IOSR Journal Of Pharmacy And Biological Sciences (IOSR-JPBS)
e-ISSN:2278-3008, p-ISSN:2319-7676. Volume 18, Issue 1 Ser. III (Jan. – Feb. 2023), PP 01-06
www.Iosrjournals.Org

Comparative Antibacterial Activity of ethanol Extract, Choloroform Fraction and n-Hexane fraction from *Tekelan's* (*Chromolaenaodorata*L.), *Kenikir's*(*Cosmos caudatus*Kunth) and *Kemangi's* (*Ocimumbacilicum*L.)Leave.

MelatiYulia Kusumastuti[1*], Suprianto[2], Devi Purnama Sari[3]
[1,3] *Department of Pharmacy, Sekolah Tinggi IlmuKesehatan Indah Medan, Medan, Indonesia*
[2] *Faculty of Pharmacy, InstitutKesehatanMedistraLubukPakam, Deli Serdang, Indonesia*
Corresponding Author: MelatiYulia Kusumastuti
Department of Pharmacy, Sekolah Tinggi IlmuKesehatan (STIKes) Indah Medan, Medan, Indonesia
STIKes Indah Medan, Jl. Saudara Ujung No. 110, SimpangLimun, Medan,20218

Abstract
Objectives:*Bacteria is one of the infectious causes. Antibacterial agents are the treatment for this infectious. The biggest source for antibacterial agent is plant. Some of plant that can be used for treat infectious was Tekelan(C. odorataL.), Kenikir(C. caudatusKunth) and Kemangi(O. bacilicumL.).Tekelan, Kenikirand Kemangiare commonly consuming as vegetable in Indonesia. They alsotraditionally were used for treat diarrhea and wound, gastritis and appetite enhancer. Objective of this study were determined best antibacterial activity and Minimum Inhibitor Concentration (MIC) of ethanol extract, chloroform fraction and n-hexane fractionfrom C. odorata. C. caudatusand O. bacilicumleaves against Staphylococcus aureusandEscherichia coli.*
Methods:*Leaves of C. odorata, C. caudatusand O. bacilicumwereextracted by maceration method using ethanol 96%. The ethanol extract continuously fractions by n- hexane and chloroform. The extracts and fraction were phytochemical screening and continued conducting antimicrobial test against S. aureusand E. coli, through disc diffusion method.*
Results:*Resultshowedethanol extracthas better activity against S. aureus than E.colicompare than chloroform fraction and n-hexane fraction. MIC of Tekelan's and Kenikir'sleaves against S. aureus were 12.5 mg/ml (ethanol extract), 50 mg/ml (chloroform fraction) and 300 mg/ml (n-hexane fraction). While for E.coli 25 mg/ml (ethanol extract), 100 mg/ml (chloroform fraction), 400mg/ml(n-hexane fraction). MIC of Kemangi's leave against S. aureus and E. coli were 12.5 mg/ml (ethanol extract), 50 mg/ml (chloroform fraction) and none on n-hexane fraction.Base on zone inhibition of concentration 500 mg/ml of ethanol extract, chloroform fraction, it showed Kemangileaves have better antibacterial activity compare to Tekelan and Kenikir leaves (> 20 mm). But n-hexane fraction of Tekelanand Kenikir still have anbacterial activity against S. aureus and E. coli.*
Conclusions:*Ethanol extract of Tekelan's, Kenikir and Kemangi'sleaves has greater antibacterial activity compare than chloroform and n-hexane fraction withMIC under 50 mg/ml against S. aureus and E. colifor all the plant. Kemangi's ethanol extract and chloroform fraction were the most growth inhibitor activity among other plant. It due to the presence of alkaloid, flavonoid, terpenoid, tannin and saponin compound in extracts and fraction.*

Keywords: *C. odorata; C. caudatus; O. sanctum; extract; fraction; antibacterial*

