Volume 06, Issue 01 (January-February2023), PP 49-57 **www.ijmsdr.org**
ISSN: 2581-902X

International Journal of Medical Science and Dental Research

Kenikir Leaf Extract (*Cosmos Caudatus*) Effectiveness against *Staphylococcus aureus* Compared to *Enterococcus faecalis* Bacteria

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Abstract

Background: *Staphylococcus aureus* and *Enterococcus faecalis* were known as the predominant bacteria in root canal infection cases. NaOCl was used as root canal irrigation to eliminate those bacteria, but it contains cytotoxic properties. Kenikir leaves have antimicrobial potential against those oral bacteria.

Purpose: To determine the antibacterial effectiveness of kenikir (*Cosmos caudatus*) leaf extract against *Staphylococcus aureus* compared to *Enterococcus faecalis* at 10% and 40% concentrations.

Methods: This research is an in vitro laboratory experiment using a post-test-only control group design. A 10% and 40% Kenikir (*Cosmos caudatus*) leaf extract were analyzed in comparison to DMSO (negative control) and NaOCl2.5% (positive control) against *Staphylococcus aureus* and *Enterococcus faecalis* using the disc diffusion method with six repetitions.

Results: ANOVA test results showed that there was a significant difference ($p<0.05$) between the diameters of the inhibition zones of 10%, 40% kenikir (*Cosmos caudatus*) leaf extract, and 2.5% NaOCl, namely 8.38 ± 1.29 mm; 11.05 ± 0.92 mm and 23.28 ± 4.12 mm in inhibiting *Staphylococcus aureus*, and 7.55 ± 0.39 mm; 8.93 ± 0.43 mm and 21.2 ± 4.65 mm in inhibiting *Enterococcus faecalis*.

Conclusion: Kenikir (*Cosmos caudatus*) leaf extract was more effective against *Staphylococcus aureus* than *Enterococcus faecalis* at both concentrations.

Keywords - *Antibacterial, Cosmos caudatus, Enterococcus faecalis, Kenikir Leaf, Staphylococcus aureus*

I. INTRODUCTION

The World Health Organization (WHO) states that caries is a primary dental and oral disease, a chronic condition generally developing worldwide[1]. Caries is a disease that affects the hard tissues of the teeth caused by bacterial metabolism, which leads to the demineralization of enamel and dentin[2]. Pulp infection will occur if caries is not given immediate treatment where the condition continues and then reaches the pulp, which, if left unchecked, will then cause pulp necrosis[3]. Root canal treatment is one of the treatment options for cases of

pulp infection[4] in dental conservation with the concept of cleaning, disinfecting, and covering the root canals thoroughly[5]. The final goal of root canal treatment is to eliminate bacteria in the pulp tissue and root canals so that the disease does not progress to secondary infection[6].

Microorganisms are one of the primary triggers of root canal treatment failure[7]. *Staphylococcus aureus* and *Enterococcus faecalis* bacteria are the dominant bacteria often found in root canal infections[8,9]. The results of a previous study conducted by Yamin and Natsir (2014) showed that 20% of *Staphylococcus aureus* bacteria were found in cases of necrotic tooth root canals[10], at relatively high numbers and these bacteria are gram-positive facultative aerobic bacteria[11]. This bacterium is one of the microorganisms often isolated during root canal treatment[12]. It is also a normal microflora in the oral cavity but could become a pathogenic bacterium resulting in infection[13].

Enterococcus faecalis bacteria are the most resistant microorganisms in periradicular lesions that have previously undergone root canal treatment[14]. These bacteria are gram-positive facultative anaerobic bacteria in the oral cavity in small amounts[15]. This bacterium exists in the oral cavity, vagina, and digestive tract and can develop and adapt well if the environment has low oxygen levels[16].

According to Prada et al. (2019), root canal treatment can be successful if the treated tooth is free from pain, inflammation, or fistulas[17]. This treatment consists of the root canal's preparation, cleaning, and obturation[18]. The persistence of microorganisms that cause intraradicular or extraradicular infections is the most significant cause of treatment failure. Therefore, it is necessary to clean debris in the root canals with root canal irrigants[2].

