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Comparison of Antibacterial Efficacy between 96% Ethanolic Extracts from *Abrus precatorius* L. and *Piper betle* L. Leaves against *Escherichia coli*

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Abstract

Escherichia coli (*E.coli*) is a frequently found infectious pathogen commonly transmitted through water. In Indonesia, the level of this pathogen exceeds the accepted standard. Several studies have shown the presence of antibiotic-resistant E.coli, making studies on alternative treatments for *E.coli* necessary. *Abrus precatorius* L. and *Piper betle* L. leaves are among herbs that have herbal antibacterial properties. This study observed and compared the antibacterial effects of *Abrus precatorius* L. and *Piper betle* L. leaves against *E.coli.* This was an in vitro experimental study performed at the Laboratory of the Department of Microbiology, Faculty of Medicine and Health Science, Atma Jaya Catholic University, from August to November 2019. *Abrus precatorius* L. and *Piper betle* L. leaves were extracted by maceration in 96% Ethanol, and further processed into concentrations of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, and 100%. Disc-diffusion on Mueller-Hinton Agar was used to identify the inhibition zones of the extracts against E.coli ATCC 25922. Ciprofloxacin disc and 96% ethanol impregnated-discs were used as positive and negative controls, respectively. Independent t-test results showed a significant difference between *Abrus precatorius* L. and *Piper betle* L. leaves effects against *E.coli* with p=0.044 and p=0.045 (p<0.05), respectively. In conclusion, *Abrus precatorius* L. and *Piper betle* L. leaves have antibacterial effects against E.coli ATCC 25922, albeit less sensitivity than Ciprofloxacin, with *Piper betle* L. presents a greater effect than *Abrus precatorius* L.

Key words: *Abrus precatorius* L., disc diffusion, *Escherichia coli*, *Piper betle* L.

Perbandingan Efektivitas Antibakteri Ekstrak Etanol 96% Daun *Abrus precatorius* L. dan Daun *Piper betle* L. Terhadap *Escherichia coli*

Abstrak

Escherichia coli (*E.coli*) adalah patogen infeksius yang sering ditemukan dan ditularkan melalui air. Di Indonesia, tingkat patogen ini melebihi standar yang diterima. Beberapa penelitian telah menunjukkan ada *E.coli* yang kebal antibiotik, membuat penelitian tentang pengobatan alternatif untuk *E.coli* diperlukan. Daun *Abrus precatorius* L. dan *Piper betle* L. berpotensi sebagai pengobatan herbal antibakteri. Penelitian ini bertujuan melihat dan membandingkan efek antimikroba kedua daun tersebut terhadap *E.coli*. Penelitian eksperimental *in vitro* ini dilakukan di Laboratorium Mikrobiologi Fakultas Kedokteran dan Ilmu Kesehatan Atma Jaya mulai dari Agustus sampai November 2019. Daun *Abrus precatorius* L. dan *Piper betle* L. dimaserasi menggunakan etanol 96%, lalu dibentuk konsentrasi 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, dan 100%. Difusi cakram pada agar Mueller-Hinton dilakukan untuk menguji zona hambat kedua ekstrak terhadap bakteri *E.coli* ATCC 25922. Cakram ciprofloksasin merupakan kontrol positif dan kontrol negatif adalah cakram yang direndam etanol 96%. Hasil uji *independent t-test* didapatkan perbedaan bermakna antara efektivitas ekstrak kedua daun terhadap *E.coli*, yaitu daun *Abrus precatorius* L. p=0.044 dan daun Piper betle L. p=0.045 (p<0.05). Simpulan, daun *Abrus precatorius* L. dan *Piper betle* L. memiliki efek antibakteri terhadap *E.coli* ATCC 25922 walaupun tidak lebih sensitif dari Ciprofloksasin dengan *Piper betle* L. memiliki efek yang lebih besar dibanding dengan *Abrus precatorius* L.