Date of Submission: 01-02-2023 Date of Acceptance: 11-02-2023

I. Introduction

Infectious diseases are still a global health problem, especially in developing countries such as Indonesia.Infectious diseases in Indonesia are still difficult to overcome, even though many efforts have been made. One of infection causes is microorganism.[1]*Staphylococcus aureus* and *Eschericia coli* are one of pathogen microorganism. *E. coli* is a Gram-negative bacterium which is a natural flora of mammals's intestine. *E. coli* which is pathogenic can cause diarrhea.[2]*S. aureus* is Gram-positive bacterium that can cause cellulitis and furunculosis lesions on injured skin.[3]

Infections caused by bacteria are generally treated or prevented by using antibacterial. Each bacterial has a different mechanism and different sensitivity to bacteria. Excessive or irrational use of antibiotics can lead to bacterial resistance to antibacterial[4].This causes the need for the discovery of new antibacterial compounds to overcome microbial resistance.

The largest source of antibacterial compounds is plants. Some of the plants that have potential as antibacterialare*Tekelan's*(*Chromolaenaodorata*L.), *Kenikir*(*Cosmos caudatus*Kunth) and *Kemangi's*(*Ocimumbacilicum*L.)plants. *Tekelan* is originally came from South America, in Indonesia tekelan plants first report in 1934 from LubukPakam's Herbarium (North Sumataera).[5]Citizen of Padang Lawas Utara-North Sumatera (Indonesia) weretraditionally used this plant as antidiarrhea, wound healing (stop bleeding), antipyretic and to treat coughing. Fresh juice of tekelan's leaves have been reported has activity to stop bleeding and decoction of their leaves and stem has antibacterial activity against *Propionibacterium acnes.*[6]

Kenikir (*Cosmos caudatus*Kunth) or also called as yellow ray flower is commonly used as vegetable in Indonesia or Malaysia. Kenikir has sweet taste and give a cold feel after consume it. It has pharmacology activity as appetite enhancer, anti-arrythmia and insect repellent. It happen because the present of flavonoids, polyphenol and volatile oil[7]. Kenikir's leaves extract also been reported to inhibit the growth of *Bacillus cereus* as it commonly contaminated rice, milk, vegetables and fish.[8]

Kemangi(*Ocimumbacilicum*L.) also known as basil is member of Lamiaceae. It is originally from Asia tropical country, one of them is Indonesia. *Kemangi* was commonly eaten raw as *ulam* in Indonesia. This plant has many benefits as a drug, pesticide, vegetables, source of volatile oil, vegetables andrefreshing drink. Kemangi's leaves contained flavonoid, phenol, saponin and volatile oil[9].

Alkaloid, flavonoid, tannin, terpenoid, steroid and volatile oil were present in leaves extract of *Tekelan, Kenikir and Kemangi.* Flavonoid is one of phenolic compound and has antimicrobial activity. One mechanism tannin as antimicrobial is act as iron deprivation, hydrogen bounding or non-specific interaction with essential protein such as enzim.[10]Indoquinoline alkaloid has been proven cause cell lysis and morphological changes of *S. aureus*[11]. Volatile oil could inhibit membrane cell growth of bacteria[12]. All phytochemicals have different polarity characteristic, so their amount in the extract also depend on the solvent that were used for extraction. Flavonoids can be polar because of sugar attached to their structure; this form tends to bemade flavonoids more soluble in water. In contrast,aglycones that less polar such asisoflavones, flavanones and methoxylated flavones and flavonols tend to be more solublein solvents such as ether and chloroform[13].Based on that, the purpose of this present study was to determined better antibacterial activity of ethanol extract, chloroform fraction and n-hexane fraction from *Tekelan, Kenikir and Kemangi's* leaves against *S. aureus* and*E. coli* with disc diffusion method.

II. Material and Method

Plant material

Tekelan's, Kenikir and Kemangi's leaves were collected from Medan, North Sumatra, Indonesia. Determination of this samples were done in Herbarium Medanense, Universitas Sumatera Utara, Medan, Indonesia.