Root canal irrigation is crucial in successful treatment[19] because it acts as a lubricant during root canal preparation, removes the smear layer, and dissolves necrotic pulp tissue, microorganisms, and their products[20]. The irrigation solution often used during endodontic treatment is sodium hypochlorite (NaOCl), with a concentration of 0.5% - 5.25%[21]. Sodium hypochlorite has an antibacterial, proteolytic, and debridement effect[22]. NaOCl could cause soft tissue to dissolve, resulting in lubrication of the canal, and it is easy to obtain at an affordable price[23]. However, a study by Ballal et al. (2019) revealed that NaOCl has cytotoxic properties, which cause an increase in pH and can act as a factor causing cell death[24]. Therefore, natural alternative materials with antibacterial properties similar to non-biological materials are highly expected.

Indonesia is known for its rich biodiversity and abundant natural resources, such as plants[25]. It has become a tradition that plants around the community are often cultivated as traditional medicine. According to Wajdi et al. (2016), plants can be used as medicine for various diseases[26]. The study by Rubinadzari et al. (2022) proved that green seed extract and roasted robusta coffee had antibacterial effectiveness against *Staphylococcus aureus*[27]. The results of Hidayat et al.'s research (2019) showed that merkubung (*Macaranga gigantea*) and mangpurang (*Macaranga triloba*)'s extracts have antibacterial effects against *Enterococcus faecalis*[28]. Other plants also found effective against oral bacteria either in micro and nanoparticles, such as rambutan peel extract (*Nephelium lappaceum L.*) and betel leaf extract (*Piper betle L.*)[29, 30].

The public widely consumes kenikir leaves because they have properties that can treat infectious diseases[31]. The phytochemical screening stated that kenikir leaves contain active compound components such as terpenoids, saponins, flavonoids, polyphenols, and essential oils[32]. These compounds have the potential as antimicrobials that can damage the permeability of cell membranes, destroy proteins and inactivate enzymes in bacterial cells, and inhibit the formation of peptidoglycan (cell wall) [33].

Based on the results of the explanation above, the researchers were encouraged to conduct research in the form of "analyzing the effectiveness of the antibacterial activity of kenikir (*Cosmos caudatus*) leaf extract against *Staphylococcus aureus* bacteria compared to *Enterococcus faecalis* bacteria at concentrations of 10% and 40% in vitro".

II. MATERIALS AND METHODS

This research design was an in vitro experimental laboratory with a post-test-only control group design. This research was conducted at the FKKGIK UNPRI Medan Integrated Laboratory from September 2022 – January 2023.

EQUIPMENTS: Blender, rotary evaporator, petri dish, funnel, sieves, autoclave, tweezers, measuring cup, cabinet dryer, erlenmeyer, scale, water bath, incubator, beaker glass, hotplate, pots, stir rods, slide calipers.

MATERIALS: Kenikir leaf extract with a concentration of 10% and 40%, 70% ethanol, 10% DMSO, 2.5% sodium hypochlorite (NaOCl), MHA, NA, *Enterococcus faecalis*, *Staphylococcus aureus*, aluminum foil, cotton, aquadest, sterile cotton swabs.

METHODS:

1,250 g of fresh kenikir leaves are washed thoroughly in running water; drain the leaves, dry them with a cabinet dryer at 45˚C, and then grind them using a blender in a container[34]. The simplicia powder was weighed, then 70% ethanol was added to it with a ratio of 1 g: 10 ml. Stir every 24 hours for three days, and cover tightly with aluminum foil to protect from light. Filter on the third day with a funnel and cotton to separate the residue and filtrate. Re-macerate the remaining residue with a new solvent as much as ½ times the amount of the first solvent. Stir every 24 hours for four days and filter again. The filtrate obtained was combined and concentrated with a rotary evaporator to separate the solvent from the filtrate. Then water bath the remaining extract to get a thick extract. Condensed extract of kenikir leaves 10 g and 40 g was diluted with 100 ml DMSO 10% to obtain kenikir leaf extract with a concentration of 10% and 40%.

