

**BACTERICIDAL ACTIVITIES OF LEAF EXTRACT OF KENIKIR
(*COSMOS CAUDATUS*) ON *SHIGELLA DYSENTERIAE* AND
*SALMONELLA TYPHI***

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ABSTRACT

Kenikir (*Cosmos caudatus* Kunth) herb has known to have antibacterial activities against several bacterial strains. In this study, we tested leaf extract of the kenikir against *Shigella dysenteriae* and *Salmonella typhi* strains. The extracts of 100%, 80%, 60%, 40%, and 20% (v/v) screened for their antibacterial activity in comparison with standard antibiotic chloramphenicol as the positive control and distilled water as the negative control using the disc-diffusion agar method. The MIC and MBC's value of the extracts was determined using broth dilution method followed by sub culturing bacterial suspension taken from the lowest concentration that completely showed no bacterial growth. The results showed all kenikir (*Cosmos caudatus* Kunth) leaf

extract concentration levels applied (20-100%) on the tested bacteria exhibited growth inhibition higher than that of standard antibiotic (chloramphenicol). MIC and MBC value of the extract against *Shigella dysenteriae* and *Salmonella typhi* consecutively are 17% and 16%. Considering the MBC values of the extract against the tested bacteria were the same as its MIC values it is suggested that the kenikir extract has bactericidal properties on *Shigella dysenteriae* and *Salmonella typhi*.

INTRODUCTION

Kenikir is Indonesian vernacular name of a perennial herb named scientifically *Cosmos caudatus* Kunth. Here in Indonesia, especially in Java, kenikir commonly being consumed as fresh vegetable and is believed to have medicinal properties.^[1]

Among medicinal benefits of kenikir that have known are lowering blood glucose levels and the total cholesterol levels^[2] and potential as anti obesity.^[3] Medicinal properties of kenikir plants are reasonable because these plants extracts known to contain medicinal antioxidants.^[4]

Kenikir also expected to develop as natural sanitizer because the leaf extract of this plant effectively reduced number of microflora such as *Bacillus cereus*, *Escherichia coli* and *Staphylococcus aureus* from raw chicken meat rinsed with such extract.^[5] Other studies found that plant extract of *Cosmos caudatus*, when mixed in gel preparation, has repellent effect on mosquito bites of *Aedes aegypti*.^[6]

In vitro test against some bacterial strains of Gram positive: *Bacillus subtilis* and *Staphylococcus aureus*, and Gram negative: *Escherichia coli* and *Pseudomonas aeruginosa* revealed that the crude extracts of *Cosmos caudatus* could be potential source of new antimicrobial agents especially to treat infections.^[7]

In order to expand knowledge about the antibacterial activities of kenikir (*Cosmos caudatus* Kunth), in this study we conducted an in vitro test the effects of leaf extracts of this plants against *Shigella dysenteriae* and *Salmonella typhi* strains. Both strains of bacteria are important because they cause infectious diseases that are very common in the tropics, especially Indonesia.

MATERIALS AND METHODS

Plant sample and extraction

Fresh leaves of kenikir (*Cosmos caudatus* Kunth) collected from Sidomekar Village, Katibung Sub district, South Lampung Regency, the province of Lampung of Indonesia. After washed with distilled water, the leaves were cut into small pieces and oven-dried for a week at 40°C. The dried small pieces of leaves then macerated using ethanol as the solvent. The extract then evaporated using rotary evaporator and freeze-dried. To make extract with

desired concentration (v/v) of 100%, 80%, 60%, 40%, and 20% the samples dissolved in DMSO.

Bacterial isolates and media

The bacteria of *Shigella dysentriae* dan *Salmonella typhi* were obtained from Microbiological Laboratory, College of Pharmacy, Bandung Institute of Technology, Bandung, Indonesia. The nutrient agar (NA, Merck) and nutrient broth (NB, Merck) used for bacterial culturing and assays.

Assay for antibacterial activity

The leaves extracts of kenikir (*Cosmos caudatus* Kunth) screened for their antibacterial activity in comparison with standard antibiotic chloramphenicol as the positive control and distilled water as the negative control. Each extract of 100%, 80%, 60%, 40%, and 20% (V/V) as well as control solution individually loaded on 3 mm sterile disc and subjected to antibacterial activity. The zone of growth inhibition surrounding the disc after 48 h of incubation at 37°C measured and recorded as the antibacterial activity of the extract.

