass oil against Isolated Fungi: The antifungal property of the extracted oil against the spoilage fungi was determined using the cup-plate agar plug diffusion method with little modification. Precisely 0.2 ml of the oil extract was thoroughly mixed with 20ml of Sabouraud-dextrose agar sterile Petri dishes. The agar plates were left to set. A cup of 10 mm in diameter was cut using a sterile cork borer in the plates. Actively growing, 48 hour old cultures of the isolated fungi were used. The cork borer was used to cut and lift a portion of the growing mycelium of isolated fungi into the cup created in the plates. Incubation of plates was at 28°C for 48hours. Control experiments without the essential lemon grass oil were also set. At the end of 48 hours, plates were examined for zones of clearance or diameter of mycelia growth; observations made were recorded (Palevitch and Yaniv, 2015).

Determination of the Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) of Lemon Essential Oil: The Minimum Inhibitory Concentration (MIC) and Minimum Fungal Concentration (MFC) of the Lemon Grass oil on the test fungi were determined by the method involving macro-dilution in culture broth, in this process, different concentrations were used (100, 50.0, 25.0, 12.5, 6.25, 3.125, 1.563, 0.7815, 0. 391, and 0.195 µL/ml) according to the method proposed by (Omori *et al*., 2012). Sabouraud Dextrose Broth was prepared and the lemon grass oil was dispensed aseptically into test tubes. Backward dilution was done to achieve the concentration used. The tubes containing the broth and oil extracts were inoculated with the 0.1 ml of spore suspension (containing approximately 1 X 10^8 spores/ml) from 48hours culture of the test isolates. The tubes were partially lidded to prevent anaerobiosis. Control experiments were set up. The tubes were incubated at 28°C for 48hours, after incubation, growth was confirmed using turbidity method with the aid of a spectrophotometer. The least concentration of the sample in which microbial growth did not occur was identified as the Minimum Inhibitory Concentration (MIC). The Minimum Fungicidal Concentration (MFC) is usually an extension from the MIC, and represents the minimum concentration where there was no viable growth from the MIC. The Minimum Fungicidal Concentration (MFC) was determined by sub-culturing from the tubes with the MIC that do not show evidence of growth. The mixture was inoculated on a freshly prepared sterile Sabouraud Dextrose Agar plate and incubated for 48 hours at 28 °C. The plate with minimum/lowest concentration of the extract that did not allow the growth of the fungi was identified as the Minimum Fungicidal Concentration (MFC) of the extract (Abdullah, 2022).

RESULTS AND DISCUSSION

Characterization and Identification of Fungal isolates: A total of five distinct fungi were obtained. The fungi isolates had septate hyphae with erect conidiophores carrying conidiospores. The colonial and microscopic characteristic of the isolated filamentous fungi are presented in Table 1. The fungi were identified as *Aspergillus niger, Penicillum* sp., *Aspergillus oryzae, Aspergillus flavus, Aspergillus terreus*.

Antifungal Activity of Lemon Grass oil against Isolated Fungi: The extracted oil from the lemon grass was cidal against the isolated food spoilage fungi. All the fungi were not able to grow on the plates with the lemon grass oil, however there were growth on the control plates where there was no lemon grass oil. The diameters of mycelia growth were measured and recorded. Result is presented in Table 2.

The Result of the GC-MS Characterization of the Essential oil from Lemon grass: The GC-MS

characterization identified more than fifteen biological components as depicted by the peaks. The biological molecules that correspond to the peak were citral, citronellol, neral, geranial, limonene and geranyl acetate, flavonoids, alcohol andterpenoids. Citral and citronellol were the most abundant constituents. The GC-MS chart is presented in Figure 1.

Minimum Inhibitory (MI) and Minimum Fungicidal Concentration (MFC) of Lemon Essential Oil: Minimum inhibitory concentration (MIC) values of the lemon grass oil against all isolated spoilage fungi is presented in Table fungi 2. The recorded MIC against *Aspergillus niger* was 0.781 µL/ml, *Penicillum* sp. 0.391µL/ml, *Aspergillus oryzae,* 0.781µL/ml *Aspergillus flavus,* 6.25µL/ml while it was 0.781µL/ml against *Aspergillus terreus.* The MFC for *Aspergillus niger* was 1.563µL/ml, 0.781µL/ml, for *Penicillum* sp. and *Aspergillusoryzae*6.25µL/ml, *Aspergillus flavus* and 1.563µL/ml *Aspergillus terreus*.