Tools and media are cleaned beforehand. Instruments and materials which cannot stand heat are sterilized by autoclaving for 15 minutes at 121°C; glassware is put into the autoclave and then fixed for 1-2 hours at 160-170ºC[35]. Mueller Hinton Agar (MHA) was used with diffusion as a growing medium for *Staphylococcus aureus* and *Enterococcus faecalis* bacteria. Pour 20 ml of MHA media into a sterilized petri dish, then allow it to solidify. A sterile cotton swab soaked in bacterial suspension is then streaked evenly onto the surface of the MHA media. Soak paper discs in the test solution for each group, wait for them to diffuse, and place it on the surface of the media. Negative control (-) with 10% DMSO and positive control (+) with 2.5% NaOCl. Incubate the media in an incubator for 18-20 hours at 36.5-37ºC. Observe and measure the diameter of the inhibition zone around the disc[36] by measuring the horizontal and vertical diameter of the transparent area using a caliper. The result is the average of the two in millimeters.

III. RESULTS

Plant Determination

The results of the determination of kenikir (*Cosmos caudatus*) leaf extract at the FKKGIK UNPRI Medan Integrated Laboratory showed that the sample studied:

Kingdom	: Plantae
Class	: Disotyledoneae
Division	: Spermatophyta
Family	: Asteraceae
Order	: Asterales
Genus	: Cosmos
Species	: *Cosmos caudatus* Kunth
Local Name	: Kenikir Leaves

Phytochemical Screening Test Results

Phytochemical screening carried out showed the presence of various components of active compounds in kenikir (*Cosmos caudatus*) leaf extract. This test is done qualitatively. Several compounds contained in kenikir (*Cosmos caudatus*) leaf extract are shown in table 1.

Table 1. Results of the Phytochemical Screening of kenikir (*Cosmos caudatus*) leaf.

Active Compound Content	Inhibition Zone Reagent (mm)	Test Result	Observation result
Flavonoids	Alkaline (NaOH)	+	Yellow color - colorless

	Synode Test (Mg+HCl)	+	Dark red color
Tannins	$FeCl_3$	+	Blackish green color
Alkaloids	Ethanol + dilute HCl + *Mayer*	-	No precipitate
	Etanol + dilute HCl + *Dragendroff*	-	No precipitate
Saponins	Foam Test	+	Formed foam> 2 cm
Triterpenoids	*Lieberman Burchard's*	+	Formed a brown ring
	Salkowski	+	Golden yellow color
Phenolic	$FeCl_3$	+	Black

Antibacterial Test Results

The inhibition zone of kenikir (*Cosmos caudatus*) leaf extract against *Staphylococcus aureus* and *Enterococcus faecalis* at concentrations of 10%, 40%, DMSO (negative control), and 2.5% sodium hypochlorite (NaOCl) (positive control) was assessed by measuring the empty area formed around the disc paper and measured with calipers (tables 2 and 3).

Table 2. The average diameter of the inhibition zone of kenikir (*Cosmos caudatus*) leaf extract against *Staphylococcus aureus* bacteria at concentrations of 10% and 40%.

Group	Inhibition Zone Diameter(mm)						
	Repetition						Mean±SD
	1	2	3	4	5	6	
10%	9,7	7,72	8,11	10,25	7,13	7,35	8,38±1,29
40%	12,20	10,27	10,27	12,1	10,29	11,18	11,05±0,92
NaOCl 2,5%	19,84	30,02	22,82	26,37	20,79	19,86	23,28±4,12
DMSO	0	0	0	0	0	0	0,00±0,00

