

Inhibitory effect of yellow cempaka leaf (*Michelia champaca* Linn.) against *Staphylococcus aureus* and *Escherichia coli*

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ABSTRACT

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Several studies have been conducted to develop herbal plants as alternatives to address infections. Yellow cempaka (*Michelia champaca* Linn.) is a natural ingredient that can serve as an alternative treatment. This study used a post-test-only control group to examine the antibacterial activity of the disc diffusion method against *Staphylococcus aureus* and *Escherichia coli*. This study included four treatment groups (10, 20, 40, and 80% ethanol extracts) and two control groups (10 µg amoxicillin for *S. aureus*, 5 µg ciprofloxacin for *E. coli*, and 96% ethanol as a negative control). The results showed that the ethanol extract of *M. champaca* leaves exhibited antibacterial activity against *S. aureus* at all concentrations tested, with mean inhibition zone diameters ranging from 13.70 mm to 23.11 mm. Statistical analysis showed that the 20% extract concentration had antibacterial activity comparable to the positive control ($p > 0.05$), whereas the 40% and 80% concentrations showed significantly different inhibition zone diameters from the positive control ($p < 0.05$). In contrast, the ethanol extract of *M. champaca* leaves showed weak antibacterial activity against *E. coli* at all concentrations tested, with mean inhibition zone diameters ranging from 6.38 mm to 7.63 mm, which were significantly different from the positive control ($p < 0.05$). These findings revealed that the ethanol extract of *M. champaca* leaves has potential as an alternative treatment for *S. aureus* infections. However, further research is needed to isolate and identify specific active compounds and to evaluate their efficacy and safety.

ABSTRAK

Cempaka kuning (*Michelia champaca* Linn.) merupakan salah satu tanaman herbal yang dapat digunakan sebagai alternatif pengobatan infeksi. Penelitian ini bertujuan untuk menganalisis aktivitas antibakteri dari ekstrak etanol daun *M. champaca*. Penelitian ini menggunakan kelompok kontrol *post-test-only* untuk menguji aktivitas antibakteri terhadap *S. aureus* dan *E. coli* dengan menggunakan metode difusi cakram. Penelitian ini melibatkan empat kelompok perlakuan (ekstrak etanol 10, 20, 40, dan 80%) dan dua kelompok kontrol (10 µg amoksisilin untuk *S. aureus*, 5 µg siprofloksasin untuk *E. coli*, dan etanol 96% sebagai kontrol negatif). Hasil penelitian menunjukkan bahwa ekstrak etanol daun *M. champaca* menunjukkan aktivitas antibakteri terhadap *S. aureus* pada semua konsentrasi yang diujikan, dengan diameter zona hambat rata-rata berkisar antara 13,70 mm hingga 23,11 mm. Analisis statistik menunjukkan bahwa konsentrasi ekstrak 20% memiliki aktivitas antibakteri yang sebanding dengan kontrol positif ($p > 0,05$), sedangkan konsentrasi 40% dan 80% menunjukkan diameter zona inhibisi yang berbeda secara signifikan dari kontrol positif ($p < 0,05$). Sebaliknya, ekstrak etanol daun *M. champaca* menunjukkan aktivitas antibakteri yang lemah terhadap *E. coli* pada semua konsentrasi yang diujikan, dengan diameter zona hambat rata-rata 6,38 mm hingga 7,63 mm, yang berbeda secara signifikan dengan kontrol positif ($p < 0,05$). Temuan ini menunjukkan bahwa ekstrak etanol daun *M. champaca* memiliki potensi sebagai pengobatan alternatif untuk infeksi yang disebabkan oleh *S. aureus*. Namun, penelitian lebih lanjut diperlukan untuk mengisolasi dan mengidentifikasi senyawa aktif spesifik dan untuk mengevaluasi efikasi dan keamanannya.

