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Antimicrobial Effects of *Ocimum gratissimum* Extracts on the Spoilage Organisms Isolated from Yoghurt Samples

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Abstract

Ocimum gratissimum (OG) is recognised for its nutritional, antioxidant, and antimicrobial properties, making it a strong candidate for natural food preservation. This study evaluated the effects of ethanolic and aqueous OG leaf extracts against spoilage organisms isolated from laboratory-prepared yoghurt samples stored at room temperature for ten days. The agar well diffusion technique was employed for antimicrobial screening, while the broth microdilution method was used to determine both minimum inhibitory concentration (MIC) and minimum cidal concentration (MCC). The isolated organisms included Bacillus subtilis, Bacillus spp., Saccharomyces cerevisiae, Saccharomyces bulderi, Pichia kudriavzevii, Aspergillus niger, and Aspergillus flavus, identified by standard microbiological methods. The aqueous extract at a concentration as low as 62.50 mg/mL inhibited Bacillus subtilis, Saccharomyces bulderi, Pichia kudriavzevii, and Saccharomyces cerevisiae, with inhibition zones ranging from 5.00±0.27 to 6.20±0.87 mm. The ethanolic extract exhibited antimicrobial effects against Bacillus subtilis, Bacillus spp., Saccharomyces bulderi, Pichia kudriavzevii, and Saccharomyces cerevisiae at the same concentration (62.50 mg/mL), but with larger inhibition zones ranging from 5.30±0.45 to 11.35±2.10 mm. The aqueous extract showed substantial inhibition of Bacillus subtilis, Saccharomyces bulderi, and Pichia kudriavzevii, with an MIC of 62.50 mg/mL. In contrast, the ethanolic extract demonstrated an MIC of 31. 25 mg/mL for Bacillus subtilis, Bacillus spp., and Aspergillus niger. The MCC of the aqueous extract was 250 mg/mL for Bacillus subtilis, Saccharomyces bulderi, Pichia kudriavzevii, Aspergillus flavus, and Saccharomyces cerevisiae, while the MCC of the ethanolic extract was 125 mg/mL for Bacillus subtilis and Bacillus spp. Overall, the OG extracts exhibited substantial antimicrobial effects, effectively inhibiting the growth of spoilage organisms isolated from stored yoghurt samples. Harnessing the potential of OG can lead to an extended shelf life for yoghurt, positioning OG as a promising natural preservative.

***Keywords:** Ocimum gratissimum, antimicrobial effects, yoghurt, minimum inhibitory concentration, minimum cidal concentration*

INTRODUCTION

Yoghurt is not merely a widely enjoyed fermented dairy product; it is a culinary staple produced through the fermentation of milk by beneficial bacteria (Mehra *et al.*, 2022; Anagboso *et al.*, 2024). Its unique flavour and creamy texture, coupled with its rich nutritional and health benefits, have earned it a cherished place in diets around the world for centuries (Shwetnisha and Nongmaithem, 2021; Mureşan *et al.*, 2020; Saleem *et al.*, 2024). However, yoghurt, like many dairy products, is susceptible to contamination due to various factors (Chimezie et al., 2015; Anagboso et al., 2024). These include inefficient milk collection methods from animals of uncertain health status, inadequate milking practices, and poor processing, transportation, and distribution systems (Onasanya *et al.*, 2019; Shu'aibu *et al.*, 2021). This contamination introduces significant health risks, making it imperative to adopt effective preservation strategies (Amenu *et al.*, 2019). Implementing proper preservation techniques is essential for safety, maintaining yoghurt's delightful flavour, extending its shelf life, and ensuring consumer satisfaction (Mafe *et al.*, 2024; Wang *et al.*, 2025). In the dairy industry, effective preservation practices are critical to maintaining the quality and safety of yoghurt (Wang *et al.*, 2025). Traditionally, the

use of synthetic chemical preservatives has been the norm. These additives prevent the growth of harmful microorganisms by interfering with their cellular functions. However, as consumers grow increasingly aware of the potential drawbacks associated with synthetic preservatives, including toxicity concerns, there is a rising demand for safer, more natural alternatives (Kovalchuk, 2021). This consumer trend signifies a major shift towards natural food preservatives, driven by a heightened consciousness around health and wellness (Atwaa *et al.*, 2022). The time has come to explore and embrace natural preservation methods that ensure safety and enhance the quality of the food we consume.

