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CHEMICAL PROFILING AND ANTIBACTERIAL ACTIVITY OF *Nannochloropsis oculata* EXTRACT AGAINST FOODBORNE PATHOGENS

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ABSTRACT

Foodborne illnesses remain a public health concern in Indonesia, prompting the search for natural antibacterial agents. This study evaluated the antibacterial activity and chemical profile of *Nannochloropsis oculata* extracts against *Listeria monocytogenes* and *Shigella flexneri*. Microalgae were cultivated, and cell density was monitored using an Ultraviolet–Visible (UV–Vis) spectrophotometer. Biomass was harvested and extracted using chloroform–methanol, followed by compound identification through Gas Chromatography–Mass Spectrometry (GC–MS). Antibacterial activity was assessed using the disc diffusion method. A total of 1.3196 g of dry biomass was obtained. At 200 mg/mL, the extract produced inhibition zones of 8.40 ± 0.17 mm against *L. monocytogenes* and 8.83 ± 0.29 mm against *S. flexneri*. GC–MS analysis revealed fatty acid derivatives, predominantly methyl palmitoleate (26.47%). These findings provide preliminary insight into the antibacterial potential of *N. oculata* extracts; however, further quantitative and application-based studies are required to determine their practical relevance.

Keywords: antibacterial activity, foodborne illness, GC-MS, microalgae, *Nannochloropsis oculata*

INTRODUCTION

Every year, contaminated food causes 600 million cases of foodborne illness and 420,000 deaths worldwide (WHO, 2024) One of the main causes of this problem is pathogenic bacterial infections, such as *Listeria monocytogenes* and *Shigella flexneri*, which cause food poisoning and pose a serious challenge to public health, especially in developing countries, including Indonesia (Binkhamis et al., 2025; Shamloo et al., 2019). This condition is exacerbated by low public awareness of food hygiene and cleanliness in food storage and processing, which increases the risk of pathogenic bacterial contamination (Fung et al., 2018). To date, the use of synthetic antibiotics remains the main method to control these infections,

but long-term use often causes side effects such as digestive system disorders, bacterial resistance, and potential damage to the liver and kidneys (Abishek & Venkateswaramurthy, 2023; Konstantinidis et al., 2020).

Various previous studies have focused on the exploration of antibacterial compounds from medicinal plants, fungi, and marine bacteria (Álvarez-Martínez et al., 2020). Microalgae, as a potential candidate, have received significant attention as a source of bioactive compounds with various benefits, including as antimicrobial, anticancer, antioxidant, and anti-inflammatory agents. The content of metabolites such as fatty acids, sulfated polysaccharides, and pigments in microalgae is known to play an important role in their biological activities, including the ability to inhibit the growth of pathogenic bacteria because they produce antimicrobial compounds through the secretion mechanism of bioactive metabolites that can disrupt bacterial cell walls or membranes. Studies conducted by Ilieva et al. (2024) have confirmed the potential of microalgae such as *Chlorellaceae* and *Scenedesmaceae* as natural antimicrobial agents, but research highlighting the specific mechanisms of action, optimization of culture conditions, and characterization of their active compounds is still very limited. This indicates a significant research gap to develop other microalgae as effective and sustainable sources of natural antibacterials.

One of the microalgae with high potential is *Nannochloropsis oculata*, a unicellular green microalgae commonly found in marine and freshwater. This species is known to have high protein, pigment, and polyunsaturated fatty acid content, as well as superior photosynthetic efficiency and lipid productivity (Ma et al., 2016; Stepanus, 2023). Several recent studies have shown that *N. oculata* has important biological activities, including as an antioxidant, antidiabetic, antihyperlipidemic, and cytotoxic agent (Deepa P. K et al., 2022; Ferdous & Yusof, 2021). Similar findings are also supported by a recent study conducted by Çağatay et al. (2021), which evaluated the antibacterial activity of *N. oculata* against several fish pathogenic bacteria such as *Lactococcus garvieae, Flavobacterium psychrophilum*, and *Yersinia ruckeri*. The results of this study indicate that *N. oculata* has high antimicrobial activity against various types of pathogens, thus strengthening the view that this microalgae has great potential to be developed as a source of natural antibacterial ingredients. However, research related to the antibacterial activity of *N. oculata* against pathogenic bacteria that cause food poisoning, especially *L. monocytogenes* and *S. flexneri*, has not yet been conducted.