Determination of MIC and MBC

The lowest concentration that completely inhibited bacterial growth in antibacterial activity test as mentioned above defined as the minimum inhibitory concentration (MIC) value of the kenikir leaf extracts. The MIC value of kenikir leaf extracts was determined using broth dilution method equipped with positive and negative cultures. The tube containing extracts and the growth medium without inoculums (antibiotic control) and the tube containing the growth medium, physiological saline and the inoculums (organism control). The lowest concentration of the extracts permitting no visible growth (no turbidity) defined as MIC when compared with the control tubes. The lowest concentration that revealed no visible bacterial growth after sub-culturing recorded as the minimum bactericidal concentration (MBC) of the extract.

Statistical analysis

The data presented as mean and analyzed using ANOVA, while and the LSD test applied in the post hoc test. Statistical significant was set at $p < 0.05$.

RESULTS AND DISCUSSION

Antibacterial activities of the kenikir leaf extract against *Shigella dysenteriae* and *Salmonella typhi* based on the inhibitory growth zone diameter of the tested bacteria presented in Table 1. The data in the Table 1 clearly revealed that all concentration levels of the kenikir extract applied (20-100%) exhibited growth inhibition on bacterial strain of *Shigella dysenteriae* and *Salmonella typhi* surfacing the effects of standard antibiotic chloramphenicol.

Table 1: Inhibitory growth zone diameter of *Shigella dysenteriae* and *Salmonella typhi* by extract concentrations of kenikir (*Cosmos caudatus* Kunth).

| Treatment | Inhibitory growth zone diameter (mm) | |
|-----------------|--------------------------------------|-------------------------|
| | <i>Shigella dysenteriae</i> | <i>Salmonella typhi</i> |
| Extract 100% | 26.21 ^a | 30.41 ^a |
| Extract 80% | 21.46 ^b | 28.26 ^a |
| Extract 60% | 20.26 ^b | 27.72 ^{ab} |
| Extract 40% | 19.45 ^b | 24.10 ^{bc} |
| Extract 20% | 17.88 ^b | 21.66 ^c |
| Chloramphenicol | 14.4 ^c | 16.69 ^d |
| Distilled water | 0 ^c | 0 ^d |

Two dilution tests using 19 series (1 – 19%) of lowest concentration of the kenikir leaf extract that completely inhibited bacterial growth of *Shigella dysenteriae* and *Salmonella typhi* resulted in the data presented in Table 2.

Table 2: Results of broth dilution test of the extract of kenikir (*Cosmos caudatus* Kunth) of lowest concentration that showed growth inhibition on *Shigella dysenteriae* and *Salmonella typhi*.

| Extract dilution series | Visible growth of bacteria | |
|-------------------------|-----------------------------|-------------------------|
| | <i>Shigella dysenteriae</i> | <i>Salmonella typhi</i> |
| 19% | - | - |
| 18% | - | - |
| 17% | - | - |
| 16% | + | - |
| 15% | + | + |
| 14% | + | + |
| 13% | + | + |
| 12% | + | + |
| 11% | + | + |
| 10% | + | + |
| 9% | + | + |
| 8% | + | + |
| 7% | + | + |
| 6% | + | + |

| | | |
|------------------|---|---|
| 5% | + | + |
| 4% | + | + |
| 3% | + | + |
| 2% | + | + |
| 1% | + | + |
| Media control | - | - |
| Extract control | - | - |
| Bacteria control | + | + |

The data in Table 2 revealed that the MIC value of kenikir extract tested on the bacterial growth of *Shigella dysenteriae* and *Salmonella typhi* are 17% and 16% respectively. Sub culturing bacteria taken from the media with negative growth above on the solid NA agar showed no bacterial colony growth (Fig 1). The results revealed that the MBC and MIC values of the extracts are the same.