Extraction and Fractionation

Dried leaves of *Tekelan*, *Kenikir*and *Kemangi*(1000 g) was extracted with ethanol 96% by maceration method in room temperature. The extracts were filtered and concentrated with rotary evaporator at temperature 50^0C.Fractionation was carried out using the LLF (Liquid-Liquid Fractionation) method with n-hexane, chloroformand ethanol as solvent continuously with polaritydifference. Fractionation was carried out as follows: The ethanol extract was dissolved in ethanoland water with a ratio of 1:1 as much as 200 ml.Then put it into a separating flask, add 200 ml of n-hexane, shake it slowly, after letting it stand there will be a separation betweenn-hexane and methanol-water fractions. The n-hexane fraction was separated, then repeated several times until the solution was clear. Fractionation was continued using chloroform with the same process asn-hexane. Liquid n-hexane fraction and liquid chloroform fraction were evaporated using a vacuumrotate, so that a viscous fraction is obtained. Viscous fractionevaporated with water bath to obtain fractionsdry. The ethanol extract and two fractions obtained were tested for activityits antibacterial[14].

Phytochemical screening

The ethanol extract, chloroform fraction and n-hexane fraction of *Tekelan, Kenikir and Kemangi*leaves were phytochemicals screening by the Indonesian Pharmacopoeia IV procedure which included examination of alkaloids, flavonoids, phenol, steroids/triterpenoid, saponins, and tannins compounds.[15]

Alkaloid Test: 4 mg samples; 0.1 mL 2N HCl; and 1.0 mL of distilled water were mixed in a test tube, heated, filtered, and divided into 2 parts. Bouchardat's reagent and Mayer's reagent were added to each part so that reddish brown and white colors were formed.

Phenol test: 4 mg of samples was dissolved in water, 1 mL of 5% $FeCl_3$ was added to form a dark blue to black color.

Flavonoid test: 4 mg samples and 3 mL ethanol were mixed in a tube, plus 0.1 mg magnesium and 10 drops of concentrated HCl to form a pink color.
Tannin Test:4 mg of samples was dissolved in 1 ml of distilled water and heated in a water bath, filtered and the filtrate was added with 3 drops of $FeCl_3$ to form a green color.
Saponin test: 4 mg samples and 10 mL hot water were mixed in a tube, shaken for 10 seconds, then added 10 drops of 2 N HCl to form foam.
Steroid/Terpenoid test: 4 mg samples and 3 mL dichloromethane were heated in a water bath, then 6 drops of CH_3COOH and H_2SO_4 were added to form a blue-green color.

Antibacterial activity

Antibacterial activity of T*ekelan, Kenikir*and*Kemangi* were studied against *S. aureus* and *E. coli*. The bacteria's strains were purchased from Universitas Sumatera Utara, Medan-Indonesia. A single colony from the stocks were transferred into Muller Hinton Agar (MHA) for bacteria and incubated at 37^0C for 24h.After incubation time, it was transferred into saline solution(NaCl 0.9%) and centrifuged. The stocks solution density was prepared same as 0.5 Mc Farland (10^8 CFU/ml).
The microbe inoculums were prepared by 0.1 ml of stock solution were diluted with 9.9 ml of saline solution to obtain 10^6 CFU/ml of microbe.

Agar disc diffusion method

The extract and fraction were dissolved in DMSO until obtained concentration 500 mg/ml, 400 mg/ml, 300 mg/ml, 200 mg/ml, 100 mg/ml, 50 mg/ml, 25 mg/ml, and 12.5 mg/ml. This test was carried out by the paper disc diffusion method. MHA was poured into a petri dish as much as 20 ml at a temperature of 45-50^0C, then 0.1 ml of *S. aureus* and*E. coli* inoculum were transferred into the media, stirred until homogeneous and waited until the media solidify. Disc paper has been immersed in the test solution at various concentrations were placed on the surface of solid media, and incubated at 37^0C for 24 hours. Chloramphenicol were used as positive control, while DMSO was used as negative control. The diameter of the inhibition area around the disc papers were measured using a caliper. All experiment was carried out in triplicate and mean value was determined. The Minimum Inhibitor Concentration (MIC) also observed based on the lowest concentration that showed inhibition zone.