It was hypothesized that the chloroform-methanol extract of *N. oculata* contains metabolites that capable of exhibiting measurable antibacterial activity against selected

foodborne pathogens, namely *L. monocytogenes* and *S. flexneri*. The methodology applied includes microalgae cultivation, cell density determination with a spectrophotometer, harvesting, crude extraction, identification of active compounds through GC–MS, and antibacterial testing using the disc diffusion method. The results of this study provide preliminary scientific information on the antibacterial potential of microalgae-derived extracts and may serve as a foundation for further investigation into natural bioactive compounds for foodborne pathogen control.

METHODS

Cultivation of *Nannochloropsis oculata*

The microalgae *N. oculata* was obtained from the Center for Brackish Water Aquaculture (BBPBAP) Jepara and prepared as a starter culture through four days of activation at the Biochemistry Laboratory of the Faculty of Mathematics and Natural Sciences, Universitas Negeri Jakarta, Indonesia. The microalgal inoculum was introduced into sterile seawater, which was enriched with Walne medium (1mL/L). The cultivation environmental conditions were controlled including: temperature 25 – 27°C, salinity 25-30 ppt (monitored with a refractometer), photoperiod 12:12 (light:dark) with a light intensity 200 µmol foton $m^{-2}s^{-1}$ and aeration (Ilhamdy et al., 2020; Wardani et al., 2022).

Determination of Cell Density Using a UV-Vis Spectrophotometer

N. oculata cell density was measured every 24 hours using a UV-Vis spectrophotometer at a wavelength of 750 nm. Cell-free culture medium served as a blank. Samples were homogenized before measurement, and if the OD value exceeded 0.8, dilution was performed until it was within the linear range. The final value was corrected based on the dilution factor and expressed as the mean ± standard deviation of three replicates (Song et al., 2024).

Microalgae Harvesting

N. oculata microalgae were harvested through centrifugation at 5.000 rpm for 15 minutes. The supernatant was then separated, and the biomass sediment was filtered using Whatman No. 1 filter paper and then weighed using an analytical balance (Song et al., 2024). Moisture content was determined from a representative 2 g wet biomass sample to estimate total dry biomass. The analysis was performed as a preliminary estimation and not as replicated quantitative measurement.

Microalgae Biomass Extraction

The harvested *N. oculata* microalgae biomass was mechanically ground to a coarse texture. The microalgae biomass was then extracted by maceration at room temperature using chloroform and methanol (2:1 v/v) at a biomass:solvent ratio of 1:10 w/v. The extraction procedure was adapted and modified from Blanco-Llamero et al. (2024). After two days of maceration, the extract was concentrated using a rotary evaporator to obtain a crude extract. The crude extract was then stored in a refrigerator at 4°C until analysis.

Gas Chromatography Mass Spectrometry (GC-MS) Analysis

To prepare the samples, crude extracts of *N. oculata* (2 mg) were mixed separately with 1 ml of 1 M HCl in methanol under a constant flow of N_2 gas for 1 minute. The mixture was then incubated at 80°C for 40 minutes and cooled to room temperature. After adding 1 mL of 0.9% sodium chloride solution and 1 mL of hexane, the samples were centrifuged at 3000 rpm for 3 minutes. The hexane phase was then transferred to a glass tube and dried. The prepared samples were then analyzed using GC-MS. GC-MS detection was performed using electron ionization at 70 eV. The column carrier gas flow rate was set at 1 mL per minute, and 1 µL of sample was injected into the column, which had been preheated to 250°C in split mode. The GC oven temperature was started at 40°C, then increased to 300°C at a rate of 5°C per minute for 52 minutes, and maintained at 300°C for 8 minutes. Mass data analysis was performed using a GC-MS post-process analysis program equipped with a database(Blanco-Llamero et al., 2024).