Ocimum gratissimum (OG), widely known as African basil or sweet basil, is an exceptional candidate for natural food preservation. This perennial herb is commercially viable and thrives across Africa, Asia, and South America (Ugbogu *et al.*, 2021). In addition to its culinary appeal, OG is rich in bioactive compounds that offer formidable antimicrobial, antioxidant, and therapeutic effects (Abu, 2024). The scientific community has firmly established OG's effectiveness as a natural preservative (Ibrahim *et al.*, 2020). Extensive research has confirmed its robust antimicrobial properties, highlighting its essential oil as a strong plant-based preservative (Melo *et al.*, 2019). The aqueous extract of OG has been shown to effectively inhibit *Pseudomonas aeruginosa*, a common spoilage organism, as well as moderately inhibit *Staphylococcus aureus*, underscoring its importance in food safety (Talabi and Makanjuola, 2017). Moreover, OG demonstrates striking antibacterial activity against *Salmonella* species (Onyebuchi and Kavaz, 2020), showcasing impressive broad-spectrum effectiveness. Oyebode *et al.* (2023) confirm that OG's aqueous and ethanolic extracts possess significant inhibitory properties against harmful bacteria, including *E. coli, Bacillus cereus, P. aeruginosa,* and *S. aureus*. This versatility is further evidenced by research by Ebabhi *et al.* (2019), which demonstrates the ability of OG to extend the shelf life of fruits, such as *Citrullus lanatus* (watermelon), through its ethanol and hexane extracts. Preliminary studies by Akwetey *et al.* (2021) successfully advocated for combining OG with common salt as effective curing agents in meat products, establishing its broad applicability within the food sector. In fruit preservation, OG's efficacy is particularly notable, with a 40% concentration effectively preserving pineapple juice for up to ten days (Sinmiat *et al.*, 2020). This establishes OG as an excellent option for maintaining the quality of various fruit-based products.

The use of OG in yoghurt preservation is sparsely documented in the literature. Apart from a study conducted in the Southeastern part of Nigeria (Ibrahim *et al.*, 2020), no documented research has focused on this topic in the Northern region, as far as the authors know. There is a distinct gap in research concerning OG's application in yoghurt preservation in the Northern region, where Kwara State is located. Hence, this study aimed to evaluate the antimicrobial effects of OG leaf extract against spoilage organisms isolated from laboratory-produced yoghurt in Malete, Kwara State, Nigeria.

MATERIALS AND METHODS

Plant Sampling Point, Identification and Authentication

The *Ocimum gratissimum* used in this study was sourced from a backyard garden located in a residential apartment on Idi-Ogede Close in the Olunlade area of Ilorin, Kwara State. The plant was identified and authenticated at the University of Ilorin Herbarium, where a voucher specimen was deposited and assigned the accession number UILH/OO1/1781/2024 (Appendix A).

Plant Extract Preparation

The pre-extraction processing of OG leaves involved harvesting the leaves, washing them, air drying, and blending. Both ethanolic and aqueous extractions (cold maceration) of the OG leaves were performed using the standard procedure described by Zhang *et al.* (2018); and Hidayat and Patricia Wulandari (2021).

Ethanolic Extraction

Ethanolic extraction was carried out using the Soxhlet extraction method. This method utilises the solvent reflux and siphon principle to continuously extract solid material using a pure solvent, which enhances the efficiency of the extraction (Zhang *et al.*, 2018). Fifty grams of the pulverised (finely ground) and sieved OG leaf sample were placed in a porous thimble made of strong filter paper and positioned in the chamber of the Soxhlet extractor. Three hundred millilitres of the extracting solvent were poured into the reservoir flask and mounted onto a heating mantle. The OG leaves were extracted for six hours at 70°C, a temperature lower than the boiling point

(78.37°C) for ethanolic extractions. The temperature of the heating mantle was adjusted to 78 °C, which is based on the boiling point of the solvent. As the solvent was heated, its vapours condensed and came into contact with the sample powder. The soluble components of the powder are mixed with the solvent for extraction. The condensed extractant dripped into the thimble containing the crude leaf powder, extracting the soluble material by contact. When the liquid in the chamber rose to the top of the siphon tube and exceeded the maximum height of the siphon, the liquid contents (the solvent containing the extract) were siphoned back into the flask (Sukhdev *et al.*, 2008). This process was repeated multiple times, ensuring the solid material was continuously in contact with the pure solvent. The extracted material was then concentrated in the flask using a rotary evaporator. The extract was stored in an airtight bottle in a refrigerator at 4°C until use. The Soxhlet extraction method is advantageous because it uses a small amount of solvent and is cost-effective.

Aqueous Extraction

In the aqueous extraction process, 50 g of the whole or coarsely powdered crude material was placed in a stoppered container with 200 ml of the solvent and allowed to stand at room temperature for at least three days, with frequent agitation, until the soluble matter dissolved. The mixture was then strained, and the marc (the damp solid material) was pressed. The combined liquids were clarified by filtration or decantation after standing (Sukhdev *et al.*, 2008; Zhang *et al.*, 2018). In this maceration method of extraction, the whole pulverised and sieved powdered material was placed in a stoppered container with the solvent for a specific period with frequent agitation; afterwards, the solvent was drained off. Water was used as the solvent for the aqueous extraction of OG leaves. The extracted material was concentrated in the flask with the aid of a freeze-dryer. The extract was stored in an airtight bottle in a refrigerator at 4°C until use.