III. Result

Phytochemical screening

It showed that *Tekelan, Kenikir*and*Kemangi*ethanol extract contained phenol, flavonoid, saponin and tannin (Table 1). Otherwise, the presence of alkaloid on ethanol extract of*Tekelan*and*Kemangi*. Steroid/triterpenoid obtained on ethanol extract of *Tekelen*and*Kenikir*. Chloroform fraction of *Tekelan*and*Kemangi* contained alkaloid, phenol, flavonoid, saponin, and tannin; steroid/triterpenoid didn't presence. While in chloroform fraction of *Kenikir* only presence flavonoid and saponin. Based on phytochemical screening on n-hexane fraction, it showed flavonoid and saponin in *Kenikir*; and steroid/triterpenoid on *Tekelan*and *Kemangi*.

Table 1. Phytochemical detected in leaves extract

No	Phytochemical	Ethanol extract			Chloroform fraction			n-hexane fraction		
		A	B	C	A	B	C	A	B	C
1	Alkaloid	+	-	+	+	-	+	-	-	-
2	Phenol	+	+	+	+	-	+	-	-	-
3	Flavonoid	+	+	+	+	+	+	--	+	--
4	Saponin	+	+	+	+	+	+	-	+	-
5	Tannin	+	+	+	+	-	+	-	-	-
6	Steroid/Triterpenoid	+	+	-	-	-	-	+	-	+

Description: + = Positive; - = Negative; A = Tekelan; B = Kenikir; C = Kemangi

Antimicrobial activity

Antibacterial activity was carried out by paper disc diffusion method. It showed presence of zone inhibition that indicated antibacterial activity of ethanol extract, chloroform fraction and n-hexane fraction.

The ethanol extract of *Tekelan*and*Kenikir* showed the greatest MIC on 12.5 mg/ml and 25 mg/ml compare to chloroform fraction (50 mg/ml and 100 mg/ml) and n-hexane fraction (300 mg/ml and 400 mg/ml) against *S. aureus* and *E. coli* (Table 2. and Table 3.). *Kemangi'*s ethanol extract also showed better MIC on 12.5 mg/ml than chloroform fraction (50 mg/ml) against both bacteria. *Kemangi'*s n-hexane fraction didn't have any antibacterial activity (Table 4). Based on table 2 and table 3, it showed ethanol extract, chloroform fraction and

n-hexane fraction of *Tekelan*and*Kenikir* more sensitive against *S. aureus* than *E. coli*. Meanwhile for *Kemangi's* ethanol extract and chloroform fraction showed same sensitivity against both bacteria (Table 4.).

Based on antibacterial activity of ethanol extract and chloroform extract from *Tekelan, Kenikir and Kemangi* on concentration 500 mg/ml, it showed that *Kemangi* have highest growth inhibitory against *S. aureus* and *E. coli* around 24.08 and 28.08 mm. While n-hexane fraction of *Tekelan* and *Kenikir* showed relatively same growth inhibitor against two bacteria (Table 5. And Figure 1.).