Table 1: Characterization and Identification of Fungal Isolates

Fungi Isolates	Color	Shape	Surface	Elevation	Types of Hyphae	Types of Spores	Isolated Fungi
F1	Black	Globose	Smooth with conidial production	Spread	Septate	Conidiospores	*Aspergillus niger*
F2	Green	Globose	Smooth with conidial production	Spread	Septate	Conidiospores	*Penicillum* sp.
F3	Green	Globose	Smooth with conidial production	Spread	Septate	Conidiospores	*Aspergillus oryzae*
F4	Green	Globose	Smooth with conidial production	Spread	Septate	Conidiospores	*Aspergillus flavus*
F5	Brown	Globose	Smooth with conidial production	Spread	Septate	Conidiospores	*Aspergillust erreus*

In this study, isolated fungi were found as species of two genera: *Aspergillus* and *Penicillium*. These two genera are notable as food spoilage agent and have been mostly isolated from spoilt food and during storage. All species of fungi are heterotrophic depending on deterioration of organic substances for their metabolism. The moulds generally release extra cellular enzymes on to the substrate upon which they grow, bringing about conversion of the organic molecules into simple substances in the form of glucose, amino acids, and minerals. The products of bioconversion are absorbed by the fungi which are osmiotrophs. Thus energy for growth, metabolism and cell wall building is derived. Similar fungi were isolated and reported earlier by Kawata *et al*. (2024). The mycelia of all the five isolates with essential oil could not growth; the lemon grass oil was totally inhibitory to the mycelia growth of all the fungi screened. This observation was similar to the report of earlier work (El-Mokhtari *et al.,* 2020). The fact that the isolated fungi grew on the control plates gave credence to the fact that the lemon grass oil contains biological molecules that were cidal to the growth of the fungi. Lemon grass oil has been documented to have antimicrobial action against many organisms including fungi. Fungicidal or fungistatic abilities of lemon grass oil against many moulds and yeast are direct products of many of the volatile and aromatic compounds in the oil (Ju *et al.,* 2019; Wani *et al.,* 2020). The biomolecules are highly toxic to numerous fungal strains (Junior *et al.,* 2020; Dong and Thuy, 2021). The cell wall gets disrupted leading to porosity and loss of ions, these can further affect signal transmission resulting into poor mycelia germination and growth. The destructions on fungal cells range from reduction in cell size, leaching of essential nutrient from cell, growth retardation, reduction in cell metabolism, and inhibition of spore germination among others (Boukhatem *et al*., 2014; Alviano *et al.,* 2017; Li *et al.,* 2020).

Table 2: Antifungal Action of the Lemon grass Oil against Isolated Food Spoilage Fungi

Isolates	Lemon grass Oil	Mean Mycelia Growth Diameter (mm)	Control
Aspergillus niger	0.00	68.33±0.5	
Penicillum sp.	0.00	34.33±0.2	
Aspergillus oryzae	0.00	27.00±0.3	
Aspergillus flavus	0.00	32.66±0.5	
Aspergillus terreus	0.00	62.33±0.5	

Table 3: Minimum Inhibitory Concentrations of Lemon grass oil on Isolated Fungi

Isolated fungi	Concentrations (µL/ml)
Aspergillus niger	0.781
Penicillum sp.	0.391
Aspergillus oryzae	0.781
Aspergillus flavus	6.25
Aspergillus terreus	0.781

Table 4: Minimum Fungicidal Concentration (MFC) and Minimum Inhibitory Concentrations of Lemon grass oil on Isolated Fungi

Isolated fungi	MFC (µL/ml)
Aspergillus niger	1.563
Penicillum sp.	0.781
Aspergillus oryzae	0.781
Aspergillus flavus	6.25
Aspergillus terreus	1.563