Preparation of Test Bacterial Cultures

A single colony of *L. monocytogenes* and *S. flexneri* on Agar was inoculated into their respective liquid media (BHI broth and MHB broth). The mixture was then homogenized by vortexing and incubated at 37°C for 24 hours. This resulted in an inoculum that could be directly used for antibacterial activity testing. The bacterial suspension was adjusted to the turbidity of a 0.5 McFarland standard by measuring the optical density at 625 nm (OD ≈ 0.08–0.10), corresponding to approximately 1×10^8 CFU/mL. The standardized inoculum was subsequently used for antibacterial activity testing (Hattab et al., 2024).

Antibacterial Test of the Microalgae *N. oculata* using Disc Diffusion Methods

The antibacterial assay was carried out according to the method of Hati et al. (2025). The bacterial isolates used in this study were *L. monocytogenes* ATCC 13932 and *S. flexneri*

ATCC 9199. Bacterial suspension 1 mL of each of *L. monocytogenes* and *S. flexneri* bacteria was poured into a petri dish, followed by 15 mL of Nutrient Agar, respectively. The discs were then homogenized and allowed to harden. Next, sterile 6 mm paper discs were dipped into the samples to be used: microalgae biomass extracts at concentrations of 200 mg/mL, 100 mg/mL, 50 mg/mL and 25 mg/mL dissolved in 10% DMSO, ampicillin for *L. monocytogenes*, and ciprofloxacin for *S. flexneri* used as a positive control, and 10% DMSO as a negative control. The discs were then placed on the surface of the agar plate inoculated with bacteria. The discs were spaced 3 cm apart from each other and 2 cm to the edge of the medium. The petri dishes were covered and incubated for 24 hours at 37ºC to observe the zone of inhibition. Antibacterial activity was determined by measuring the diameter of the inhibition zone. This inhibition zone measurement was performed three times.

Statistical Analysis

The antibacterial activity results were presented as the mean ± deviation standard. Data were analyzed statistically with analysis of variance (One-Way Anova; $\alpha = 0.05$), followed by *Tukey's Post Hoc* test to see more clearly the differences between each group. The level of statistical significance was defined as $p < 0.05$.

RESULTS AND DISCUSSION

Biomass Yield and Recovery

The cultivation of the microalgae *N. oculata* was conducted for 12 days to obtain sufficient biomass and to determine the optimal harvesting phase for bioactive compound extraction. Cultivation refers to the controlled growth of microalgae under regulated environmental conditions to maximize biomass productivity and metabolite accumulation for downstream applications. Recent studies report that *Nannochloropsis* species can accumulate total lipids of dry cell weight under stress conditions, particularly nitrogen limitation (Palanisamy et al., 2023; Udayan et al., 2023)

The growth curve of *N.oculata* (Figure 1**)** showed a gradual increase in optical density (OD) from day 0 to day 12, indicating continuous cell proliferation. The OD value increased from approximately 0.10 on day 0 to 0.58 on day 12, indicating a progressive rise in cell density during cultivation. The increase observed on day 2 marked the transition from the lag phase to the exponential phase. Rapid cell division occurred between days 2 and 8, corresponding to the exponential phase. From day 10 to day 12, growth stabilized, indicating the stationary phase. During this phase, nutrient depletion—especially nitrogen limitation—triggers lipid

biosynthesis and the accumulation of fatty acid derivatives such as palmitoleic acid and its methyl esters (Liang et al., 2019).

Environmental factors including light intensity, temperature, nutrient availability, salinity, and aeration significantly influence both biomass yield and lipid composition (Navalho et al., 2025). Higher biomass does not always correlate with higher bioactive compound concentration, as lipid accumulation is often enhanced under stress-induced stationary conditions. Therefore, harvesting time plays a crucial role in determining both the quantity of biomass and the qualitative composition of antibacterial compounds present in the extract.