Table 2. Zone inhibition of*Tekelan* leaves

Levels (mg/mL)	Zones of inhibition (mm)					
	Ethanol extract		Chloroform fraction		n-hexane fraction	
	S. aureus	*E.coli*	*S. aureus*	*E.coli*	*S. aureus*	*E.coli*
500	19.00 ± 0.25	18.08 ± 0.18	14.45 ± 0.02	13.83 ± 0.11	11.34 ± 0.12	9.56 ± 0.21
400	16.58 ± 0.18	15.83 ± 0.15	13.23 ± 0.01	11.08 ± 0.22	9.17 ± 0.20	8.31 ± 0.32
300	14.16 ± 0.22	14.00 ± 0.25	11.57 ± 0.02	10.95 ± 0.10	8.39 ± 0.12	-
200	13.25 ± 0.23	13.25 ± 0.25	10.05 ± 0.01	9.70 ± 0.32	-	-
100	11.66 ± 0.22	11.25 ± 0.10	9.45 ± 0.02	8.57 ± 0.21	-	-
50	10.25 ± 0.20	10.25 ± 0.13	8.33 ± 0.02	-	-	-
25	8.91 ± 0.14	7.13 ± 0.38	-	-	-	-
12.5	7.58 ± 0.18	-	-	-	-	-
Control (+)	10.14 ± 0.01	15.10 ± 0.43	10.27 ± 0.45	15.21 ± 0.13	10.28 ± 0.07	15.13 ± 0.28
Control (-)	0	0	0	0	0	0

Table 3. Zone inhibition of*Kenikir* leaves

Levels (mg/mL)	Zones of inhibition (mm)					
	Ethanol extract		Chloroform fraction		n-hexane fraction	
	S. aureus	*E.coli*	*S. aureus*	*E.coli*	*S. aureus*	*E.coli*
500	17.50 ± 0.15	18.43 ± 0.05	13.65 ± 0.32	12.03 ± 0.41	12.43 ± 0.21	10.26 ± 0.61
400	16.28 ± 0.18	17.08 ± 0.57	12.32 ± 0.11	10.08 ± 1.20	9.47 ± 0.09	9.53 ± 0.81
300	15.16 ± 0.21	16.71 ± 0.43	10.17 ± 0.21	9.6 ± 1.00	7.32 ± 0.54	-
200	13.45 ± 0.27	14.55 ± 0.23	9.32 ± 0.51	8.52 ± 2.13	-	-
100	10.34 ± 0.02	12.74 ± 0.19	7.51 ± 0.12	6.39 ± 1.41	-	-
50	9.04 ± 0.29	10.34 ± 0.05	6.29 ± 0.92	-	-	-
25	7.41 ± 0.54	5.62 ± 0.36	-	-	-	-
12.5	6.52 ± 0.36	-	-	-	-	-
Control (+)	10.25 ± 0.91	15.13 ± 0.47	10.32 ± 0.54	15.35 ± 0.03	10.15 ± 0.39	15.63 ± 0.20
Control (-)	0	0	0	0	0	0

Table 4. Zone inhibition of*Kemangi* leaves

Levels (mg/mL)	Zones of inhibition (mm)					
	Ethanol extract		Chloroform fraction		n-hexane fraction	
	S. aureus	*E.coli*	*S. aureus*	*E.coli*	*S. aureus*	*E.coli*
500	24.08 ± 0.25	28.08 ± 0.58	23.45 ± 0.72	24.55 ± 0.72	-	-
400	22.58 ± 0.08	24.13 ± 0.15	20.33 ± 0.61	22.13 ± 0.32	-	-
300	19.18 ± 0.52	21.30 ± 0.75	17.57 ± 0.52	19.07 ± 0.51	-	-
200	16.34 ± 0.27	18.24 ± 0.71	15.05 ± 0.31	17.19 ± 0.93	-	-
100	14.24± 0.12	15.42 ± 0.10	13.42 ± 0.02	14.48 ± 0.12	-	-
50	10.15 ± 0.53	12.06 ± 0.17	10.30 ± 0.32	11.47 ± 0.09	-	-
25	9.10 ± 0.62	8.70 ± 0.80	-	-	-	-
12.5	7.24 ± 0.82	6.14 ± 0.22	-	-	-	-
Control (+)	10.15 ± 0.39	15.25 ± 0.17	10.48 ± 0.69	15.55 ± 0.13	10.05 ± 0.63	15.57 ± 0.26
Control (-)	0	0	0	0	0	0

Table 5. Inhibition zone of extract (500 mg/ml)

