Antifungal and Antibacterial Activities of *Kenikir* Leaf (*Cosmos caudatus* Kunth) Essential Oil Against Common Foodborne Pathogens

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Abstract. Natural antimicrobial agents are increasingly attracting interest as safer alternatives. *Cosmos caudatus* Kunth., an edible plant native to tropical regions, contains bioactive compounds such as polyphenols, flavonoids, tannins, saponins, alkaloids, and essential oils, all of which have been reported to exhibit potential as natural antimicrobial food preservatives. This study aimed to evaluate the antibacterial and antifungal activities of *C. caudatus* leaf essential oil against various foodborne pathogens. The findings showed that the fungal strains were more susceptible to *C. caudatus* leaf essential oil than the bacterial strains. Moreover, the essential oil exhibited stronger activity against Gram-positive bacteria than Gram-negative bacteria. The minimum inhibitory concentration (MIC) values for *A. niger* FNCC 6114 and *A. flavus* FNCC 6181 were 6.25 and 12.5 $\mu L \cdot mL^{-1}$, respectively, with both fungi showing minimum fungal concentration (MFC) values of 50 $\mu L \cdot mL^{-1}$. For *S. aureus* ATCC 25923, the MIC and minimum bactericidal concentration (MBC) values were 31.25 and 250 $\mu L \cdot mL^{-1}$, respectively. Additionally, *P. aeruginosa* ATCC 27853 and *E. coli* ATCC 25922 exhibited MBC and MIC values of 500 and 62.5 $\mu L \cdot mL^{-1}$, respectively. These results suggest that *C. caudatus* leaf essential oil holds promise as a natural antimicrobial agent.

1 Introduction

Food safety is a critical consideration prior to consuming food and represents a global concern affecting both developed and developing nations. Foodborne illnesses, which account for a significant number of deaths globally, are primarily caused by pathogenic bacteria, toxin-producing molds, enteric viruses, and protozoa. These illnesses can occur when sufficient quantities of pathogenic microbes or their toxins are ingested through raw or cooked food [1].

Among the various microorganisms that contaminate food, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Aspergillus niger*, and *Aspergillus flavus* are among the most prevalent. The enterotoxigenic Gram-positive *Staphylococcus aureus* has been shown to contaminate meat, poultry, dairy products, and baked goods. The Gram-negative bacterium *Escherichia coli* is naturally present in the digestive tracts of birds, warm-blooded animals, and humans. Different strains of *E. coli* are linked to a range of intestinal diseases, such as infant diarrhea (enteropathogenic *E. coli*), gastroenteritis (enterotoxigenic *E. coli*), dysentery (enteroinvasive *E. coli*), and hemorrhagic colitis (enterohemorrhagic *E. coli*). The Gram-negative bacterium *Pseudomonas aeruginosa* is the most common spoilage organism among psychrotrophic bacteria, particularly in high-moisture foods with a natural pH that are stored under aerobic conditions, such as meat, poultry, fish, and dairy products. *Aspergillus niger* is a storage fungus that infects harvested crops and produces mycotoxins, including Ochratoxin A (OTA), which has been associated with carcinogenic, neurotoxic, embryotoxic, immunosuppressive, teratogenic, and nephrotoxic effects. *Aspergillus flavus* is a well-known producer of aflatoxins and is considered a major global food contaminant [1].

Cosmos caudatus Kunth (wild cosmos), popularly known as *kenikir* in Indonesia, is an edible plant found across tropical countries worldwide. *C. caudatus* has been shown to be rich in minerals and vitamins and contains a high total phenolic content. This aromatic plant is considered to offer medicinal benefits due to its anti-inflammatory, anti-diabetic, anti-hypertensive, antioxidant, antibacterial, and antifungal properties. Terpenoids, fatty acids, flavonoids, alkaloids, tannins, and saponins are among the bioactive compounds

identified in the ethanol extract of *C. caudatus* and may contribute to its antibacterial activity [2].