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INTRODUCTION

Infectious diseases are among the most prevalent causes of morbidity in tropical climates, such as Indonesia. Morbidity and mortality rates associated with infectious diseases are significantly elevated in various countries.^{1,2} The World Health Organization (WHO) states that approximately 20% of deaths in children aged <5 years in Indonesia are caused by infectious diseases.³ In addition, data from 2020 show that pneumonia and diarrhea are still the main causes of death in children aged 29 days to 11 months, at 14.5% (pneumonia) and 9.8% (diarrhea), respectively.⁴

Infectious diseases result from interactions among the agent, host, and environment. Microorganisms that cause bacterial infectious diseases include *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*).⁵ *S. aureus* is a commensal microorganism that is present in the majority of healthy individuals. *S. aureus* predominantly colonizes the skin and mucous membranes, particularly in the nasal region. This bacterium typically does not cause infection; however, if it enters the bloodstream or internal tissues, it can cause severe invasive infections.^{5,6} *E. coli* are gram-negative bacteria that typically reside in the human gastrointestinal tract. However, when subjected to various predisposing factors, *E. coli* may become pathogenic and potentially cause disease.⁷

The high prevalence of infectious diseases requires appropriate treatment, including the administration of antimicrobials such as antibacterials, antibiotics, antifungals, antivirals, and antiprotozoals. Antibiotic therapy is used to treat diseases caused by bacterial infection. Based on a study of antibiotic quality in various hospitals, approximately 30-80% of antibiotic use was not based on indications. The increasing rate of improper antibiotic use can lead to the development of antibiotic resistance.⁴

The WHO in Antimicrobial Resistance: Global Report on Surveillance reported that Southeast Asia has the highest number of antibiotic-resistant cases worldwide, especially infections caused by methicillin-resistant *S. aureus*.⁸ Antimicrobial Resistance in Indonesia (AMRIN-Study) 2000-2005 reported that 43% of *E. coli* strains were resistant to several types of antibiotics, including ampicillin (34%), cotrimoxazole (29%), and chloramphenicol (25%).⁹ These conditions provide an opportunity for the use of natural substances as alternative treatments for infectious diseases.¹⁰

Yellow cempaka (*Michelia champaca* Linn.) is native to the Indo-Malayan region which includes South Asia, Indochina, and southern China.¹¹ Traditionally, *M. champaca* has been used by the local community to treat diarrhea, cough, bronchitis, dyspepsia, fever, rheumatic disease, abscess, dysmenorrhea, and inflammation.¹² The ethanol extract of *M. champaca* contains various compounds, including alkaloids, flavonoids, tannins, polyphenols, and essential oils, which have various biological activities, including antioxidant, antidepressant, and antimicrobial activities.¹³ Previous studies have reported antibacterial activity of *M. champaca* using different plant parts, including the bark and flowers, but the reported potency varies depending on the plant part, extraction method, and test organism.

Although previous studies have reported the antibacterial activity of *Michelia champaca* using different plant parts, evidence regarding the antibacterial potential of its ethanolic leaf extract remains limited. In particular, data on concentration-dependent inhibitory effects and comparative activity against Gram-positive and Gram-negative bacteria are still underexplored. Therefore, this study aimed to evaluate the antibacterial activity of ethanolic leaf extract of *M. champaca* against *S. aureus* and *E. coli* using a disc diffusion assay. This study is expected to provide preliminary evidence regarding the

potential of *M. champaca* leaf extract as a natural antibacterial agent.

METHODS

Plant materials

M. champaca leaves were collected from the Darussalam Sub-District Office, Banda Aceh, at coordinates 5.5884° N and 95.3973° E. *M. champaca* leaves had morphological characteristics of ovoid leaves that were up to 30.5 cm long and 10.2 cm wide, narrowing to a smooth point at the apex with a stalk length of 1-3 cm.

Preparation of ethanol extract of *M. champaca* leaves

The thick *M. champaca* leaf extract used in this study was prepared as described by Waisul et al (2023).¹⁴ For antibacterial testing, the thick crude extract was treated as the 100% stock extract. Working concentrations of 10%, 20%, 40%, and 80% were prepared by dilution with ethanol using the formula $N1 \times V1 = N2 \times V2$, where N1 is the concentration of the stock extract, V1 is the volume of stock extract required, N2 is the target concentration, and V2 is the final volume.