Sample	Zones of inhibition (mm)					
	S. aureus			*E.coli*		
	Tekelan	*Kenikir*	*Kemangi*	*Tekelan*	*Kenikir*	*Kemangi*
Ethanol	19.00 ± 0.25	17.50 ± 0.15	24.08 ± 0.25	18.08 ± 0.18	18.43 ± 0.05	28.08 ± 0.58
Chloroform	14.45 ± 0.02	13.65 ± 0.32	23.45 ± 0.72	13.83 ± 0.11	12.03 ± 0.41	24.55 ± 0.72
n-Heksan	11.34 ± 0.12	12.43 ± 0.21	0	9.56 ± 0.21	10.26 ± 0.61	0

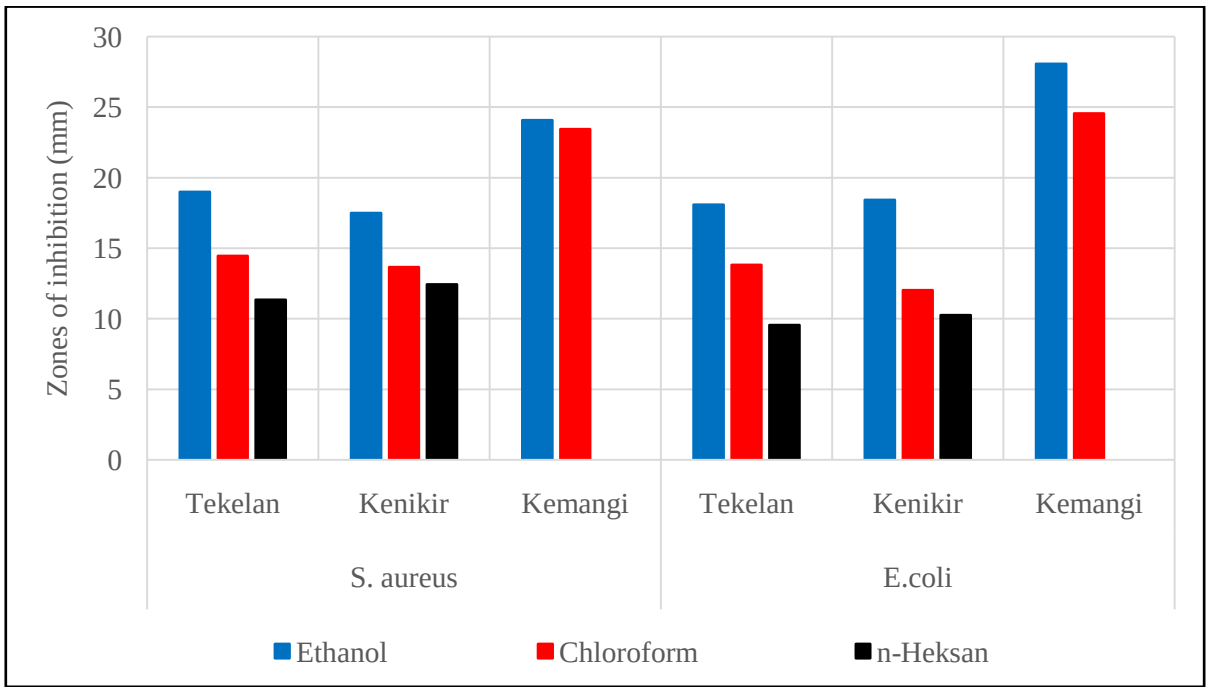

Figure 4. Inhibition zone of extract (500 mg/ml)