Kata kunci: *Abrus precatorius* L., difusi cakram, *Escherichia coli, Piper betle* L.

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Introduction

Indonesia is a developing country that still has to deal with various infectious diseases, such as diarrhea. According to the Ministry of Health of the Republic of Indonesia, 180,000 new cases of diarrhea were observed in 2014,[1] and 100,000 toddlers died each year due to diarrhea.[2] *Escherichia coli* (*E.coli*) bacteria is the most common pathogen to cause bacterial diarrhea and is linked to disease severity.[2]

E.coli is mainly transmitted through food and water contaminated by this bacteria. With many areas in Indonesia still lacking proper sanitation and clean water, *E.coli* transmission is common. According to the United States Environmental Protection Agency, *E.coli* in Indonesia's bodies of water exceed the set standard, especially in crowded urban areas.[3] Considering that Indonesia has a very large population, and is going through urbanization, it is necessary to find methods to prevent and treat *E.coli* infections. Aside from the prevalent amount, studies also showed that antibiotic-resistance amongst *E.coli*, just like in other types of *Enterobacteriaceae,* is common. Therefore, studies on alternative medicines that could treat *E.coli* infections are required.[4]

Indonesia is rich with plants used for treating a variety of diseases. *Abrus precatorius* L. and *Piper betle* L. leaves are examples of potentially beneficial herbs that could be used as an alternative to treat bacterial infections. Both vinous plants have been shown to have inhibitory effects *on Staphylococcus aureus*.[5] According to Haryuni[6] *Piper betle* L. leaf has inhibitory effects towards *E.coli*, so does *Abrus precatorius* L. leaf.[7] Some active compounds thought to have antibacterial properties in *Abrus precatorius* L. are polyphenol, flavonoid, and saponin,[5] while the *Piper betle* L. contains 4.2% essential oils.[6]

Since *E.coli* is still a common cause of many infections, it also poses a high risk of being antibiotic-resistant, and research on the use of alternative medicine towards the treatment of *E.coli* infection will be beneficial. This study aimed to observe and compare the effects of *Abrus precatorius* L. and *Piper betle* L. leaves on *E.coli* bacteria.

Methods

This was an in vitro experimental study approved by the Ethics Research Committee, Faculty of Medicine, Atma Jaya University (No. 01/09/KEP-FKUAJ/2019). Data collection was performed at the Laboratory of the Department of Microbiology, Faculty of Medicine and Health Science, Atma Jaya Catholic University from August to November 2019. One thousand two hundred and fifty grams of *Abrus precatorius* L. and *Piper betle* L. leaves already identified by the Bogor Institute of Agriculture were weighed using an analytical scale, rinsed with water, and then air-dried. The dried leaves were ground with a blender into powder form. *Abrus precatorius* L. and *Piper betle* L. leaf powder were extracted by maceration by putting them into separate containers and each was soaked by one liter of 96% ethanol. After five days of soaking, the macerate was filtered and then added with new sets of 96% ethanol to sit for another five days. This process was repeated three times. The product of maceration from both leaves were collected and evaporated using a rotary evaporator and then diluted into 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, and 100% concentration. Duplication was performed using the formula (t-1) (n-1)≥15.[9]

Disc-diffusion on Mueller-Hinton Agar was used to test the inhibition zones of the extract concentration variables towards *E.coli* ATCC 25922, which was the isolate collection from the Laboratory of the Microbiology Department, Faculty of Medicine and Health Science, Atma Jaya Catholic University. The isolate was then standardized into McFarland 0.5. Blank discs infused with the various concentrations of *Abrus precatorius* L. and *Piper betle* L. extracts and 96% ethanol, which was the negative control. Ciprofloxacin disc was used as a positive control. The infused blank discs and ciprofloxacin discs were implanted in Mueller-Hinton agar already inoculated with *E.coli* ATCC 25922. Afterward, incubation was performed for 16–18 hours at 37°C. The inhibition zones created by the positive control were classified according to the Clinical and Laboratory Standards Institute (CLSI),[9] in which ≤16 mm was interpreted as resistance, 17–20 mm as intermediate, and ≥21 mm as susceptible. The inhibition zones created by the extracts and negative control were classified according to Ouchari et al.,[10] in which >20 mm was interpreted as very strong, 10–20 mm as strong, 5–10 mm as intermediate, and <5 mm as weak.