Several studies have shown the antimicrobial properties of *Cosmos caudatus*. Faradila and Rahmawati (2022) [3] reported that essential oil from *C. caudatus* leaves inhibits the growth of the Gram-negative bacterium *Klebsiella pneumoniae*. Additionally, Lutpiatina et al. (2017) and Dwiyanti et al. (2014) [4, 5] demonstrated the antibacterial activity of its ethanolic extract against Gram-positive bacteria, *Staphylococcus aureus* and *Bacillus cereus*. Furthermore, Wasilah *et al.* (2022) [6] demonstrated the antifungal activity of *C. caudatus* essential oil by inhibiting the growth of *Candida albicans*, with a Minimum Fungal Concentration (MFC) of 2 % ($v \cdot v^{-1}$). However, studies specifically investigating the essential oil of *C. caudatus* against a broader range of foodborne pathogens, particularly *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Aspergillus niger*, and *Aspergillus flavus* remain scarce. Therefore, this study aims to evaluate the antibacterial and antifungal activities of *C. caudatus* essential oil against selected foodborne pathogens.

2 Material and methods

2.1 Essential oil preparation

The essential oil of *Cosmos caudatus* leaves was obtained from the Medical Laboratory Technology at Poltekkes, Ministry of Health, Yogyakarta, Indonesia. Isolation was performed using the water vapor distillation method, in which the volatile oil content of *C. caudatus* was separated based on the difference in the partial pressure between the volatile compound and the water vapor phase [6]. The essential oil was then dissolved in pure dimethyl sulfoxide to prepare stock solutions with concentrations of 1000 $\mu L \cdot mL^{-1}$, for bacterial MIC testing and 200 $\mu L \cdot mL^{-1}$, for fungal MIC testing.

2.2 Bacterial culture

The bacterial cultures used in this investigation included the Gram-positive bacterium *Staphylococcus aureus* ATCC 25923 and the Gram-negative bacteria *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853. To revive the parent cultures of *P. aeruginosa* ATCC 27853, *S. aureus* ATCC 25923, and *E. coli* ATCC 25922, 100 µL of each culture was transferred into Mueller-Hinton Broth (MHB) and incubated for 18 hours at 37 °C. Subsequently, the cultures were inoculated onto Mueller-Hinton Agar (MHA) and incubated for an additional 18 hours at 37 °C. Bacterial colonies from MHA were collected using a sterile loop and suspended in 2 mL of 0.85 % NaCl solution to prepare the bacterial suspension for the antibacterial test. The suspension was standardized to the 0.5 McFarland standard, equivalent to 1.5×10^8 $CFU \cdot mL^{-1}$.

2.3 Antibacterial susceptibility assay

Using the well diffusion method described by Magaldi *et al.* (2004) [7], the antibacterial activity of *C. caudatus* leaf essential oil against *E. coli*, *P. aeruginosa*, and *S. aureus* was evaluated. One milliliter of the bacterial suspension, standardized using the McFarland standard, was added to 20–25 mL of sterile Mueller-Hinton Agar (MHA) medium (OXOID, UK), which had been preheated to 55 °C. The agar was then poured into a Petri dish and allowed to solidify. Once solidified, wells with a diameter of 6 mm were created using a cork borer. Into each well, 25, 50, and 75 µL of *C. caudatus* essential oil were added. A 5 µg ciprofloxacin paper disc was placed on the agar medium as a control. All samples were incubated for 18 hours at 37 °C

2.4 Determination of the Minimum Inhibitory Concentrations (MIC) and Minimum Bactericidal Concentrations (MBC)

The microdilution method, following the guidelines of the National Committee for Clinical Laboratory Standards' (2001) [8], was employed to determine the Minimum Inhibitory Concentration (MIC). For the MIC assessment, a bacterial culture solution standardized to the 0.5 McFarland standard, 100 µL of Mueller-Hinton Broth (MHB), and *C. caudatus* leaf essential oil were added to each well of a 96-well microplate. The essential oil was tested at varying concentrations, beginning with 1000 $\mu L \cdot mL^{-1}$, and serially diluted by half in each successive well, reaching 1.9 $\mu L \cdot mL^{-1}$, in the 11^{th} column. The microplate was then incubated for 18 hours at 37 °C. The lowest concentration at which no visible microbial growth was observed is recognized as the MIC value. Each sample was tested in triplicate.

Following the MIC test, bacteria from the microplate were inoculated onto Mueller-Hinton Agar (MHA) medium to conduct the Minimum Bactericidal Concentration (MBC) test. The plates were incubated for 18 hours at 37 °C. Every sample was tested in triplicate.