Preparation of Yoghurt and Storage

The yoghurt sample used for this research was prepared in the Microbiology Laboratory of the Kwara State University, Malete, strictly following aseptic conditions under a biosafety cabinet. The steps followed in the processing include the standardisation of milk, homogenisation, heat treatment, inoculation of starter culture (level), fermentation (temperature/time), cooling and packaging (Nor-Khaizura *et al.*, 2018; Faraz, 2022) Figure 1. The prepared yoghurt samples were stored at room temperature for ten days.

Isolation and Identification of Spoilage Organisms from Yoghurt

Aliquots of laboratory-prepared yoghurt samples, stored for ten days, were daily homogenised and transferred into test tubes containing sterile distilled water for serial dilution. The serial dilution of the samples was performed as described by the reference source. One millilitre of the appropriate dilution was then transferred into previously prepared molten agar, which included nutrient agar, Sabouraud Dextrose agar (SDA), yeast extract agar, MacConkey agar, and Eosin Methylene Blue agar (EMB). Nutrient agar, MacConkey agar, and EMB were incubated at 37°C for 24 hours, while the SDA plates were incubated at both room temperature (25°C) and in an incubator at 37°C for 2 to 5 days. To identify the organisms, various staining techniques (Gram staining and spore staining), motility tests, and assessments of colonial/morphological and biochemical characteristics were performed on pure cultures. The biochemical tests included the urease test, catalase test, methyl red test, Voges-Proskauer test, indole test, citrate utilisation test, sugar fermentation test, hydrogen sulfide production test, and starch hydrolysis, as described by Nijris (2021).

Inoculum Preparation

The inoculum suspension was prepared by transferring pure colonies of isolated spoilage organisms (bacteria, moulds, and yeasts) from 24-hour cultures into the sterile nutrient broth and sterile saline solution (0.85%). These were then incubated at 37°C for 18 hours and standardised to the 0.5 McFarland standard (Tordzagla *et al.*, 2024).

Antimicrobial Screening of *O. gratissimum* Extract

Screening of OG leaf extracts (both ethanolic and aqueous) for antimicrobial effects was done using the agar well diffusion method, as described by (Ginovyan *et al.*, 2017; Gajic *et al.*, 2022). This method relies on the diffusion of antimicrobial agents from wells into the surrounding agar medium, thereby inhibiting the growth of test microorganisms inoculated on the agar surface. In brief, the Mueller Hinton Agar

(MHA) medium was autoclaved at 15 psi and 121°C for 15 minutes. After autoclaving, the agar was cooled to 50-55°C and poured into sterile Petri dishes to a uniform depth of 4 mm (approximately 25 mL). Once solidified, the culture (prepared inoculum) was applied to the agar. The inoculum's density was adjusted, and within 15 minutes, 1 mL of the organism suspension was inoculated onto the agar medium. A sterile cotton swab was employed to ensure even distribution of the inoculum across the surface. The plates were allowed to sit undisturbed for 3 to 5 minutes to facilitate the absorption of any excess moisture. Using a sterile cork borer; wells approximately 6 mm in diameter were punched into the inoculated agar, and these wells were labelled according to the concentration of the plant extract (250 mg/mL, 125 mg/mL, 62.5 mg/mL, and 31.25 mg/mL) based on a two-fold serial dilution. Each well was filled with varying concentrations of ethanolic and aqueous leaf extracts of OG (250 mg/mL, 125 mg/mL, 62.5 mg/mL, and 31.25 mg/mL). A volume of 100 µL of each diluted compound stock solution was placed into the corresponding wells, and 100% DMSO was used as a control. The plates were then incubated at 37°C for 24 hours, after which the zones of inhibition on the medium were measured in millimetres. Ofloxacin and Fluconazole served as standard antibiotics for evaluating the bacterial and fungal test organisms.