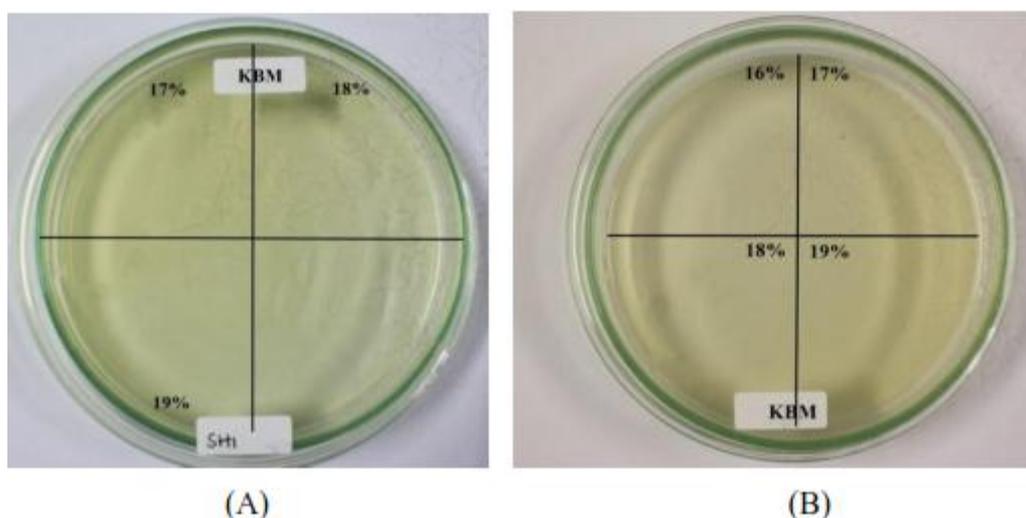


Figure 1: Sub cultures showing negative growth of *Shigella dysenteriae* (A) and *Salmonella typhi* (B) taken from media containing leaf extract of *Cosmos caudatus* Kunth of minimum inhibitory growth concentration.

The high medical benefits of kenikir plant (*Cosmos caudatus* Kunth) are reasonable considering this plant is rich in bioactive compounds. Aerial part of this plant contains monoterpenes, sesquiterpenes, and diterpenes. While its leaves are rich in flavonoids (catechin and quercetin) and phenolic acid (chlorogenic acid, neochlorogenic acid, cryptochlorogenic acid, caffeic acid, ferulic acid).^[8]

Some of the phenolic compounds extracted from kenikir showed antioxidant activities that make them useful for health maintenance.^[9] Test on male mice showed that aqueous extract of this plant increased the sperm quality and fertility without affects body weight of the

animal.^[10] Another test on animal revealed that leaves extract of *C. caudatus* potential for the treatment of hyperlipidemia.^[11]

The research findings showed the leaf extracts of kenikir (*Cosmos caudatus* Kunth) possess strong antibacterial effects and bactericidal properties on both strain of *Shigella dysenteriae* and *Salmonella typhi*. The anti-bacterial properties of kenikir plants are known to apply to several strains of bacteria, both of Gram-positive (*Bacillus subtilis*, *Staphylococcus aureus*) and Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*) groups.^[12] Other bioassay tests on aquatic organisms show that extracts of kenikir plants not only inhibit bacteria (*Aeromonas hydrophila*) but also lethal to brine shrimp.^[13]

Among bioactive chemicals obtained from the leaf extract of *Cosmos caudatus* supposed to be anti-bacterial are caffeic acid (phenolic acid), quercetin and catechin (flavonoids). There are numerous of green leafy vegetables exhibiting antibacterial properties against *S. aureus*, *S. pyogenes*, *B. subtilis*, *E. coli* and *P. aeruginosa* revealed to contain flavonoids.^[14] Other plants which is also revealed to have antibacterial effects due to its flavonoid contents include *Cleome spinosa* Jaqc^[15], *Pterospartum tridentatum* and *Mentha pulegium*^[16], and *Asparagus suaveolens* aerial parts.^[17]

There are several mechanism that cause growth inhibition in bacteria by phytochemicals extracted from plants, among others: cell division inhibitor, protein and DNA synthesis inhibitor, destruction of bacterial membrane, ATP synthase inhibitor, rigidifying bacterial membrane, damage to the cytoplasmic membrane. Among bioactive compound belonging to the flavonoid group contained in the crude extract of *Cosmos caudatus* is quercetin, which has known to act as efflux pump inhibitor in bacterial membrane.^[18]

CONCLUSION

All kenikir (*Cosmos caudatus* Kunth) leaf extract concentration levels applied (20-100%) on the tested bacteria exhibited growth inhibition higher than that of standard antibiotic (chloramphenicol). MIC value of the extract against *Shigella dysenteriae* and *Salmonella typhi* consecutively are 17% and 16%. Considering the MBC values of the extract against the tested bacteria were the same as its MIC values it is suggested that the kenikir extract has bactericidal properties on *Shigella dysenteriae* and *Salmonella typhi*.

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