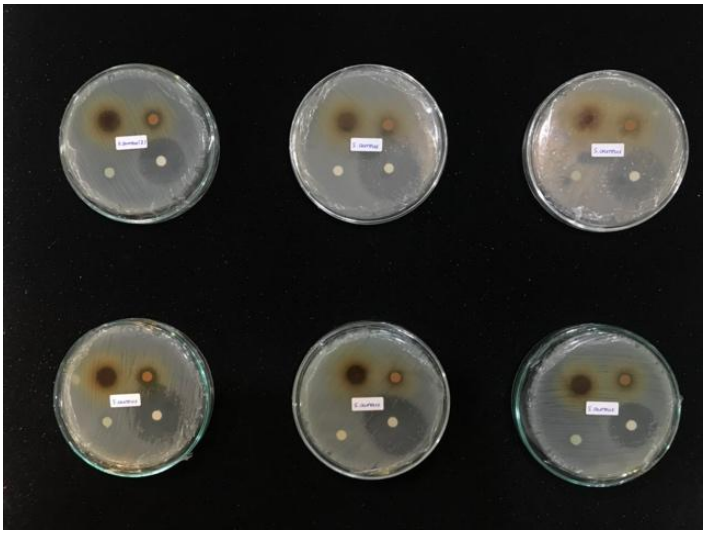

Fig 1. Antibacterial test results of kenikir (*Cosmos caudatus*) leaf extract against *Staphylococcus aureus* bacteria.

Table 3. The average diameter of the inhibition zone of kenikir (*Cosmos caudatus*) leaf extract against *Enterococcus faecalis* bacteria at concentrations of 10% and 40%.

Group	Inhibition Zone Diameter(mm)						Mean±SD
	Repetition						
	1	2	3	4	5	6	
10%	7,27	8,10	7,17	7,21	7,73	7,84	7,55±0,39
40%	8,2	8,73	9,23	9,28	8,83	9,33	8,93±0,43
NaOCl 2,5%	15,83	20,99	28,53	16,69	21,82	23,34	21,2±4,65
DMSO	0	0	0	0	0	0	0,00±0,00

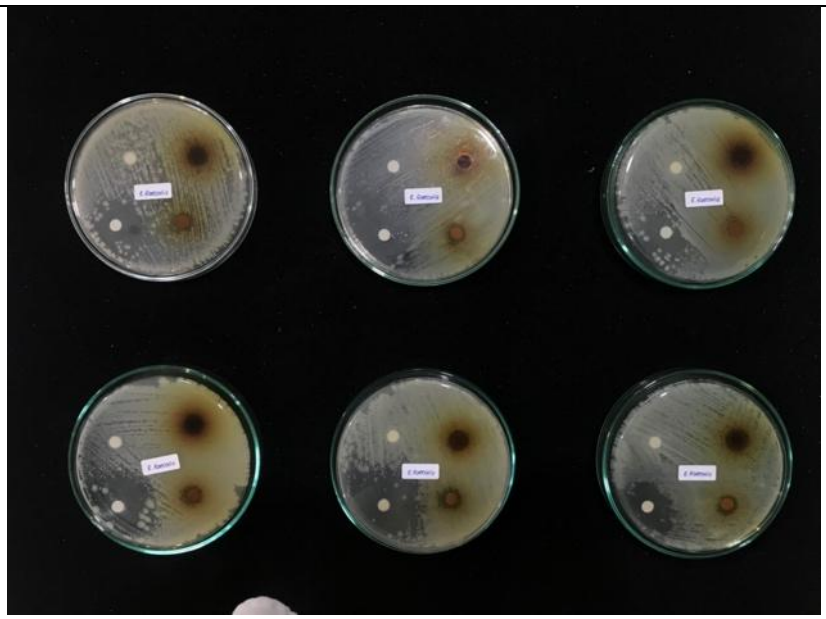

Fig 2. Antibacterial test results of kenikir (*Cosmos caudatus*) leaf extract against *Enterococcus faecalis* bacteria.

Based on the study's results, it was found that 2.5% NaOCl had the highest average inhibition zone, namely 23.28 ± 4.12 against *Staphylococcus aureus* bacteria and 21.2 ± 4.65 against *Enterococcus faecalis* bacteria.

Statistical Test Results

Levene's homogeneity test and the Shapiro-Wilk normality test showed that the data was homogeneous and regular (p>0.05). Then one-way ANOVA statistical test was performed, and the results obtained showed p>0.05 value, which indicated that there was a significant difference between the average diameter of the inhibition zone kenikir (*Cosmos caudatus*) leaf extract 10% and 40% in inhibiting *Staphylococcus aureus* and *Enterococcus faecalis* (table 4). LSD posthoc test results found that there was a significant difference (p<0.05) between kenikir (*Cosmos caudatus*) leaf extract against *Staphylococcus aureus* bacteria at concentrations of 10% and 40%, and there was no significant difference (p>0.05) between kenikir (*Cosmos caudatus*) leaf extract against *Enterococcus faecalis* bacteria at concentrations of 10% and 40% (table 5).