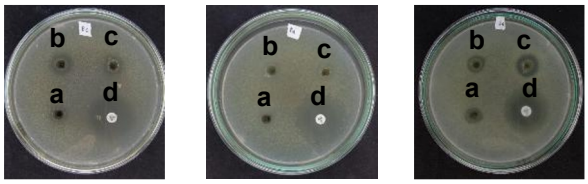
2.5 Fungal culture

The fungal cultures used in this study were *Aspergillus niger* FMCC 6114 and *Aspergillus flavus* FNCC 6181. The parent cultures of *A. niger* FNCC 6114 and *A. flavus* FNCC 6181 were rejuvenated by collecting spores from each fungal culture with a sterile loop and dissolving them in 2 mL of 0.05 % tween 80 solution. The fungal suspension was then collected using a sterile cotton swab and inoculated onto Potato Dextrose Agar medium using the streak plate method. The cultures were incubated at 30 °C for 48 hours. After incubation, 10–20 µL of the *A. niger* and *A. flavus* culture suspension was placed onto a haemocytometer to count the number of cells. The observations were performed using a microscope (Olympus, Japan) at 100× magnification.

Table 1. Antibacterial susceptibility assay

Bacterial strains	Inhibition zone diameter (mm)			
	C. caudatus leaf essential oil (µL)			Positive control
	25	50	75	Ciprofloxacin 5 µg
Gram-negative				
E. coli ATCC 25922	11.57 ± 1.305[a]	12.88 ± 1.125[a]	14.77 ± 1.504[a]	32.07 ± 3.742[b]
P. aeruginosa ATCC 27853	11.33 ± 1.53[a]	12.43 ± 0.93[a]	14.067 ± 1.604[a]	20.550 ± 3.309[b]
Gram-positive				
S. aureus ATCC 25923	11.60 ± 0.85[a]	12.55 ± 0.28[a]	14.78 ± 0.21[b]	22.67 ± 3.06[c]

The diameter of the inhibition zone (mm) is presented as the mean (± SD), including the 6-mm paper disk, with three replications.
[a,b,c] Values within each fungal strain that share the same superscript across volumes are not significantly different ($P < 0.05$).



***Escherichia coli* ATCC 25922** ***Pseudomonas aeruginosa* ATCC 27853** ***Staphylococcus aureus* ATCC 25923**

Fig.1 Inhibitory activity of *C. caudatus* leaf essential oil against *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, and *S. aureus* ATCC 25923. a 25 µL; b 50 µL; c 75 µL; d positive control (ciprofloxacin 5 µg).

2.6 Antifungal susceptibility assay

The antifungal assay for *Cosmos caudatus* leaf essential oil was assessed using the Kirby-Bauer diffusion method, following the guidelines of the Clinical and Laboratory Standards Institute (2015) [9]. This method is designed to evaluate the sensitivity or resistance of microorganisms to antimicrobial agents. A culture suspension of *A. niger* FNCC 6114 and *A. flavus* FNCC 6181, standardized to 10^6 CFU·mL^{-1}, was collected using a sterile cotton bud and inoculated onto Potato Dextrose Agar (PDA) medium using the streak plate method. Subsequently, 100 % *C. caudatus* leaf essential oil was applied to 6 mm diameter blank paper discs (Oxoid, UK) in volumes of 5, 10, and 15 µL. These samples were compared to a positive control consisting of 33 µg Nystatin paper disc (Oxoid, UK). The prepared paper discs were then placed on the PDA medium previously inoculated with the fungal cultures. The plates were incubated at 30 °C for 48 hours, after which the inhibition zones around the paper discs were measured to assess antifungal activity.

2.7 Determination of the Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC)

Following the National Committee for Clinical Laboratory Standards (2001) [8] guidelines, the microdilution method was employed to determine the MIC for fungi. The assay was conducted in a 96-well microplate containing 100 µL of RPMI medium (Advanced Pharmaceutical Sciences Learning Center UGM), 10 µL of *A. niger* and *A. flavus* culture suspension at 10^6 CFU·mL^{-1}, and varying concentration of *C. caudatus* leaf essential oil. The essential oil was tested at an initial concentration of 200 µL·mL^{-1} and serially diluted by half in each subsequent well, down to 0.39 µL·mL^{-1}, in the eleventh column. The 12th well served as a negative control, containing RPMI medium supplemented with a fungal suspension. The microplate was incubated for 48 hours at 30 °C. Fungal growth was indicated by turbidity in the well, whereas a clear well signified fungal inhibition. All samples were tested in triplicate.