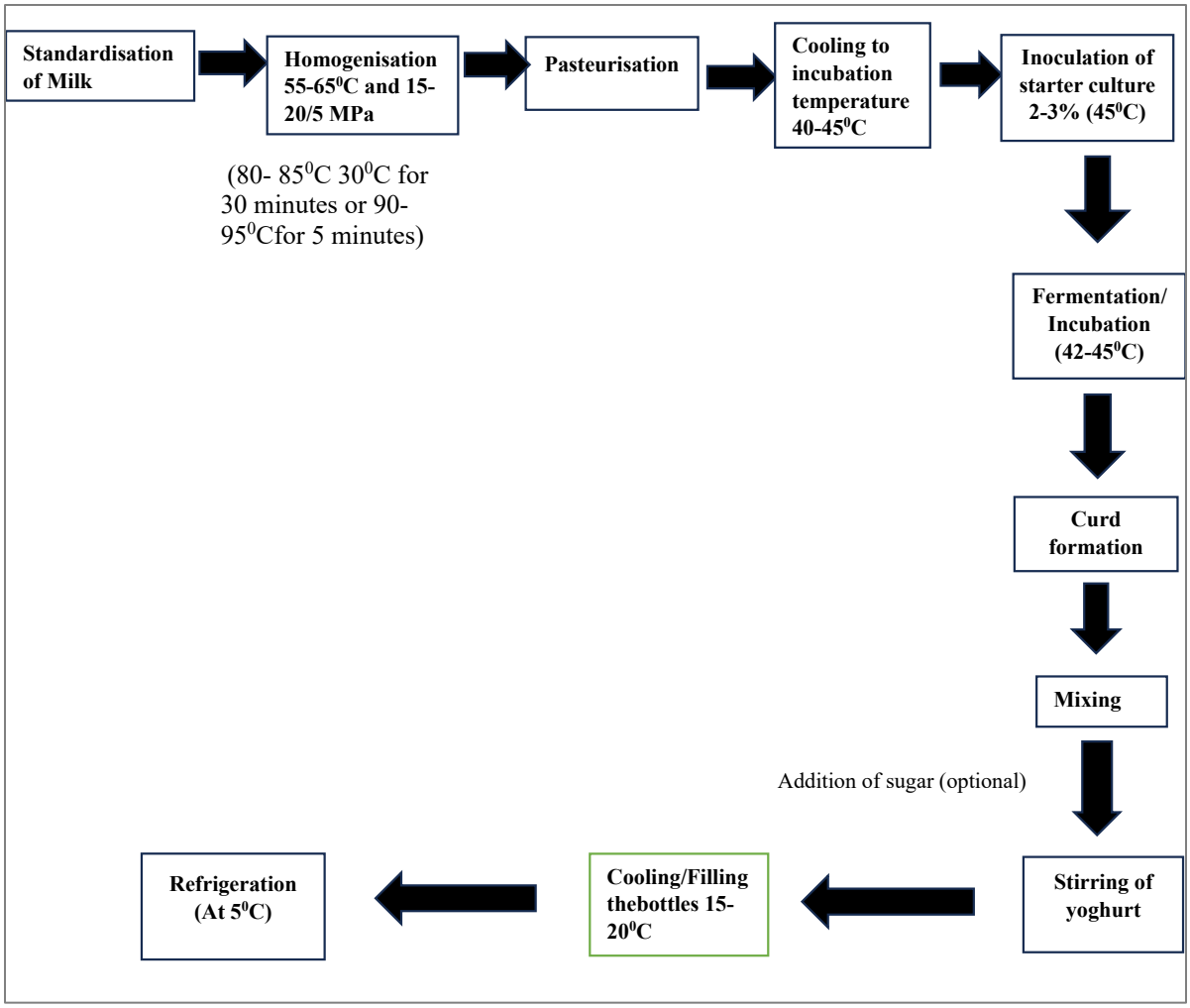

Fig 1. Yoghurt preparation steps (Le Ba *et al.*,2025)

Determination of Minimum Inhibitory Concentration

The minimum inhibitory concentration (MIC) of OG was evaluated using the microdilution broth method, as detailed by Akinduti et al. (2019), Rodríguez-Melcón et al. (2022), Swebocki et al. (2023). This technique involves preparing a liquid broth medium containing various concentrations of antimicrobial agents, into which a defined inoculum of microorganisms is inoculated and then incubated to observe for growth. A standard micro-tube dilution bioassay in a 96-well microtiter plate was utilised to

determine the MIC of ethanolic and aqueous leaf extracts of OG against bacterial and fungal strains. To each of the wells (wells 2 to 10), 100 µL of sterile 1% peptone water was added. In wells 1 and 2, 100 µL of the ethanolic and aqueous leaf extracts of OG were introduced, and serial two-fold dilutions were performed from well 2 to well 10, discarding 100 µL from well 10. An equal volume of 100 µL of a 0.5 MacFarland turbid culture of bacteria or fungi was added to the dilution range from well 1 to well 10. Overnight broth cultures of 100 µL were placed in well 11, with 100 µL of sterile 1% peptone water as the control, and 100 µL of sterile 1% peptone water was added to 100 µL of sterile water in well 12 (the blank). The turbidity of each well (as measured by absorbance, known as optical density) was assessed at time zero (T_0) using a Jenway 6405 UV/Vis spectrophotometer set to 250 nm. The plate was incubated at 37°C in ambient air for 24 hours on a shaker. After incubation, the optical density of each well was again measured (T_1), with the absorbance correlating to the turbidity due to bacterial growth. Each test was performed in triplicate. Upon completion of the incubation, the microplate was removed from the incubator, and the lid and protective film were taken off, allowing the plate to cool for 15 minutes at room temperature. The microplate was then placed in a plate reader for analysis. The readings of the wells were recorded within 24 hours to avoid contamination. Alongside visual endpoint readings, the optical density of each microplate well was measured again after 48 hours of incubation using a microplate spectrophotometer set at 250 nm. Spectrophotometric MIC values were calculated based on the growth control density.