Preparation of growth media

Mueller Hinton Agar powder (60 g) was added to an Erlenmeyer flask and dissolved in 1000 mL of distilled water. The resulting suspension was heated to boiling. The flask was then autoclaved at 121°C for 15 min. The flasks were then transferred to an incubator at 37°C for 24 h and stored in a refrigerator.¹⁵ Nutrient agar media were prepared as culture media for bacterial isolates. The agar medium was formulated by combining 2 g of nutrient broth (1%) and 4 g of agar (2%) with 200 mL of water in an Erlenmeyer flask. After thorough homogenization of the ingredients,

the Erlenmeyer flask was sealed using cotton and aluminum foil and sterilized in an autoclave at 121°C for 15 min. The sterilized media were subsequently aseptically transferred to Petri dishes to ensure complete coverage with agar media. The agar medium was allowed to solidify at room temperature. Once solidification was complete, the medium was incubated at room temperature to determine the presence or absence of contaminants.¹⁶

Preparation of bacterial suspensions

S. aureus and *E. coli* were cultured on nutrient agar (NA) media for 18-24 hours at 37°C in an incubator. Bacterial suspensions were prepared by aseptically dissolving the bacterial cultures in sodium chloride (NaCl) solution. Subsequently, the absorbance was measured using a spectrophotometer at 625 nm, with an absorbance range of 0.08–0.13.

Identification of bacteria

A series of tests was conducted to identify the bacteria used, including Gram staining, the coagulase test, and the IMViC test.¹⁷ The isolation of gram-positive *Staphylococcus aureus* revealed the morphology of cocci-shaped bacteria with a purple coloration. Conversely, Gram-negative bacteria, such as *E. coli*, exhibited bacillus-shaped bacterial morphology with pink coloration. Subsequently, a catalase test was performed to differentiate the genus *Staphylococcus sp.* from *Streptococcus sp.*¹⁸ The coagulase test was performed to detect the formation of the coagulase enzyme bound to the bacterial cell wall, thereby confirming whether the *Staphylococcus* species examined was *Staphylococcus aureus* or another species. Finally, the IMViC test was implemented to identify bacteria through four distinct tests: the indole, methyl red, Voges-Proskauer, and citrate tests.^{19,20}

Disc diffusion method

Sterile cotton swabs were immersed in the bacterial suspension (0.1 mL each of *S. aureus* and *E. coli*) and subsequently homogenized in separate petri dishes. A sterile cotton swab containing the bacterial suspension was inoculated onto the surface of Mueller-Hinton agar (MHA) to ensure uniform distribution. Four different concentrations (10, 20, 40, and 80%) of the plant extracts were applied in four replicates. Plant extracts (25 µL) were applied in quadruplicate on paper discs, which were then aseptically placed on Mueller-Hinton agar plates. Commercially available standard amoxicillin antibiotic discs (10 µg) were used as positive controls for *S. aureus*, standard ciprofloxacin antibiotic discs (5 µg) were used as positive controls for *E. coli*, and ethanol (96%) was used as the negative control. Each paper disc was positioned at a predetermined distance to prevent the overlap of inhibition zones. The Petri dishes were incubated at 37°C for 18-24 hours without inversion. The diameters of the inhibition zones surrounding the discs, where bacterial growth was absent (in millimeters), were measured with a caliper.

Data analysis

The SPSS software was used to analyze quadruplicate data, and the results were expressed as the mean ± standard deviation. Data were analyzed using one-way ANOVA followed by the Games–Howell post hoc test because the assumption of homogeneity of variances was not met. Statistical significance was set at $p < 0.05$.

RESULT AND DISCUSSION

Bacterial identification was performed to verify and confirm the validity of *S. aureus* and *E. coli* strains. The results of the bacterial identification in this study confirmed that the examined bacteria were indeed *S. aureus* and *E. coli*, as evidenced by positive Gram staining, positive catalase test, and positive coagulase test (TABLE 1).

The zone of inhibition of the ethanol extract of *M. champaca* leaves on *S. aureus* was determined using the disc diffusion method on MHA media previously inoculated with bacterial strains. The results are presented in TABLE 2 and FIGURE 1.