Statistical analysis was performed using the Saphiro-Wilk normality test and Independent t-test statistical analysis in SPSS software (version 22.0) to determine the normality of distribution of data and significant difference among inhibition zones created by *Abrus*

Table 1 Inhibition Zones of *Abrus precatorius* L., Negative Control, and Positive Control against *E.coli* ATCC25922

Concentration (%)	Disc Diffusion Result (mm)			
	I	II	Mean±SD	Classification
10	0	0	0.00±0.00	Weak
20	7	0	3.50±4.95	Weak
30	8	8	8.00±0.00	Medium
40	12	10	9.50±1.41	Medium
50	11	7	9.0±2.83	Medium
60	10	7	8.50±2.12	Medium
70	11	11	11.0±0.00	Strong
80	13	14	13.5±0.70	Strong
90	14	14	14.0±0.00	Strong
100	14	16	15.0±1.41	Strong
Ethanol 96%	0	0	0.00±0.00	Weak
Ciprofloxacin	32	35	33.5±2.12	Susceptible

precatorius L. and *Piper betle* L. leaves.

Results

Antibacterial susceptibility testing using various extract concentrations of *Abrus precatorius* L. and *Piper betle* L. leaf against *E.coli* ATCC 25922 was performed in Mueller-Hinton Agar.

As shown in Table 1 and Table 2, both leaf extracts started to show inhibition of *E.coli* in 20% concentration with the average minimum inhibition zones towards *E.coli* ATCC 25922 were 3.5 mm (weak) and 9.5 mm (medium) for *Abrus precatorius* L. and *Piper betle* L. respectively. Meanwhile, the average maximum inhibition zones against E.coli ATCC 25922 were 15 mm (intermediate) for *Abrus precatorius* L. leaf and 20.5 mm (very strong) for *Piper betle* L. leaf, both at a 100% concentration. *Piper betle* L. leaf started

Table 2 Inhibition Zones of *Piper betle L.*, Negative Control, and Positive Control against *E.coli* ATCC25922

Concentration (%)	Disc Diffusion (mm)			
	I	II	Mean±SD	Classification
10	0	0	0.00±0.00	Weak
20	11	8	9.50±2.12	Medium
30	12	13	12.50±0.70	Strong
40	14	14	14.00±0.00	Strong
50	16	15	15.50±0.70	Strong
60	17	17	17.00±0.00	Strong
70	18	19	18.50±0.70	Strong
80	18	18	18.00±0.00	Strong
90	19	19	19.00±0.00	Strong
100	21	20	20.50±0.70	Very Strong
Ethanol 96%	0	0	0.00±0.00	Weak
Ciprofloxacin	30	33	31.50±2.12	Susceptible

to show strong inhibitory capability towards *E.coli* ATCC 25922 at the concentration of 30%, which was much earlier than *Abrus precatorius* L. that started to show strong inhibitory capability at 70%. Furthermore*, Piper betle* L. showed a very strong ability to inhibit *E.coli* ATCC 25922 in 100% concentration.

The Saphiro-Wilk normality test of *Abrus precatorius* L. and *Piper betle* L. presented normally distributed data, where p=0.157 and p=0.073 (p>0.05) for *Abrus precatorius* L. and *Piper betle* L. respectively. An independent t-test statistical analysis was performed after the data collection and the p-value for Abrus precatorius L. was 0.044 and 0.045 for *Piper betle* L., meaning that both had a p-value of more than 0.05. Hence, there was a significant difference in the mean zones of inhibition between *Piper betle* L. and *Abrus precatorius* L. leaves for E.coli ATCC 25922, with a stronger effect created by the *Piper betle* L.