IV. Discussion

Tekelan, *Kenikir*and*Kemangi*leaves ethanol extract, chloroform and n-hexane has potential antibacterial against *Staphylococcus aureus*,and*Escherichia coli*. The ethanol extract from *Tekelean*, *Kenikir*and*Kemangi* leaves has better antibacterial activity was related to the results of phytochemical screening which indicated the presence most of phytochemical namely alkaloids, flavonoids, saponins, tannins and triterpenoids. Alkaloids have antibacterial activity by inhibiting DNA synthesis through topoisomerase inhibition.[16] The indolequinoline alkaloids (cryptolepine) cause cell lysis and change the morphology of *S. aureus*.[11] Flavonoids function as antimicrobials by inhibiting nucleic acid synthesis, cytoplasmic membrane function and energy metabolism.[17] Saponins are active substances that can increase membrane permeability so that cell hemolysis occurs when saponins interact with bacterial cells, the bacteria will break or lysis.[18] Tannins have chelating properties that have the activity of shrinking cell walls or cell membranes so that they interfere with the permeability of the cell itself. Impaired permeability can cause cell growth to be inhibited or even die.[19] The mechanism of terpenoids as antibacterial is by bonding with porins (transmembrane proteins) on the outside of the bacterial cell wall, so that strong polymer bonds are formed which result in protein damage.[20]

Based on the MIC results shown in Table 2 and Table 3, Ethanol extract, chloroform fraction and n-hexane fraction of *Tekelan* and *Kenikir* were more sensitive on *S. aureus*than*E. coli*. This is due to structure of microbe are different. *S. aureus* is Gram-positive bacteria which are they don't have the outer membrane and periplasmic space while in Gram-negative such as *E. coli* have both of them.[21,]Outer membrane of Gram-negative bacterial are knownhas a function as a barrier to the penetration of many antibiotic molecules. On the other hand, the periplasmic space also contains enzymes that can break downforeign molecules from outside cell.[22]

Kemangi's ethanol extract and chloroform fraction have best growth inhibitory activity compare other plant. It happen because *Kemangi* extract has rich flavor compound and volatile oil which have antibacterial activity[23]. Volatile oil could inhibit membrane cell growth of bacteria[12]. Volatile oil of *Kemangi* consist of linaool, epi-α-cardinol, α-bergamotene, ϒ-cadinene, monoterpene and sesquiterpene hydrocarbon. Linaool has antibacterial activity against *S. aureus, E. coli, Bacillus subtilis, Pasteurella multocida*[24].

V. Conclusion

Ethanol extract of *Tekelan*'s(*C. odorata*L.), *Kenikir*(*C. caudatus*Kunth) and *Kemangi*(*O. bacilicum*L.)leaves has betterantibacterial activity against *S. aureus* and*E. coli*than chloroform fraction and n-hexane fraction from same plant. *Tekelan*and *Kenikir*extract and fraction were more sensitive against *S. aureus* than *E. coli* .*Kemangi's*ethanol extract and chloroform fraction showed better antibacterial against *S. aureus* and *E. coli* than *Tekelan*and *Kenikir*.

Acknowledgment

The author would like to thank you Sekolah Tinggi IlmuKesehatan (STIKes) Indah Medan for accommodation (laboratories) to conduct this research and all colleague to support the author.