TABLE 1. Identification of *S. Aureus* and *E. Coli*

Test for bacterial identification	Media	Results
<i>S. aureus</i>		
Gram staining	Not applicable (direct purple-colored cocci-shaped bacteria from culture)	
Catalase test	Gram staining	(+)
Coagulase test		(+)
<i>E. coli</i>		
Gram staining	Not applicable (direct Gram staining from culture)	
Indol test	Sulfide indol motility	(+)
Methyl red test	Methyl red	(+)
Voges Proskauer test	Voges Proskauer	(-)
Citrate test	Simmons citrate agar	(-)
TSIA test	Triple sugar iron agar	Color change on the <i>slant</i> and <i>butt</i> from orange to yellow. There was a gas production at the base of the media and no H ₂ S was observed.
Urea test	Urea broth	(-)

TABLE 2. The inhibition zone of the ethanol extract of *M. champaca* leaves on *S. aureus*

Group	Mean±SD	Inhibitory Response*
Control negative	6±0.00	Weak
Amoxicillin 10 µg	19.20±0.91 ^b	Active
Extract 10%	13.70±1.00 ^{a,b}	Active
Extract 20%	15.83±2.57 ^b	Active
Extract 40%	23.06±0.82 ^{a,b}	Very Active
Extract 80%	23.11±0.70 ^{a,b}	Very Active

*Morales criteria weak 6-10 mm; active 11-20 mm; very active 21-30 mm.

^ap<0.05 VS amoksisilin; ^bp<0.05 vs control negative

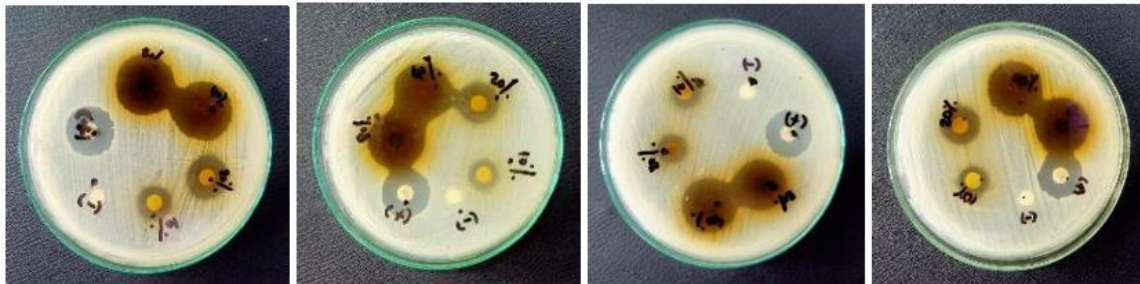


FIGURE 1. Inhibition zones formed by the antimicrobial activities of the ethanol extracts of *M. champaca* against *S. aureus*.

The ethanolic extract of *M. champaca* leaves exhibited antimicrobial properties against *S. aureus*, with inhibition zones observed at a minimum extract concentration of 10%. In this study, amoxicillin (10 µg) was used as a positive control, which generated an average inhibition zone diameter of 19.20 mm. Notably, the mean inhibition zones produced by 40% and 80% extract concentrations exceeded those of the positive control (23.06 mm and 23.11 mm, respectively). Evaluation of inhibition responses using the Morales criteria revealed that 10% and 20% extract concentrations had strong inhibitory effects, whereas 40% and 80% concentrations displayed very strong inhibitory effects, indicating a positive correlation between extract concentration and the size of the inhibition zone.

ANOVA revealed a statistically significant difference in the inhibition zone diameter among the treatment groups ($p = 0.000$). The significant difference between the 10% concentration group and the positive control group ($p = 0.002$) indicated that the 10% concentration extract exhibited antibacterial activity against *S. aureus* but was not as effective as the positive control. The 20% concentration group showed no significant difference from the positive control ($p = 0.319$), suggesting comparable antibacterial activity to 10 µg of amoxicillin. Compared with the positive control, the 40% and 80% concentrations showed significant differences in inhibition zone diameter ($p = 0.006$ and $p = 0.005$, respectively).

The inhibition zone measurements of the ethanol extract of *M. champaca* leaves on *E. coli* growth are shown in

Table 3. The ethanolic leaf extract of *M. champaca* showed only weak inhibitory activity against *E. coli* at all tested concentrations. The mean inhibition zone diameters ranged from 6.38 ± 0.34 mm to 7.63 ± 0.72 mm, which were close to the negative control value. According to the Morales criteria, all extract concentrations were categorized as weak.