Table 4. One-Way ANOVA statistical test against *Staphylococcus aureus* and *Enterococcus faecalis*.

Group	*Staphylococcus aureus*		*Enterococcus faecalis*	
	Mean±SD	p-*value*	Mean±SD	p-*value*
10%	8,38±1,29	0.000	7,55±0,39	0.000
40%	11,05±0,92		8,93±0,43	
NaOCl 2,5%	23,28±4,12		21,2±4,65	
DMSO	0,00±0,00		0,00±0,00	

Table 5. LSD post hoc statistical test against *Staphylococcus aureus* and *Enterococcus faecalis*.

Group		p-*value*	
		Staphylococcus aureus	*Enterococcus faecalis*
10%	40%	0.049	0.319
	+	0.000	0.000
	-	0.000	0.000
40%	10%	0.049	0.319
	+	0.000	0.000
	-	0.000	0.000
+	10%	0.000	0.000
	40%	0.000	0.000
	-	0.000	0.000

	10%	0.000	0.000
-	40%	0.000	0.000
	+	0.000	0.000

IV. DISCUSSION

This study used kenikir (*Cosmos caudatus*) leaf as research material to compare the effectiveness of inhibition of kenikir leaf extract against *Staphylococcus aureus* and *Enterococcus faecalis* bacteria at concentrations of 10% and 40%. The results of the phytochemical screening test showed that kenikir (*Cosmos caudatus*) leaf extract contains active compounds such as saponins, flavonoids, tannins, terpenoids, and phenolics. The results of this study follow previous research by Herlina (2021), where the components of these compounds have been shown to inhibit bacterial growth[37].

Flavonoids are active compounds that form complex compounds bound to proteins to inhibit cell membrane function. Tannins can inhibit the plasma membrane, which affects the growth of bacteria. Saponins reduce surface tension, so the bacterial cell membrane breaks[38]. Terpenoids damage proteins by forming polymeric bonds from reactions with proteins outside the cell wall membrane, inhibiting bacterial growth. Phenolics bind to bacterial cell proteins to prevention and macromolecular imbalances so that cells become lysed[3].

From this study, it was found that the inhibition of kenikir (*Cosmos caudatus*) leaf extract on the growth of *Staphylococcus aureus* bacteria at concentrations of 10%, 40%, 2.5% NaOCl, and DMSO had an average diameter of the inhibition zone sequentially, namely 8.38±1.29; 11.05±0.92; 23.28±4.12 and 0.00±0.00. These results are consistent with Astutiningrum's (2016) research, where kenikir (*Cosmos caudatus*) leaf extract has antibacterial properties against *Staphylococcus aureus* at a concentration of 30%[38].

Kenikir (*Cosmos caudatus*) leaf extract has an inhibitory effect on *Enterococcus faecalis* bacteria at concentrations of 10%, 40%, 2.5% NaOCl, and DMSO with the average diameter of the inhibition zone sequentially, namely 7.55±0.39; 8.93±0.43; 21.2±4.65 and 0.00±0.00. The results showed that 2.5% NaOCl is more effective than kenikir (*Cosmos caudatus*) leaf extract at 10% and 40% concentrations.

V. CONCLUSION

From the results of this study, it can be concluded:

1. Kenikir (*Cosmos caudatus*) leaf extract at a concentration of 10% and 40% has inhibitory activity against *Staphylococcus aureus* bacteria with mean and standard deviation of 8.38±1.29 and 11.08±0.96 and *Enterococcus faecalis* bacteria with mean and standard deviation of 7.67±0.61 and 8.93±0.43.
2. The results of the phytochemical screening test showed that kenikir (*Cosmos caudatus*) leaf extract contained saponins, flavonoids, tannins, terpenoids, and phenolic compounds.
3. There is a difference in the effectiveness of inhibition of kenikir (*Cosmos caudatus*) leaf extract on the growth of *Staphylococcus aureus* and *Enterococcus faecalis* at concentrations of 10% and 40%.

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