Determination of Minimum Cidal Concentration

Following the minimum cidal concentration (MCC) assessment, 20 µL from each well that showed no turbidity was directly plated onto Petri dishes containing MHA and incubated overnight. The MCC value was determined from the wells where no growth was observed (Swebocki *et al.*, 2023).

Statistical Analysis

Data collected from this study were uploaded to the Statistical Package for Social Sciences (SPSS) version 23 for analysis. The antimicrobial effects of OG extracts were evaluated using a one-sample T-test at a significance level of 0.05. Additionally, Analysis of Variance (ANOVA) was employed to compare the antimicrobial effects of OG extracts with those of Ofloxacin and Fluconazole at a significance level of 0.05.

RESULTS

Seven spoilage organisms were isolated from laboratory-prepared yoghurt during the storage period. These organisms included *Bacillus subtilis*, *Bacillus* spp., *Saccharomyces cerevisiae*, *Saccharomyces bulderi*, *Pitchiakudriavzevii*, *Aspergillus niger*and *Aspergillus flavus*. Appendices B and C.

The screening of leaf extracts of OG (both ethanol and aqueous) for antimicrobial effect against yoghurt spoilage organisms is summarised in Tables 1 and 2. The results indicated that OG extracts have the potential to suppress the microbial growth of the tested spoilage organisms. Five fungi and two bacteria were tested against the aqueous extract of OG at various concentrations, using fluconazole and ofloxacin as standard antimicrobials. For the aqueous extract at the concentration of 62.50 mg/mL, antimicrobial effect was demonstrated against *B. subtilis*, *S. bulderi*, *P. kudriavzevii* and *S. cerevisiae*, resulting in inhibition zones of 5.00±0.27 mm, 6.10±0.81 mm, 5.30±0.45 mm, and 6.20±0.87 mm, respectively as in Table 1. At a concentration of 62.50 mg/mL of the ethanolic extract, antimicrobial effect was observed against *B. subtilis*, *B.* spp., *S. bulderi*, *P. kudriavzevii* and *S. cerevisiae*, yielding inhibition zones of 7.55±0.32 mm, 10.30±2.05 mm, 21.20±5.72 mm, 19.45±3.85 mm, and 17.95±3.28 mm, respectively as in Table 2.

The MIC of the leaf extracts is reported in Table 3. For the ethanolic extract, growth inhibition was observed at a MIC of 31.25 mg/mL for *Bacillus subtilis*, *Bacillus* spp.and *Aspergillus niger*. The MIC for *Saccharomyces bulderi* was 62.50 mg/mL, while for *P. kudriavzevii* and *A. flavus*, it was 125 mg/mL. In the case of the aqueous extract, a MIC of 62.50 mg/mL was recorded for *B. subtilis*, *B.* spp., *S. bulderi*and *P. kudriavzevii*. For *S. cerevisiae*, *A. flavus* and *A. niger*, the MIC was 125 mg/mL.

The minimum concentrations required to kill the spoilage organisms using the ethanolic and aqueous leaf extracts of OG are shown in Table 4. For the ethanolic extract, the MCC was 125 mg/mL for *Bacillus subtilis* and *Bacillus* spp., 250 mg/mL for *Saccharomyces bulderi*, *P. kudriavzevii* and *S. cerevisiae*; and 500 mg/mL for *Aspergillus flavus* and *Aspergillus niger*. For the aqueous extract, the MCC was determined to

be 250 mg/mL for *Bacillus subtilis*, *S. bulderi*, *P. kudriavzevii* and *S. cerevisiae*. The MCC was 500 mg/mL for both *Bacillus* spp. and *Aspergillus niger*.

Table 1 Antimicrobial screening of aqueousextract of *Ocimum gratissimum*against spoilage organisms isolated from Yoghurt

	Zones of inhibition (mm) at varied concentrations			
Test Organisms	**250 mg/mL**	**125 mg/mL**	**62.5 mg/mL**	**31.25 mg/mL**
B. subtilis	14.75 ± 3.21^{d}	8.55 ± 1.02^{c}	5.00 ± 0.27^{b}	0.00 ± 0.00^{a}
B. spp	13.60 ± 2.53^{c}	5.30 ± 0.72^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
S. bulderi	19.25 ± 4.13^{d}	11.30 ± 2.07^{c}	6.10 ± 0.81^{b}	0.00 ± 0.00^{a}
P. kudriavzevii	17.55 ± 3.21^{d}	9.70 ± 1.23^{c}	5.30 ± 0.45^{b}	0.00 ± 0.00^{a}
A. flavus	5.80 ± 0.75^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
A. niger	7.55 ± 1.02^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
S. cerevisiae	18.30 ± 5.10^{d}	13.75 ± 2.52^{c}	6.20 ± 0.87^{b}	0.00 ± 0.00^{a}
Standard Antimicrobials Used				
Ofloxacin	23.50±3.57^{d}	17.25±1.75^{c}	11.55±1.02^{b}	6.20±0.73^{a}
Fluconazole	17.85±2.75^{d}	12.30±2.01^{c}	7.85±0.85^{b}	0.00±0.00^{a}

Values are expressed as mean ± standard error of the mean (SEM) for triplicate readings. Values with the same superscript letter in the same column are not significantly different by one-way ANOVA followed by Duncan test.