The positive control, ciprofloxacin 5 μ g, produced a substantially larger inhibition zone than all extract

concentrations. Although one-way ANOVA showed a statistically significant difference among groups ($p = 0.001$), the inhibition zones produced by the extract remained minimal and close to the negative control, indicating weak antibacterial activity against *E. coli* under the present experimental conditions. Therefore, the statistical difference observed in the overall analysis should be interpreted cautiously in light of the very small absolute differences among the extract-treated groups.

TABLE 3. The inhibition zone of the ethanol extract of *M. champaca* leaves on *E. coli*

Group	Mean \pm SD	Inhibitory Response*
Control negative	6.38 \pm 0.37	Weak
Ciprofloxacin 5 μ g	35.34 \pm 0.98 ^b	Active
Extract 10%	6.48 \pm 0.37 ^a	Weak
Extract 20%	6.38 \pm 0.34 ^a	Weak
Extract 40%	6.71 \pm 0.27 ^a	Weak
Extract 80%	7.63 \pm 0.72 ^a	Weak

*Morales criteria weak 6-10 mm; active 11-20 mm; very active 21-30 mm.

^a $p < 0.05$ VS ciprofloxacin; ^b $p < 0.05$ vs control negative

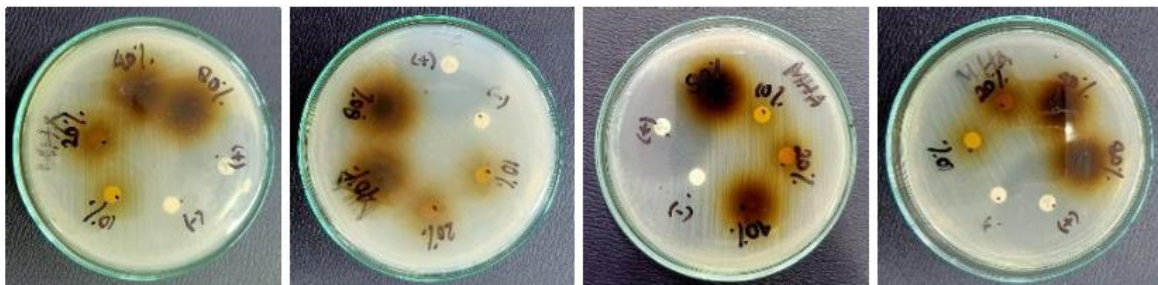


FIGURE 2. Inhibition zones formed by the antimicrobial activities of the ethanol extracts of *M. champaca* on *E. coli*.

This study aligns with Palgunadi *et al.*,² who demonstrated that the ethanol extract of *M. champaca* bark exhibited moderately effective antibacterial activity against *S. aureus*. Conversely, the ethanol extract of *M. champaca* bark displayed no antibacterial activity against *E. coli*. The antibacterial effect of *M. champaca* extract against the growth of *S. aureus* and *E. coli* was attributed to its constituent metabolites. Different plant parts produce different secondary metabolites. According to the phytochemical analysis conducted by Waisul *et al.*,¹⁴ the *M. champaca* leaf extract used in this study contained secondary metabolites, such as alkaloids, saponins, tannins, flavonoids, steroids, quinones, and polyphenols. Another study by Ruwali *et al.*,²¹ showed that phytochemical screening of *M. champaca* leaf extract yielded secondary metabolites such as alkaloids, saponins, tannins, flavonoids, sterols, and triterpenoids. Aditya *et al.*,¹¹ reported that *M. champaca* leaves contain alkaloids, glycosides, carbohydrates, amino acids, and flavonoids. Flowers contain alkaloids, tannins, glycosides, carbohydrates, amino acids, flavonoids, and sterols.¹¹ In contrast, the stem bark contains flavonoids and sterols. Variation in compound composition can be attributed to the geographic conditions under which the plants grow. Furthermore, secondary metabolite production is influenced by a plant's adaptability and survival mechanisms.