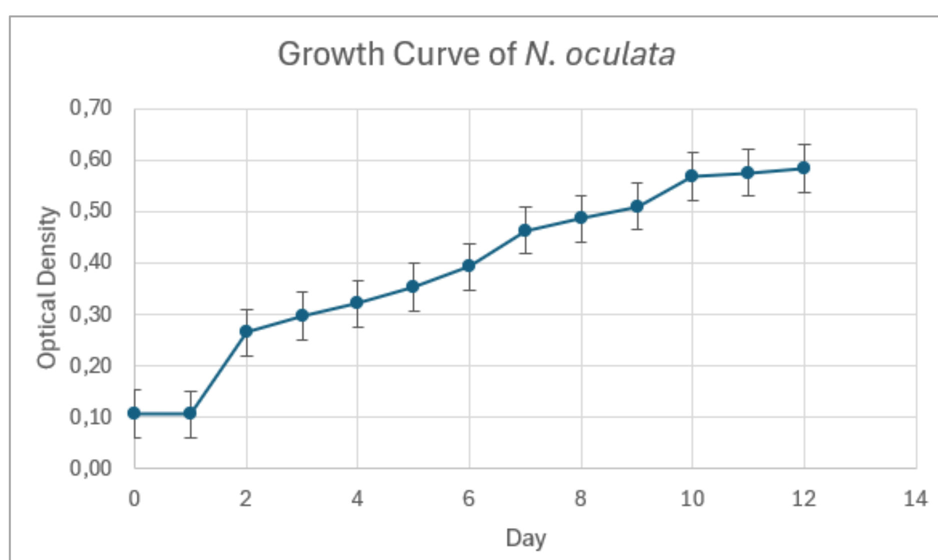

Figure 1. Growth Curve of Microalgae *N. Oculata* Measured Spectrophotometrically for 12 Days to Determine Harvest Phase for Biomass Collection (Mean ± SD, N=3)

Cell density was monitored indirectly using optical density (OD) measurements obtained by UV–Visible spectrophotometry. In this study, cell concentration was not converted into cells/mL because no direct counting method, such as a haemocytometer or flow cytometry, was employed. Therefore, OD values were used as a relative indicator of cell density. An increase in optical density corresponds to increased culture turbidity, which reflects higher microalgal biomass concentration. Although OD measurements do not provide absolute cell numbers, they are widely accepted for monitoring microalgal growth trends and determining optimal harvesting time (Pessoa et al., 2025).

The cultivation process of *Nannochloropsis oculata* under controlled environmental conditions is presented in Figure 2. At the initial stage (day 0), the culture appeared light green, indicating a relatively low cell density following inoculation (Figure 2 (a)). After 12 days of

cultivation, a noticeable increase in microalgal density was observed, as evidenced by the darker green coloration of the culture medium (Figure 2 (b)). This visual change suggests active cell growth under the applied culture conditions. Throughout the cultivation period, the cultures were maintained at a salinity of 25–30 ppt, a 12:12 h light/dark photoperiod, and continuous aeration to ensure adequate mixing and carbon dioxide supply.

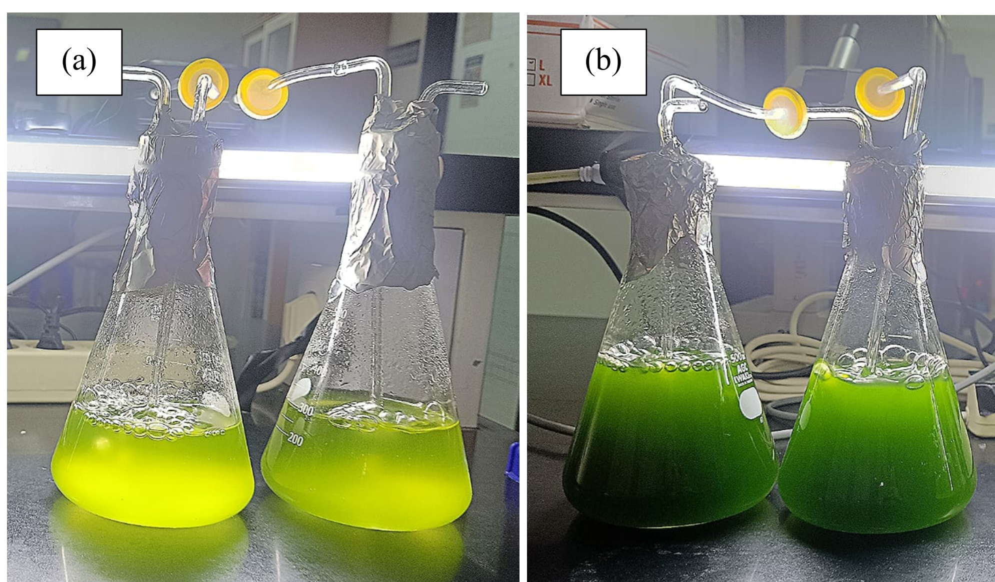

Figure 2. Cultivation Of *N. Oculata* in Controlled Environmental Conditions: (a) Initial Inoculation at Day 0; and (b) Microalgae Density After 12 Days of Cultivation. The Cultures Were Maintained Salinity 25–30 Ppt, 12:12 Light/Dark Photoperiod and Continuous Aeration