Table 2 Antimicrobial screening of ethanolic extract of *Ocimum gratissimum* against spoilage organisms isolated from Yoghurt

	Zones of inhibition (mm) at varied concentrations			
Test Organisms / Agents	**250 mg/mL**	**125 mg/mL**	**62.5 mg/mL**	**31.25 mg/mL**
B. subtilis	15.35 ± 3.10^{d}	11.10 ± 1.75^{c}	7.55 ± 0.32^{b}	0.00 ± 0.00^{a}
B. spp	18.50 ± 2.81^{d}	13.75 ± 2.32^{c}	10.30 ± 2.05^{b}	0.00 ± 0.00^{a}
S. bulderi	21.20 ± 5.72^{d}	11.30 ± 2.07^{c}	11.35 ± 2.10^{b}	0.00 ± 0.00^{a}
P. kudriavzevii	19.45 ± 3.85^{d}	9.70 ± 1.23^{c}	5.30 ± 0.45^{b}	0.00 ± 0.00^{a}
A. flavus	8.75 ± 1.03^{c}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
A. niger	10.15 ± 2.13^{c}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
S. cerevisiae	17.95 ± 3.28^{d}	13.75 ± 2.52^{c}	6.20 ± 0.87^{b}	0.00 ± 0.00^{a}
Standard Antimicrobials Used				
Ofloxacin (standard)	23.50 ± 3.57^{d}	17.25 ± 1.75^{c}	11.55 ± 1.02^{b}	6.20 ± 0.73^{a}
Fluconazole (standard)	17.85 ± 2.75^{d}	12.30 ± 2.01^{c}	7.85 ± 0.85^{b}	0.00 ± 0.00^{a}

Values are expressed as mean ± standard error of the mean (SEM) for triplicate reading. Values with the same superscript letter in the same column are not significantly different by one-way ANOVA followed by Duncan test.

Table 3 Minimum inhibitory concentration of ethanolic and aqueous extracts of *Ocimum gratissimum* against spoilage organisms isolated from Yoghurt

Test organisms	**Minimum Inhibitory Concentration (MIC)**	
	Ethanolic MIC (mg/mL)	**Aqueous MIC (mg/mL)**
Bacillus subtilis	31.25	62.50
Bacillus spp	31.25	125.00
Saccharomyces bulderi	62.50	62.50
Pichia kudriavzevii	125.00	62.50
Aspergillus flavus	125.00	125.00
Aspergillus niger	31.25	125.00
Saccharomyces cerevisiae	-	-

Table 4 Minimum cidal concentration of ethanolic and aqueous extracts of *Ocimum gratissimum* against spoilage organisms isolated from Yoghurt

Test organisms	Minimum Cidal Concentration (MCC)	
	Ethanolic MIC (mg/mL)	Aqueous MIC (mg/mL)
Bacillus subtilis	125.00	250.00
Bacillus spp	125.00	500.00
Saccharomyces bulderi	250.00	250.00
Pichia kudriavzevii	250.00	250.00
Aspergillus flavus	500.00	250.00
Aspergillus niger	500.00	500.00
Saccharomyces cerevisiae	250.00	250.00

DISCUSSION

This study illustrates that *Ocimum gratissimum* (OG) extracts exhibit impressive antimicrobial effects against spoilage organisms isolated from yoghurt. Both ethanolic and aqueous OG extracts demonstrated remarkable inhibitory effects against the bacteria and fungi isolated from the stored yoghurt samples, including *Bacillus subtilis*, *Bacillus* spp., *Saccharomyces cerevisiae*, *Saccharomyces bulderi*, *Pichia kudriavzevii*, *Aspergillus niger*, and *Aspergillus flavus*. Although the effectiveness of OG extracts may not reach the levels of the well-established conventional antimicrobials tested (Ofloxacin and Fluconazole), it is clear that OG shows remarkable antimicrobial effects deserving of attention. This study revealed that the inhibition of microbial growth by OG extracts is concentration-dependent, supporting the notion that OG can play a pivotal role in inhibiting microbial growth and enriching the growing body of evidence endorsing its use in food preservation. Our findings align with those documented by Oyebode *et al.* (2023) in Ado-Ekiti, Nigeria, confirming that OG's aqueous and ethanolic extracts possess notable inhibitory properties against harmful bacteria. Similar antimicrobial effects of both the ethanolic and aqueous extracts of OG were reported by Azuamah *et al.* (2024), on the isolates tested in Owerri, Nigeria. Other researchers documented similar findings, including Okeke *et al.* (2023), Talabi and Makanjuola (2017), Alexander (2016), Soola *et al.* (2021), and Edo *et al.* (2023). Studies outside Nigeria have also revealed the remarkable antimicrobial effects of OG extracts on tested microorganisms in some countries, such as Kenya (Matasyoh *et al.*, 2008), Brazil (Silva *et al.*, 2022) and Morocco (Zouine *et al.*, 2024).