Alkaloids are organic heterocyclic nitrogen compounds that form water-soluble salts. These compounds exhibit antibacterial activity by damaging DNA and inhibiting the activity of DNA and RNA polymerases, thereby inhibiting bacterial DNA and RNA synthesis. Additionally, alkaloids impede cell wall formation by disrupting peptidoglycan synthesis, subsequently leading to bacterial cell lysis.²² Flavonoids and tannins, which are a class of phenolic compounds containing hydroxyl groups, can neutralize free radicals.²³

The antibacterial activity of flavonoid compounds occurs via multiple mechanisms, including the inhibition of nucleic acid synthesis, alteration of cytoplasmic membrane function, disruption of energy metabolism, reduction of cellular adhesion and biofilm formation, modification of membrane permeability, and degradation of the bacterial cytoplasmic membrane. Furthermore, flavonoids inhibit sortase, an enzyme that catalyzes protein synthesis on the surface of Gram-positive bacteria. This mechanism further substantiates the antibacterial activity of flavonoid compounds. Tannins are secondary metabolites that inhibit bacterial cell adhesion and enzymatic activity. Additionally, these compounds can impede protein transport across the inner cell membrane, thereby inhibiting bacterial growth.²⁴

The mechanism of action of steroids as antibacterial agents involves lipid membranes and their susceptibility to steroid components, leading to leakage from bacterial liposomes. Steroids can interact with phospholipid membranes that are permeable to lipophilic compounds, leading to decreased membrane integrity, alterations in cell membrane morphology, and ultimately, cell lysis. In contrast, the mechanism of action of saponins involves inducing protein and enzyme leakage from bacterial cells. Saponins are bioactive compounds that can enhance membrane permeability and, consequently, cause hemolysis of bacterial cells.²⁵

Another compound present in the ethanol extract of *M. champaca* leaves is quinone. Quinones exhibit antibacterial effects by forming irreversible complex compounds with nucleophilic amino acid residues on transmembrane proteins in the plasma membrane, cell wall polypeptides, and enzymes located on the cell membrane surface, thereby disrupting bacterial cell functions. Additionally, polyphenolic compounds that act as toxins in bacterial protoplasm are also present. Polyphenols can

compromise bacterial cells, resulting in protein denaturation and cellular leakage.²⁶

The secondary metabolites present in *M. champaca* may have mediated the antibacterial effects observed in this study. The limited antibacterial activity against *E. coli* may be attributed to the more complex structure of the bacterial cell wall than that of gram-positive bacteria. Gram-negative bacteria have a high lipid content and are composed of multiple polymer layers: lipoproteins in the outer layer, lipopolysaccharides in the middle layer, peptidoglycan in the inner layer, and an outer membrane consisting of a lipid bilayer that serves as a protective barrier against antibiotics. The presence of an efflux mechanism in *E. coli* can reduce permeability and enhance the expulsion of antibacterial substances. The efflux pump on the bacterial membrane pumps antibacterial substances out of the cell before they can damage it. The cell walls of gram-positive bacteria consist of polysaccharides, peptidoglycans, teichoic acid, and teichuronic acid. Polysaccharide-based cell walls are more susceptible to denaturation than cell walls containing phospholipids.² This phenomenon may explain why the antibacterial compounds in the ethanol extract of *M. champaca* leaves encounter greater difficulty penetrating the cell walls of gram-negative bacteria, resulting in comparatively weaker antibacterial activity against them than against gram-positive bacteria.

A limitation of this study is that the minimum inhibitory concentration (MIC) test was not performed; therefore, the minimum effective concentration of the ethanol extract of *M. champaca* leaves against *S. aureus* and *E. coli* was not determined.

CONCLUSIONS

The ethanolic leaf extract of *Michelia champaca* demonstrated promising *in vitro* antibacterial activity against *S.*

aureus in the disc diffusion assay, while its activity against *E. coli* was weak. These findings suggest the extract's potential as a preliminary natural antibacterial candidate against Gram-positive bacteria. However, the absence of MIC testing and the use of a single *in vitro* assay limit the interpretation of efficacy, and further studies are needed to isolate active compounds, determine MIC values, and evaluate safety.

DECLARATIONS

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AUTHOR'S CONTRIBUTION

All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by S. M. The first draft of the manuscript was written by F.H. and T. H., and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

ETHICAL STATEMENT

Not applicable.

CONFLICT OF INTEREST

None.

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