To determine the water content and dry biomass content, a 2-gram sample of wet biomass was tested, which was then dried in an oven at 40°C to a constant weight. The drying results showed a dry weight of 0.1762 grams, resulting in a dry biomass content of 8.81% and a water content of 91.19%. These values indicate that the microalgae biomass has a high water content, as is common in marine microalgae. The estimated total dry biomass of the 14.98 g of wet biomass was 1.3196 grams. The overall data can be seen in Table 1. The moisture content analysis was conducted using a single representative wet biomass sample to estimate dry biomass prior to extraction. As no replicated measurements were performed, the calculated dry biomass value should be considered an estimation rather than a precise quantitative determination. Variability related to harvesting efficiency and drying conditions may influence the actual moisture content.

Table 1. Estimation of Moisture Content and Dry Biomass of Microalgae *N. oculata*

Parameter	Value
Total wet biomass	14.98 g
Wet biomass samples for testing	2.00 g
Dried biomass from the sample	0.18 g
Moisture content (based on sample)	91.19%
Dry biomass rate (based on samples)	8.81%
Estimate of total dry biomass	1.32 g

Fatty Acid Profile from GC-MS Analysis

Lipid extraction was performed using chloroform:methanol (2:1 v/v) solvent. The extraction yield was not quantitatively determined in the present study, as the primary objective focused on antibacterial screening and chemical profiling rather than process efficiency evaluation. The extraction resulted in a lipid extract that was then analyzed using Gas Chromatography–Mass Spectrometry (GC-MS) after derivatization to methyl esters. GC-MS results revealed the presence of seven main compounds, as indicated by the peaks in the chromatogram, as shown in Figure 3. The major peak appeared at a retention time below 2 minutes, corresponding to solvent residues or volatile impurities with low molecular weight.

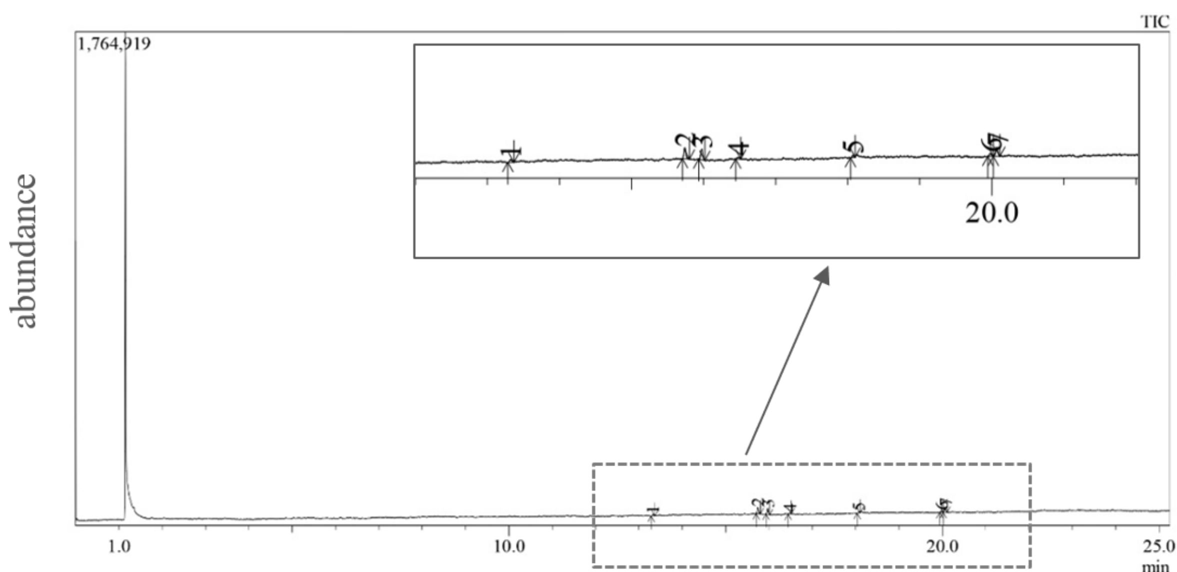

Figure 3. Chromatogram Results Of GC-MS Analysis Of Microalgae *N. Oculata* Lipid Extract After Derivatization into Methyl Ester. The Inset Presents An Enlarged Chromatogram of The Retention Time Region (12.0–22.0 Min), Showing Peaks (17) Detected in This Interval