This study posits OG as a potential natural preservative for yoghurt, demonstrating remarkable antimicrobial effects against spoilage organisms found in laboratory-prepared yoghurt samples. Thus, OG could be an excellent alternative to chemical preservatives, which often raise safety concerns and potential toxicity with extensive consumption. The antimicrobial effectiveness of OG on microorganisms is firmly supported by existing literature documenting the diverse bioactive compounds present within its extracts (Pavithra, 2019; Xie *et al.*, 2023). Flavonoids, terpenoids, alkaloids, and phenolic compounds are integral to OG's antimicrobial action, as they interact with microbial cell membrane enzymes and proteins to disrupt their integrity. This disruption creates a considerable flow of protons out of the cell, potentially resulting in cell death or inhibiting essential enzymes needed for amino acid biosynthesis.

Furthermore, the hydrophobic nature of OG extracts allows them to engage effectively with microbial cell membranes and mitochondrial proteins, compromising their structure and membrane permeability (Abu-Zaid *et al.* 2022). Central to OG's antibacterial efficacy is eugenol, a well-known phytoconstituent renowned for its potent antimicrobial properties. The presence of eugenol not only enhances the overall effectiveness of OG extracts but also confirms their essential role in inhibiting microbial growth (Unegbu *et al.*, 2020; Xie *et al.*, 2023).

The findings from this study suggest that OG possesses antimicrobial potential that can be harnessed as an effective natural preservative. This approach aims to mitigate the toxicity concerns that may arise from using synthetic chemical preservatives in yoghurt preservation, which has been the norm. Moreover, in dairy products such as yoghurt, where pH and moisture content encourage microbial proliferation, OG extracts may provide a natural means to extend shelf life without compromising product safety or sensory quality.

CONCLUSION

This study showed that ethanolic and aqueous OG extracts possess remarkable antimicrobial

effects, inhibiting the growth of spoilage organisms isolated from stored yoghurt samples. These findings revealed OG's potential to extend the shelf life when used in yoghurt preservation, suggesting it as a promising natural and effective preservative. By incorporating OG extracts, manufacturers can significantly enhance the quality and safety of their products while reducing dependence on synthetic preservatives, ultimately ensuring fresher, longer-lasting food products for consumers. *Ocimum gratissimum*'s proven efficacy makes it a promising asset in modern food preservation strategies.

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DATA AVAILABILITY

Data sets used during the current study are available from the corresponding author.

DECLARATION ETHICAL APPROVAL

All authors confirm that this manuscript meets the journal's ethical standards and requirements.

CONSENT TO PARTICIPATE

This research paper did not involve any animal testing or human participants.

CONSENT TO PUBLISH

All authors consent to the publication of this work.

COMPETING INTEREST

All the Authors declare that they have no competing interests.

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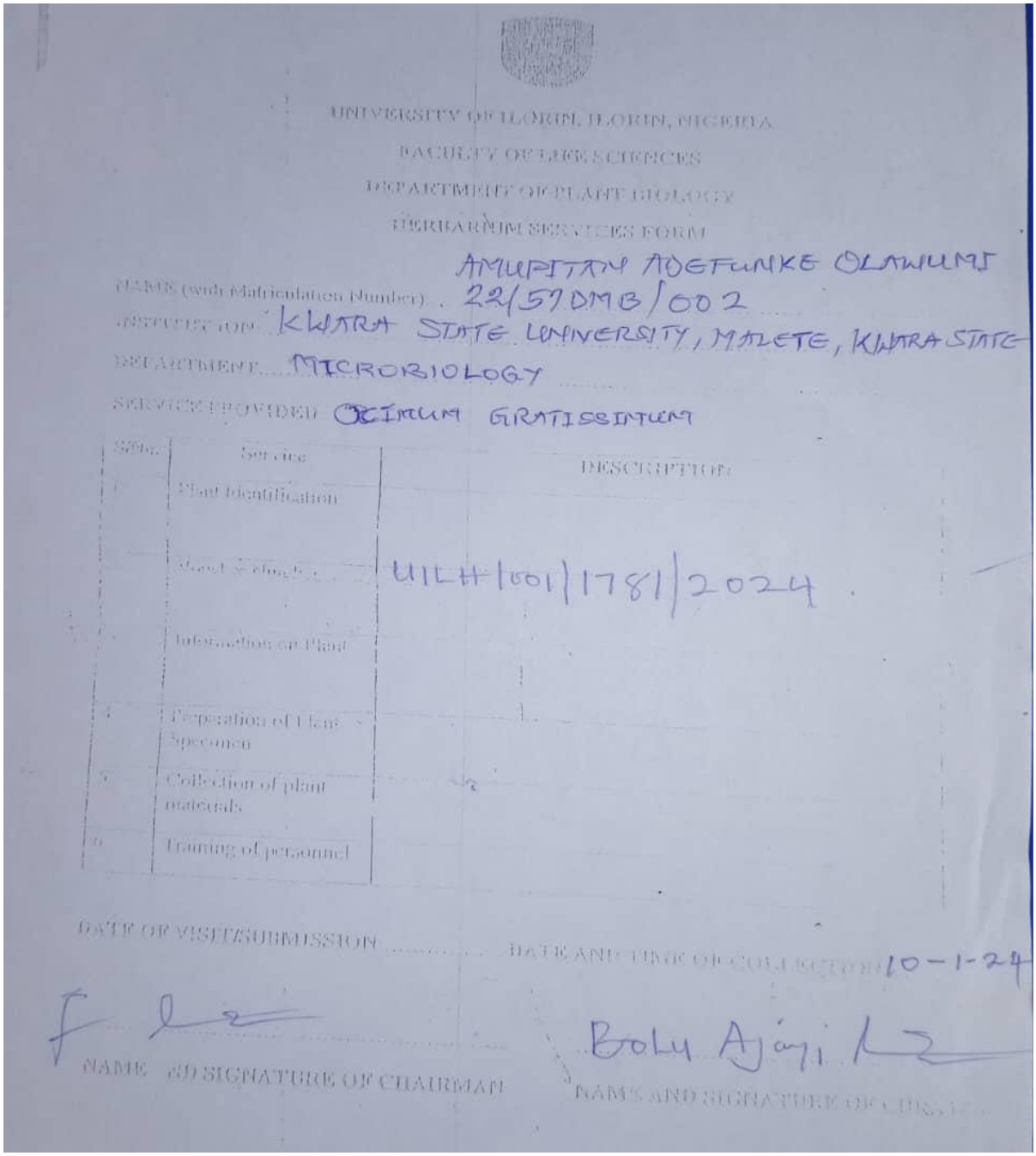
APPENDICES