Following the MIC assay, fungi from the microplate were inoculated onto Potato Dextrose Agar (PDA) medium to determine the Minimum Fungicidal Concentration (MFC). The inoculated plates were then incubated for 48 hours at 30 °C. The MFC value was defined as the lowest concentration of essential oil at which no fungal growth appeared on the PDA medium. Every sample was analyzed in triplicate.

3 Results

3.1 Antibacterial activity of *C. caudatus* leaf essential oil

Following the method of Magaldi *et al.* (2004) [8], the well diffusion technique was used to assess the antibacterial susceptibility of *C. caudatus* leaf essential oil against Gram-positive (*S. aureus* ATCC 25923) and Gram-negative (*E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853) bacteria. The mean diameters of the inhibition zones (DIZs) produced by *C. caudatus* essential oil are shown in Table 1, while representative clear zones on Mueller-Hinton Agar (MHA) are depicted in Fig. 1.

Overall, increasing volumes of essential oil resulted in larger DIZs across all bacterial strains; however, these differences were not statistically significant. Among the tested bacteria, the Gram-positive *S. aureus* ATCC 25923 displayed the highest sensitivity to the essential oil. Nevertheless, the positive control, consisting of 5 µg of ciprofloxacin, exhibited significantly greater inhibitory effects against all bacterial strains compared to *C. caudatus* essential oil.

Table 2 displays the results of the MIC and MBC tests conducted on *C. caudatus* leaf essential oil against *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, and *S. aureus* ATCC 25923. The essential oil showed higher MIC and MBC values against the Gram-negative bacteria, with MIC and MBC values of 62.5 ± 0 µL·mL^{-}

[1], and 500 ± 0 µL/mL, respectively, for *P. aeruginosa* ATCC 27853 and *E. coli* ATCC 25922. In contrast, the Gram-positive *S. aureus* ATCC 25923 exhibited lower MIC and MBC values, measured at 31.25 ± 0 µL·mL^{-1}, and 250 ± 0 µL·mL^{-1}, respectively.

Table 2. MIC and MBC values of *C. caudatus* leaf essential oil against bacterial strains

Bacterial strains	*C. caudatus* leaf essential oil	
	MIC (µL/mL)	MBC (µL/mL)
Gram-negative		
E. coli ATCC 25922	62.5 ± 0	500 ± 0
P. aeruginosa ATCC 27853	62.5 ± 0	500 ± 0
Gram-positive		
S. aureus ATCC 25923	31.25 ± 0	250 ± 0

3.2 Antifungal activity of *C. caudatus* leaf essential oil

Table 3. Antifungal susceptibility assay

Fungal strains	Inhibition zone diameter (mm)			
	C. caudatus leaf essential oil (µL)			Positive control
	5	10	15	Nystatin 100 U (0.033 mg)
A. niger FNCC 6114	9.23 ± 1.76[a]	11.57 ± 2.32[a]	13.77 ± 1.81[a]	20.07 ± 2.30[b]
A. flavus FNCC 6181	8.37 ± 2.97[a]	11.13 ± 2.15[a]	12.68 ± 1.14[a]	13.38 ± 0.83[b]

The diameter of the inhibition zone (mm) is presented as the mean (± SD), including the 6-mm paper disk, with three replications.

[a,b,c] Values within each fungal strain that share the same superscript across volumes are not significantly different ($P < 0.05$).

***Aspergillus niger* FNCC 6114** ***Aspergillus flavus* FNCC 6181**

Fig. 2 Inhibitory activity of *C. caudatus* leaf essential oil against *A. niger* FNCC 6114 and *A. flavus* FNCC 6181. a 5 µL; b 10 µL; c 15 µL; d positive control [nystatin 100 U (0.033 mg)]

Table 4. MIC and MFC values of *C. caudatus* leaf essential oil against fungal strains

Fungal strains	*C. caudatus* leaf essential oil	
	MIC (µL·mL^{-1})	MFC (µL·mL^{-1})
A. niger FNCC 6114	6.25 ± 0	50 ± 0
A. flavus FNCC 6181	12.5 ± 0	50 ± 0

The antifungal activity of *C. caudatus* leaf essential oil against *A. niger* FNCC 6114 and *A. flavus* FNCC 6181 was evaluated using the Kirby-Bauer disc diffusion method. The mean diameters of the inhibition zones (DIZs) observed following exposure to the essential oil are reported in Table 3, and the clear zones formed on the fungal cultures are illustrated in Fig. 2. Generally, large DIZs were observed with increasing volumes of *C. caudatus* essential oil applied to the paper discs. Statistical analysis indicated a modest but significant difference in DIZs with each incremental 5 µL increase in the applied volume. *A. niger* FNCC 6114 demonstrated higher sensitivity to the antifungal agents. The positive control, consisting of 0.033 mg of Nystatin, produced the largest DIZs compared to *C. caudatus* essential oil.