The seven detected compounds were shown in Table 2. Some of the compounds detected were methyl esters because they were the result of fatty acid methylation prior to GC-MS testing, while Z3,Z6,E8-dodecatriene-1-ol compounds which are unsaturated alcohol categories were detected on the chromatogram likely because they are secondary metabolites

in microalgae or by-products of the extraction process. The composition of this sample shows the most dominant components are methyl palmitoleate (26.47%) and methyl palmitate (25.88%) as shown in **Figure 3**. Both of these compounds are long-chain fatty acid derivatives known to possess good biological activity, including antibacterial properties. Identification was based on Wiley 7 library matching. Only compounds with similarity index ≥80% were considered acceptable. Values represent relative peak area normalization and do not indicate absolute concentration.

Table 2. Compounds identified by GC–MS analysis in the sample, including retention time (RT) and relative percentage

Peak	Retention Time (min)	Similarity Index (%)	Name of Compound	Percentage in sample (%)
1	13.325	86	Methyl heptadecanoate	7.76
2	15.740	94	Methyl palmitoleate	26.47
3	15.972	95	Methyl palmitate	25.88
4	16.485	62	Palmitic acid	5.87
5	18.073	76	Methyl linolelaidate	4.23
6	19.978	83	Methyl arachidonate	7.27
7	20.068	85	Z3,Z6,E8-dodecatrien-1-ol	22.52

Note: Compounds with SI <80% should be considered low-confidence tentative identifications.

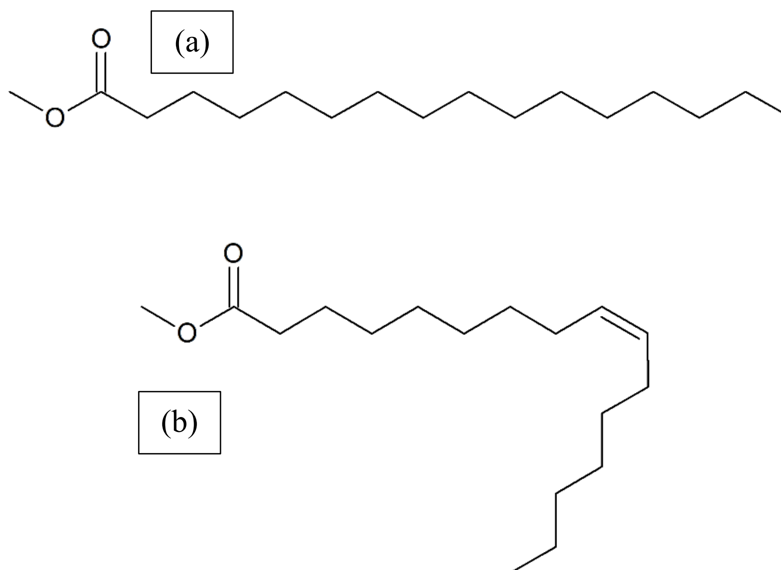

Figure 3. Chemical Structures of Dominant Components: (a) Methyl Palmitate (Peak 3, and (b) Methyl Palmitoleate (Peak 2) Identified By GC–MS Analysis.

Furthermore, the presence of methyl arachidonic acid is of particular interest because arachidonic acid is a well-known precursor of eicosanoids, a group of biologically active lipid mediators involved in inflammatory and antimicrobial responses (Calder, 2020; Zhang et al., 2023). Eicosanoids derived from arachidonic acid include prostaglandins, leukotrienes, and thromboxanes, which play important roles in cellular signaling and host defense mechanisms.