Appendix A

Plant Identification VoucherNumber



UNIVERSITY OF ILORIN, ILORIN, NIGERIA
FACULTY OF LIFE SCIENCES
DEPARTMENT OF PLANT BIOLOGY
HERBARIUM SERVICES FORM

NAME (with Matriculation Number) AMUPITAN ADEFUNKE OLAWUMI 22/57DMB/002
INSTITUTION KWARA STATE UNIVERSITY, MALETE, KWARA STATE
DEPARTMENT MICROBIOLOGY
SERVICE PROVIDED OPTIMUM GRATISSIMUM

S/No.	Service	DESCRIPTION
1	Plant Identification	UILH/001/1781/2024
2	Seed Extract	
3	Information on Plant	
4	Preparation of Plant Specimen	
5	Collection of plant materials	
6	Training of personnel	

DATE OF VISIT/SUBMISSION ..... DATE AND TIME OF COLLECTION 10-1-24

NAME AND SIGNATURE OF CHAIRMAN

Bolu Ajayi
NAME AND SIGNATURE OF [illegible]

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Appendix B

Morphological and biochemicalcharacteristics of bacterial isolates from Yoghurt samples

Characteristic	B1	B2
Shape	Rod	Rod
Cell Arrangement	S	S/C
Pigmentation	–	–
Gram Reaction	+	+
Motility	+	+
Endospore	+	+
Catalase	+	+
Gelatin Hydrolysis	+	–
Methyl Red	–	+
Voges Proskauer	+	–
Glucose Fermentation	+	+
Glycerol Fermentation	+	–
Sucrose Fermentation	+	+
Lactose Fermentation	+	+
Starch Hydrolysis	+	+
Gas Production	–	–
Haemolysis on Blood Agar	+	–
Oxygen Relationship	FA	FA
Probable Organism	*Bacillus subtilis*	*Bacillus* spp.

KEY:
B1 and B2 = Bacterial isolates; S = Single; C = Chains; + = Positive; - = Negative; FA = Facultative Aerobes

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Appendix C

Morphologic and microscopic features of fungal isolates from Yoghurt samples

Fungal Isolates	Colony Appearance	Microscopic feature	Fungal species
F1	White, later becoming black and some pale yellow	Septate hyphae with simple thick walls. Conidiophores bearing conidial heads containing conidia were seen	*Aspergillus niger*
F2	Granular, flat, yellow at first, but quickly becoming brownish green with age	Septate hyphae, having thick-walled, roughened conidiophores, bearing vesicles	*Aspergillus flavus*
F3	Off-white, entire margin, convex elevation, smooth surface, and opaque density	Round to ovoid budding yeasts	*Saccharomyces cerevisiae*
F4	White, raised, entire margin, smooth surface	Spherical to ellipsoidal yeast cells with multilateral budding and some asci-containing ascospores	*Saccharomyces bulderi*
F5	Spreading colonies with rough whitish-yellow surface, wrinkled and flat	Spherical yeasts with “Long grain rice” appearance	*Pichia kudriavzevii*

KEY: F1 to F5 = Fungal isolates