Discussion

In this study, ethanol extract of *Abrus precatorius* L. is shown to have a strong ability to inhibit *E.coli* starting from the concentration of 70% up to 100%, with the largest inhibition zone of 15 mm. This shows that even though *Abrus precatorius* L. could inhibit bacterial growth, it is not as strong as the positive control. In another study by Yuswantina et al.,[7] aquadest extract of *Abrus precatorius* L. with a concentration of 25%, 50%, and 100% create an inhibition zone of 27.7, 32.7, and 33 mm, respectively, against *E.coli*. This is categorized as a very strong inhibitory capability. The study also looked at the minimum bactericidal concentration (MBC) of *Abrus precatorius* L. leaf towards *E.coli* using the broth dilution method, which showed that the minimum concentration needed to kill the *E.coli* bacteria is 50%.

Several studies also observed the antibacterial effects of other parts of the *Abrus precatorius* L. plant, such as stem, root, and seed. These studies show that the seeds seem to have a stronger effect in creating inhibition zones against a variety of bacteria.[11,12] However, the use of this seed carries a risk since it has a toxic compound called abrin which could cause mydriasis, tremor, tachycardia, and other symptoms if it is accidentally ingested.[13]

In this present study, *Piper betle* L. leaf extract starts to show a strong ability to inhibit *E.coli* ATCC 25922 at 30% concentration all the way to 90% and shows a very strong ability at 100% with the maximum inhibition zone of 20.5 mm. This is in line with the finding of Pinatik et al.[14] where one gram of 96 % ethanol extract of *Piper betle* L. creates a 20.5 mm inhibition zone. According to Syahrinastiti et al.,[15] 10% ethanol extract of *Piper betle* L. does not show any ability to create an inhibition zone against *E.coli*, which is consistent with this study.

Both leaf extracts showed the ability to prevent the growth of E.coli with *Piper betle* L. leaf extract seems to show a stronger capacity. Likewise, a study conducted by Usemahu[5] showed a stronger capacity of *Piper betle* L. leaf to inhibit *Staphylococcus aureus* bacteria in comparison to *Abrus precatorius* L. leaf.

The antibacterial property observed in *Piper betle* L. leaf is presumed to be the effects of the phenolic compounds and its derivatives contained in the leaf. Klavikol and eugenol are some examples of phenol derivatives. These compounds inhibit the formation of the bacterial cell wall by binding proteins and sulfhydryl groups, which then could initiate lysis of bacteria.[6,16] Another active compound found in *Piper betle* L. and *Abrus precatorius* L. is flavonoids. Flavonoid is a polyphenolic compound that could hinder bacterial nucleic acid formation and halt the cytoplasmic function.[15] In addition to phenolic compounds, other active compounds that have antibacterial effects contained in *Piper betle* L. are tanin, amino acids, and fatty acids while saponin and alkaloids are found in Abrus precatorius L. a*nd Piper betle* L.[14,17]

In conclusion, both *Abrus precatorius* L. and *Piper betle* L. leaf extracts have antibacterial effects against *E.coli* ATCC 25922 with *Piper betle* L. leaf seems to show greater effects. Further studies need to be done to confirm the antibacterial effects of both *Abrus precatorius* L. and *Piper betle* L. leaves, preferably using the dilution method to confirm their MBC against different types of bacteria.