Antibacterial Activity of *N. oculata* Against *L. monocytogenes* dan *S. flexneri*

The antibacterial activity of the chloroform extract of the microalgae *N. oculata* was evaluated against *L.monocytogenes* and *S. flexneri* using the disc diffusion method, as shown in Figure 4. The antibacterial assay was performed in three biological replications using three independent agar plates for each concentration. The extract exhibited measurable inhibitory effects with inhibition zones of 8.40 ± 0.17 mm against *L. monocytogenes* and 8.83 ± 0.29 mm against *S. flexneri* at the highest tested concentration (200 mg/mL). Although the inhibition zones were smaller than those of positive controls (ampicillin and ciprofloxacin, >12 mm). The negative control (10% DMSO) showed no inhibition zone, confirming that the observed antibacterial effect was attributable to the extract rather than the solvent. These findings suggest the presence of bioactive lipid compounds that may contribute to the observed effects. The extract exhibited mild antibacterial activity under agar diffusion conditions. This minimal inhibition suggests that the extract may contain very low concentrations of antibacterial compounds or compounds with poor diffusion in the agar medium.

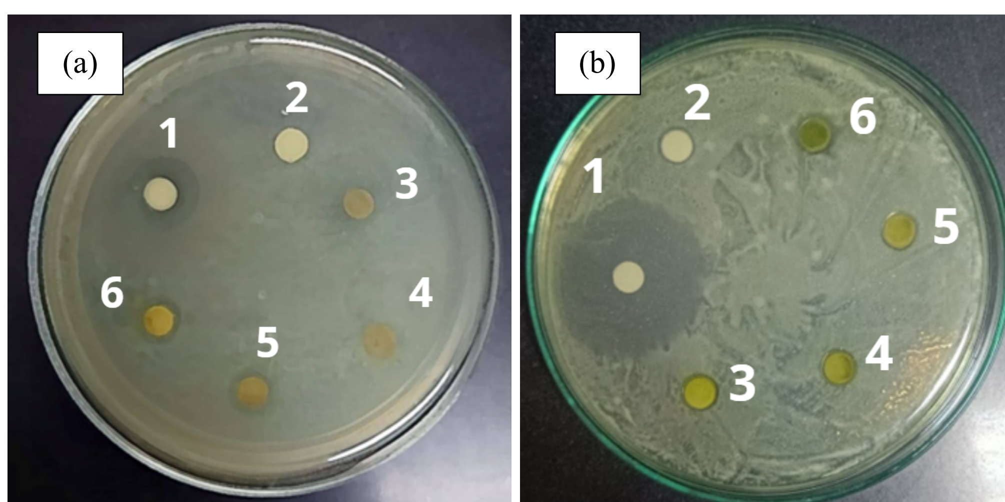

Figure 4. The Zone of Inhibition of *N. Oculata* Extracts Against: (a) *L. Monocytogenes*, and (b) *S.Flexneri* with Positive Control for *L. Monocytogenes* Was Ampicillin (10 µg/mL), Whereas for *S. Flexneri* Was Ciprofloxacin (5 µg/mL) (1), DM as A Blanko (2), Microalgal Extract Concentration Variation Of 25 mg/mL (3),

50 mg/mL (4), 100 mg/mL (5) and 200 mg/mL (6). The Diameter of Disc Paper Was About 6 mm. The Image Represents One Of Each Three Replicates

The GC-MS analysis of the chloroform extract of *N. oculata* showed the presence of several fatty acid derivatives, including methyl palmitate and methyl palmitoleate, which is the methyl ester form to palmitic acid and palmitoleic acid, respectively. The presence of methyl palmitoleate was particularly significant, since its palmitoleic acid has been known to exert antibacterial effects by increasing the fluidity and permeability of bacterial membranes (Han et al., 2024). The membrane disruption can lead to ion leakage and destabilization of membrane homeostasis, which may explain the inhibitory activity against *L. monocytogenes* and *S. Flexneri* as shown in Table 3**.** Therefore, the observed antibacterial activity of *N. oculata* was likely related to its lipid profile, especially the presence of unsaturated fatty acid and their methyl esters.