The MIC and MFC test results for *C. caudatus* essential oil against *A. niger* FNCC 6114 and *A. flavus* FNCC 6181 are shown in Table 4. Based on visual observation of turbidity, the MIC represents the lowest concentration of *C. caudatus* essential oil that inhibited fungal growth, while the MFC denotes the minimum concentration required to eliminate fungal cells. The MIC for *A. niger* FNCC 6114 was determined to be 6.25 ± 0 µL·mL^{-1}, whereas *A. flavus* FNCC 6181 required twice that concentration at 12.5 ± 0 µL·mL^{-1}. Both strains, however, exhibited identical MFC values of 50 ± 0 µL·mL^{-1}.

4 Discussion

This study confirms the antimicrobial properties of *C. caudatus* leaf essential oil against foodborne pathogenic microorganisms. The antifungal activity was tested against *Aspergillus niger* FMCC 6114 and *Aspergillus flavus* FNCC 6181, while the antibacterial activity was evaluated against *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Staphylococcus aureus* ATCC 25923.

No significant differences were observed in the antibacterial activity of *C. caudatus* leaf essential oil across the tested bacterial strains. *S. aureus* ATCC 25923 exhibited the largest inhibitory zone, followed by *P. aeruginosa* ATCC 27853 and *E. coli* ATCC 25922. However, the positive control (5 µg ciprofloxacin) produced a larger inhibition zone compared with the *C. caudatus* essential oil. This is attributed to the fact that ciprofloxacin is a single antibacterial chemical compound, whereas essential oils comprise a complex mixture of various constituents. Additionally, the semipolar nature of essential oils contrasts with the polar nature of the medium, significantly affecting their diffusion capacity in the media and resulting in smaller DIZs.

Although the difference was not statistically significant, this study revealed that the Gram-positive bacterium *S. aureus* ATCC 25923 exhibited a larger inhibitory zone than the Gram-negative bacteria *P. aeruginosa* ATCC 27853 and *E. coli* ATCC 25922. The simpler structure of the Gram-positive bacterial cell wall facilitates the entry of antibacterial compounds, whereas

the thicker and more complex cell wall of Gram-negative bacteria acts as a barrier, limiting compound penetration [10].

The inhibitory mechanism against bacterial cells is likely attributed to the penetration of antibacterial compounds present in the *C. caudatus* leaf essential oil. These compounds penetrate and damage the bacterial cell walls before interacting with intracellular components and disrupting cellular metabolism. This process impairs the formation of cell membrane structures, alters cell permeability, and interferes with other metabolic activities, ultimately leading to cell death [10].

P. aeruginosa ATCC 27853 produced a smaller inhibition zone compared to *E. coli* ATCC 25922. According to Skočibušić et al. (2016) [11], *P. aeruginosa* is highly resistant to various antimicrobial compounds and antibiotics. However, both *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 2785 exhibited identical MIC and MBC values of 62.5 and 500 $\mu L \cdot mL^{-1}$, respectively. In contrast, the Gram-positive *S. aureus* ATCC 25923 demonstrated greater susceptibility, as reflected by lower MIC and MBC values of 31.25 and 250 $\mu L \cdot mL^{-1}$, respectively. This result aligns with the findings of Fournomiti et al. (2015) [12], who reported that Gram-negative bacteria exhibit greater resistance to thyme essential oil compared to Gram-positive bacteria.

As previously mentioned, the primary reason for differences in resistance to antibacterial agents between Gram-positive and Gram-negative bacteria lies in their cell wall structures. The cell membrane of gram-negative bacteria consists of a thin peptidoglycan layer, an outer membrane, and an external layer of lipopolysaccharides (LPS). In contrast, gram-positive bacteria are more susceptible to antibacterial agents because they possess a thicker peptidoglycan layer without an outer membrane or LPS. LPS is a complex molecular structure composed of lipids and polysaccharides that block the entry of macromolecular substances, including antibacterial compounds, into the cells. The LPS and outer membrane in Gram-negative bacteria are covered by a hydrophilic outer layer, which prevents the penetration of hydrophobic materials such as *C. caudatus* leaf essential oil [10].