References

1. Irawan E, Mulyana H. Faktor-faktor penyebab infeksi saluran kemih (ISK) (literature review). Prosiding Seminar Nasional dan Diseminasi Penelitian Kesehatan STIKes Bakti Tunas Husada Tasikmalaya; 2018 April 21; Tasikmalaya. Tasikmalaya: STIKes Bakti Husada. 2018.
2. Halim F, Warouw S, Rampengan N, Salendu P. Hubungan jumlah koloni escherichia coli

dengan derajat dehidrasi pada diare akut. Sari Pediatri. 2017;19(2):81–5.
3. Widmer K, Van HN, Vinitnantharat S, Sthiannopkao S, Wangsaatmaja S, Prasetiati M et al. Prevalence of Escherichia coli in surface waters of Southeast Asian cities. World J Microbiol Biotechnol. 2013;29(11):2115–24.
4. Brooks G, Carroll KC, Butel J, Morse S, editors. Jawetz Melnick & Adelberg's medical microbiology. 27th ed. New York: McGraw-Hill Medical; 2016. p. 229–36.
5. Usemahu R. Perbandingan efektivitas ekstrak abrus precatorius l. dan daun sirih terhadap bakteri staphylococcus aureus [Undergraduate]. Makassar: Fakultas Kedokteran Gigi Universitas Hassanudin; 2015
6. Haryuni N, Widodo E, Sudjarwo E. Aktivitas antibakteri jus daun sirih (Piper bettle linn) terhadap bakteri patogen dan kualitas telur selama penyimpanan. ternak tropika. J Ternak Tropika. 2015;16(1):48–54.
7. Yuswantina R, Karminingtyas SR, Akhmad R. Uji efektivitas antibakteri abrus precatorius l. (abrus precatorius l.) terhadap bakteri staphylococcus aureus dan escherichia coli. Program Studi Farmasi Fakultas Kesehatan Universitas Ngudi Waluyo. 2017.
8. Marhamah M. Perbandingan kemampuan ekstrak daun saga (abrus precatorius linn.) dengan madu hitam dalam menghambat pertumbuhan bakteri pseudomonas aeruginosa. Jurnal Analis Kesehatan. 2015; 4(2):412–9
9. CSLI. Performance standards for antimicrobial susceptibility testing. CLSI supplement M100S. Wayne, PA: Clinical and Laboratory Standards Institute; 2019.
10. Ouchari L, Boukeskasse A, Bouizga B, Ouhdouch Y. Antimicrobial potential of actinomycetes isolated from the unexplored hot Merzouga desert and their taxonomy diversity. Biol Open. 2019;8(2):bio035410.
11. Arora R, Grill NS, Kaur S, Jain AD. Phytopharmacological evaluation of ethanolic extract of the seeds of Abrus precatorius Linn. J Pharmacol Toxicol. 2011; 6:580–8.
12. Sunday OJ, Babatunde SK, Ajiboye AE, Adedayo RM, Ajao MA, Ajuwon BI. Evaluation of phytochemical properties and in-vitro antibacterial activity of the aqueous extracts of leaf, seed and root of Abrus precatorius Linn. against Salmonella and Shigella. Asian Pac J Trop Biomed. 2016;6(9):755–59.
13. Okhale SE, Nwanosike EM. Abrus precatorius Linn (Fabaceae): phytochemisty, ethnomedicinal uses, ethnopharmacology, and pharmacological activities. Int J Pharmaceutical Sci Res. 2016;1(6):37–43.
14. Pinatik NJ, Joseph W, Akili RH. Efektivitas daun sirih hijau (Piper betle linn.) dalam menghambat pertumbuhan bakteri escherichia coli. KESMAS. 2017;6(4):1–9.
15. Syahrinastiti TA, Djamal A, Irawati L. Perbedaan daya hambat ekstrak daun sirih hijau (Piper betle l.) dan daun sirih merah (piper crocatum ruiz & pav) terhadap pertumbuhan escherichia coli. Jurnal Kesehatan Andalas. 2015;4(2):421–4.
16. Saraswati D. Pengaruh konsentrasi ekstrak daun sirih terhadap daya hambat Escherichia coli. Jurnal Health Sport. 2011; 3(2):331–8
17. Patil RS, Harale PM, Shivangekar KV, Kumbhar PP, Desai RR. Phytochemical potential and in vitro antimicrobial activity of Piper betle Linn. leaf extracts. J Chem Pharm Res. 2015;7(5):1095–1101.