References

[1]. Penyakit Infeksi di Indonesia Solusi Kini & Mendatang Edisi Kedua: Solusi kini dan mendatang. (Pusat Penerbitan dan Percetakan Unair, 2019).
[2]. Rahayu, P. D. W. P., Dr. Siti Nurjanah, S. T. P. M. S. & Ema Komalasari, S. T. P. M. S. Escherichia coli: Patogenitas, Analisis, dan Kajian Risiko. (PT Penerbit IPB Press, 2021).
[3]. Hidayati, A. N. et al.Infeksi Bakteri di Kulit. (Airlangga University Press, 2019).
[4]. Vanilssen, A. Patogen dalam Mikrobiologi. (Cambridge Stanford Books).
[5]. THTROSEMITO, S. The establishment of Procecidochares connexa in West Java, Indonesia: a biological control agent of Chromolaena odorata. BIOTROPIA-The Southeast Asian J. Trop. Biol. (1999).
[6]. Chakraborty, A. K., Rambhade, S. & Patil, U. K. Chromolaena odorata (L.): An overview. J Pharm Res**4**, 573–576 (2011).
[7]. Hariana, H. A. Tumbuhan Obat dan Khasiatnya 2. (Niaga Swadaya).
[8]. Dwiyanti, W., Ibrahim, M. & Trimulyono, G. Pengaruh Ekstrak Daun Kenikir (Cosmos caudatus) terhadap Pertumbuhan Bakteri Bacillus cereus secara In Vitro. LenteraBio**3**, 1–5 (2014).
[9]. Cahyani, N. M. E. Daun Kemangi (Ocinum Cannum) Sebagai Alternatif Pembuatan Handsanitizier. J. Kesehat. Masy.**9**, 136–142 (2014).
[10]. Scalbert, A. Antimicrobial properties of tannins. Phytochemistry**30**, 3875–3883 (1991).
[11]. Sawer, I. K., Berry, M. I. & Ford, J. L. The killing effect of cryptolepine on Staphylococcus aureus. Lett. Appl. Microbiol.**40**, (2005).
[12]. Harborne, J. B. Metode fitokimia: Penuntun cara modern menganalisis tumbuhan. Bandung Penerbit ITB**78**, (1987).
[13]. Andersen, O. M. & Markham, K. R. Flavonoids: Chemistry, Biochemistry and Applications. (CRC Press, 2005).
[14]. Salni, Marisa, H. & Mukti, R. W. Isolasi Senyawa Antibakteri Dari Daun Jengkol (Pithecolobium lobatum Benth) dan Penentuan Nilai KHM-nya. J. Penelit. Sains**14**, 38–41 (2011).
[15]. Departemen Kesehatan RI. Farmakope Indonesia. (Departemen Kesehatan RI, 1995).
[16]. Karou, D. et al. Antibacterial activity of alkaloids from Sida acuta. African J. Biotechnol.**5**, (2006).
[17]. Cushnie, T. P. T. & Lamb, A. J. Antimicrobial activity of flavonoids. International Journal of Antimicrobial Agents vol. 26 (2005).
[18]. Poeloengan, M. Uji Aktivitas Antibakteri Ekstrak Kulit Buah Manggis (Garcinia mangostana Linn). UJI Akt. ANTIBAKTERI EKSTRAK KULIT BUAH MANGGIS (Garcinia mangostana Linn)**20**, (2012).
[19]. Ajizah, A. Sensitivitas Salmonella Typhimurium Terhadap Ekstrak Daun Psidium Guajava L . Sensitivitas Salmonella Typhimuriumterhadap Ekstrak Daun Psidiumguajava L**1**, (2004).
[20]. Cowan, M. M. Plant products as antimicrobial agents. Clinical Microbiology Reviews vol. 12 (1999).
[21]. H., N. Outer Membran. in Escherichia coli and Salmonella: Cellular and Molecular Biology (ed. Neidhardt FC) 29–47 (ASM Press, 1996).
[22]. Duffy, C. F. & Power, R. F. Antioxidant and antimicrobial properties of some Chinese plant extracts. Int. J. Antimicrob. Agents**17**, 527–529 (2001).
[23]. Aarthi, N. & Murugan, K. Larvicidal and repellent activity of Vetiveria zizanioides L, Ocimum basilicum Linn and the microbial pesticide spinosad against malarial vector, Anopheles stephensi Liston (Insecta: Diptera: Culicidae). J. Biopestic.**3**, 199 (2010).
[24]. Hussain, A. I., Anwar, F., Sherazi, S. T. H. & Przybylski, R. Chemical composition, antioxidant and antimicrobial activities of basil (Ocimum basilicum) essential oils depends on seasonal variations. Food Chem.**108**, 986–995 (2008).

MelatiYulia Kusumastuti, et. al. "Comparative Antibacterial Activity of ethanol Extract, Choloroform Fraction and n-Hexane fraction from Tekelan's (ChromolaenaodorataL.), Kenikir's(Cosmos caudatusKunth) and Kemangi's (OcimumbacilicumL.)Leave." *IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)*, 18(1), (2023): pp. 01-06.