The inhibition of bacterial growth observed in this research was caused by the antimicrobial compounds present in the *C. caudatus* leaf essential oil, primarily monoterpenes and sesquiterpenes. *C. caudatus* leaves contain various bioactive compounds with antibacterial properties, including flavonoids, phenols, tannins, and saponins [4]. Wasilah et al. (2022) [6] previously identified the chemical constituents of *C. caudatus* leaf essential oil using gas chromatography. These compounds were predominantly from the monoterpene and sesquiterpene groups, with 1,3,6-Octatriene, 3,7-dimethyl-, (E)- (CAS).BETA (40.28 %) being the most abundant monoterpene and germacrene d (14.77 %) being the most abundant sesquiterpene. Terpenes are hydrocarbon compounds with the chemical formula $(C5H8)n$ known for their antimicrobial properties [13].

The antifungal susceptibility assay of *C. caudatus* leaf essential oil against *A. niger* FNCC 6114 and *A. flavus* FNCC 6181 demonstrated inhibitory effects. Larger volumes of essential oil resulted in wider DIZs for both strains, with *A. niger* FNCC 6114 showing greater sensitivity, as evidenced by the larger DIZ. The positive control, 0.033 mg of nystatin (100 U), exhibited superior inhibition, producing a wider DIZ. This finding is further supported by the MIC test, where *A. niger* FNCC 6114 had a lower MIC value compared to *A. flavus* FNCC 6181. However, the MFC values for both strains were identical, at 50 ± 0 $\mu L \cdot mL^{-1}$.

This study indicates that *A. niger* FNCC 6114 displayed higher sensitivity to antifungal compounds than *A. flavus* FNCC 6181. This may be attributed to differences in the components and structure of the fungal cell membranes. The monoterpene and sesquiterpene concentrations in *C. caudatus* leaf essential oil may also contribute to its antifungal properties. According to Contreras Martínez et al. (2022) [13], isoespintanol, a monoterpene, efficiently inhibits the growth of *Candida tropicalis*.

Terpenes have reported to have the ability to interfere with fungal cell wall structure. This compound can rupture the plasma membrane through permeabilization, wherein essential oils penetrate and destroy fungal cell membranes, thereby increasing their permeability. Furthermore, by binding to the active sites of enzymes, essential oils can interact with membrane proteins, disrupt ion transport mechanisms, and reduce enzyme activity [13].

The use of terpenes as antimicrobial agents holds significant promise. Terpenes are widely applied across various industries, including cosmetics, food, and healthcare products [13]. As natural food preservatives, essential oils containing terpenes are regarded as eco-friendly alternatives. They have been used to preserve a variety of food products, including meat, bread, grains, fruits, vegetables, milk, and dairy products.

The US Food and Drug Administration has approved several essential oils and their components as Generally Recognized as Safe (GRAS) for use as food preservatives and flavorings [13]. Although *C. caudatus* leaf essential oil is not yet included on this list, studies have demonstrated that its consumption is safe and well-tolerated in humans. Owing to its antimicrobial properties, this essential oil shows strong potential for incorporation into bioactive packaging, providing both safe and environmentally friendly alternatives to conventional packaging. By combining it with natural polymer, for example porang glucomannan, antimicrobial packaging can feasibly be developed [14]. Nevertheless, further research is required to evaluate its practical applications in food packaging and products, particularly regarding to its stability and consumer acceptance.

5 Conclusion

This study demonstrated that *Cosmos caudatus* leaf essential oil possesses antimicrobial activity against both Gram-negative (*Escherichia coli* ATCC 25922,

Pseudomonas aeruginosa ATCC 27853) and Gram-positive (*Staphylococcus aureus* ATCC 25923) bacteria, as well as fungi (*Aspergillus niger* FNCC 6114 and *Aspergillus flavus* FNCC 6181). *S. aureus* showed the highest sensitivity, with MIC and MBC values of 31.25 $\mu L \cdot mL^{-1}$ and 250 $\mu L \cdot mL^{-1}$, respectively, likely due to its simpler cell wall structure. Among the fungi, *A. niger* was more susceptible than *A. flavus*, potentially due to higher ergosterol content in its membrane. Owing to its natural origin and antimicrobial activity, *C. caudatus* essential oil shows potential as a natural food preservative. Nevertheless, further research is needed to assess its stability, safety, and effectiveness in food applications.

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