Table 3. Average Inhibition Zone Diameters (Mm) of *N. Oculata* Extract Against *L. Monocytogenes* and *S. Flexneri* After 24 hours Incubation at 37°C. Zones Were Measured Including The 6 mm Disc. Ampicillin (10 µg/mL) And Ciprofloxacin (5 µg/ml) Served as Positive Controls and 10% DMSO as Negative Control. The Antibacterial Assay Was Performed in Three Biological Replicate

Sample	Concentration	Mean ± SD Inhibition Zone (mm), N=3	
		L. monocytogenes	*S. flexneri*
Control (+) (Ampicillin / Ciprofloxacin)	10 / 5 µg/mL	18.0 ± 0.50[a]	33.33 ± 0.58[a]
Control (-) (DMSO)	-	6.00 ± 0.00[e]	6.00 ± 0.00[d]
Microalgae extract	200 mg/mL	8.40 ± 0.17 [b]	8.83 ± 0.29[b]
	100 mg/mL	7.83 ± 0.29[b,c]	7.00 ± 0.50[c]
	50 mg/mL	7.33 ± 0.29[c,d]	6.90 ± 0.26[c,d]
	25 mg/mL	6.83 ± 0.15[d]	6.60 ± 0.17[c,d]

Note: a,b,c,d and e value was grouped in analytical statistics one-way ANOVA and post-hoc Tukey with $p < 0.001$. Different values showed significant differences statistically.

Table 3 shows that one-way ANOVA revealed a significant difference among treatments against *L. monocytogenes* ($F(5,12) = 759.57$, $p < 0.001$). Tukey's post hoc test indicated that the positive control exhibited significantly larger inhibition zones than all extract concentrations. While the 200 mg/mL concentration demonstrated significantly higher activity compared to 50 and 25 mg/mL, it did not differ significantly from the 100 mg/mL group.

Similarly, a significant difference was observed against *S.flexneri* ($F(5,12) = 2720.93$, $p < 0.001$). Tukey's post hoc analysis showed that the positive control exhibited significantly larger inhibition zones than all extract concentrations ($p < 0.001$). Although the 200 mg/mL concentration demonstrated significantly higher antibacterial activity compared to lower concentrations, no significant differences were observed among 100, 50, and 25 mg/mL groups. Overall, the antibacterial potency of the extracts was markedly lower compared to the commercial antibiotic, which aligns with the low abundance of fatty acid methyl esters detected in the GC-MS profile.

The chloroform–methanol (2:1) extraction system primarily isolates lipophilic compounds such as fatty acids and their methyl esters. Since the disc diffusion assay depends on compound diffusion through an aqueous agar matrix, hydrophobic constituents may diffuse poorly (Balouiri et al., 2016). Therefore, the small inhibition zones observed may partly reflect diffusion limitations rather than the absence of antibacterial activity. The absence of minimum inhibitory concentration (MIC) determination represents a limitation of this study, as disc diffusion results alone do not allow precise quantification of antibacterial potency or detailed dose–response evaluation. Therefore, the present findings should be interpreted as preliminary screening data. Future studies incorporating broth-based MIC assays are necessary to more accurately assess antibacterial efficacy and enable comparison with existing literature.

Furthermore, the synergistic interaction of compounds in the crude extracts may not be sufficient to elicit a strong antibacterial effect until further purification or concentration increases. Overall, although the extracts demonstrated relatively low antibacterial activity, these findings provide preliminary evidence that the chloroform fraction of microalgae may contain bioactive metabolites, possibly at low concentrations. Optimization of extraction parameters, solvent polarity, and concentration procedures could potentially enhance the antibacterial potential of the extract, as extraction efficiency and bioactive compound recovery are strongly influenced by solvent polarity and processing conditions (Kopp & Lauritano, 2025).

CONCLUSION

This study showed that the crude extract of the microalgae *N. oculata* exhibited mild antibacterial activity against *L. monocytogenes* and *S. Flexneri* under disc diffusion condition. GC–MS analysis identified the presence of various bioactive compounds, with the main component being methyl palmitoleate which may contribute to the observed inhibitory effects. Although the antibacterial activity was modest compared to standard antibiotics, the results

suggest that the crude extract of *N. oculata* contains bioactive metabolites worthy of further investigation. Future studies should focus on determining the minimum inhibitory concentration (MIC), improving compound purification, and applying in silico approaches to better understand the potential molecular interactions and mechanisms underlying the antibacterial effects.

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