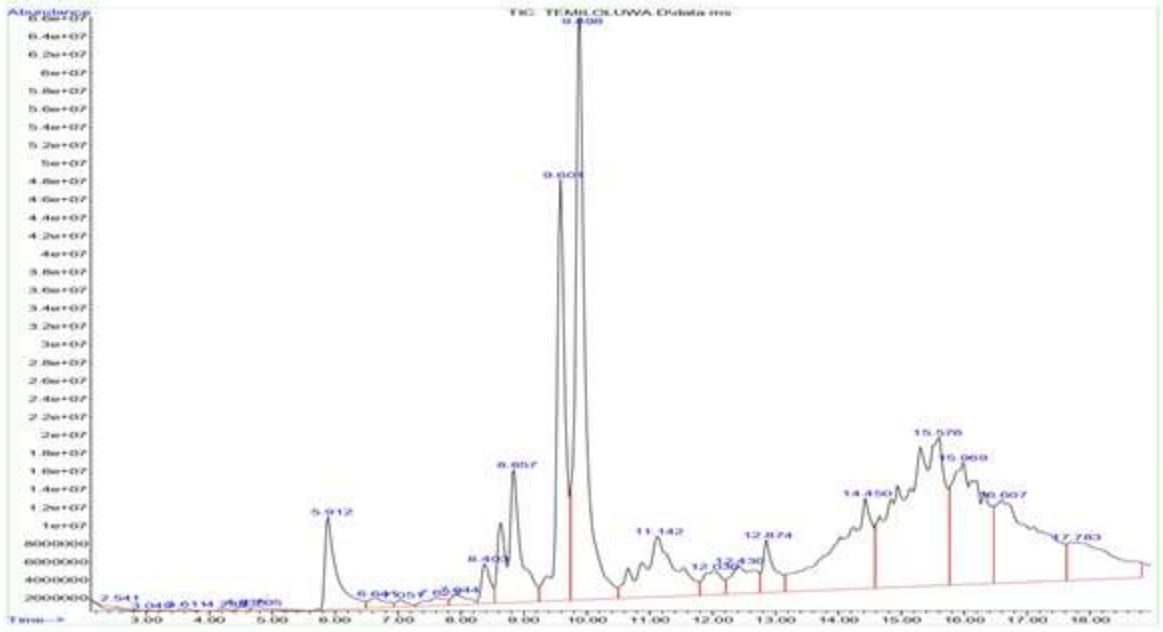

Fig 1: Identified Peak in GC-MS that corresponds to Essential Biocompounds in Lemon grass Oil

Antifungal action of lemon grass oil could also be due to its ability to induce formation of toxic oxygen leading oxidative stress that damages the cell irreparably (Lee *et al.,* 2020). Anaerobiosis created by the presence of oil is also inhibitory to fungal metabolism; fungi being mostly aerobes. The observed antifungal property demonstrated by the lemongrass oil in this research suggests that the oil can be used as a biopreservative and sustainable alternative to chemical in the food and allied food industries, it could also be part of the components of edible coating on fruit and vegetables (Oh *et al.,* 2017; Muhammad *et al.,* 2017, Artiga-Artigas *et al.,* 2017). Preservation with lemongrass oil is project to enhance food qualities in addition to forestalling deterioration occasioned by fungi (Frazao *et al.,* 2017). The minimum inhibitory and cidal concentrations obtained in this study suggests that very little quantity of the oil will be required to achieve preservation of food during storage. The oil is naturally edible and well accepted with good taste and aroma and hence, could be considered as a safe and readily available alternative preservative in the food industries (Frazao *et al.,* 2017). The oil could possibly be added to biopreservative formulations against storage fungi to enhance effectiveness.

Conclusion: It was seen in this research that lemongrass oil inhibited the growth of the isolated fungi which are among the commonly encountered spoilage organisms of agricultural products during marketing and storage. Therefore, the inclusion of lemongrass oilin stored food could prevent growth of spoilage fungi. It is recommended that further research be carried out on the use of lemon grass oil as food biopreservative or as precursor in formulations to be used in extending food shelf life. The findings of the research could justify the use of the oil in food processing, packaging and storage.

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