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THE DIFFERENCE IN ANTIBACTERIAL POTENCY OF THE INFUSION OF AKAR KUNING (*Fibraurea tinctoria* Lour.) AGAINST *Shigella dysenteria* AND *Salmonella typhi* IN VITRO

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Abstract: ***Akar kuning (Fibraurea tinctoria Lour.), an original plant from Kalimantan, is often used by the community as traditional medicine.*** *Previous studies showed that akar kuning contains active compounds such as alkaloids, flavonoids, saponins, and terpenoids that have antibacterial properties. This study aims to analyze the difference in antibacterial potency of the infusion of akar kuning against the growth of Shigella dysenteriae (S. dysenteriae) and Salmonella typhi (S. typhi) in vitro. This was a true experimental study with a post-test method only with control group design using the infusion of akar kuning with concentrations of 16%, 32%, and 64%, 5 µg ciprofloxacin as the positive control and aquadest as the negative control. The data were analyzed using the One-Way ANOVA test, LSD's Post-hoc test, and independent T test with a 95% of confidence level. This study showed that the higher the concentration of the infusion of akar kuning, the larger the inhibition zone of the infusion at the concentration of 64% had a larger inhibition zone for S. typhi (16,32 mm) than S. dysenteriae (15,59 mm). However, there was no statistical difference in antibacterial potency of the infusion of akar kuning against S. dysenteriae and S. typhi.*

Keywords: *antibacterial potential, infusion, akar kuning, Fibraurea tinctoria Lour., Shigella dysenteriae, Salmonella typhi*

INTRODUCTION

The province of South Kalimantan is mostly composed by rivers and wetlands. The community often uses fresh water for daily activities. Bacteria causing systemic infection and gastrointestinal infections such as *Shigella dysenteriae (S. dysenteriae)* and *Salmonella typhi (S. typhi)* are frequently found in polluted waters.[1] *S. dysenteriae* causes basilary dysentery and *S. typhi* causes mild gastroenteritis to typhoid fever.[2] According to Riset Kesehatan Dasar (RISKESDAS), the prevalence of diarrhea in Indonesia and specifically in South Kalimantan are about 8% and 7%, respectively.[3]

Antibiotic fluoroquinolone group such as ciprofloxacin is often used to treat *S. dysenteria* and *S. typhi* infection. Unfortunately, some studies showed the increased number of resistance against these bacteria.[4,5,6]

Kalimantan island has wide plant diversity with potency as herbal medicine, including akar kuning (*Fibraurea tinctoria* Lour.). Local community has used the stew of this fresh plant's root as herbal medicine. It is then consumed by local community.[7]

A study conducted by Heriyani and Nugroho demonstrated that water extract of akar kuning's root inhibits the growth of *S. thypi*.[8] Another study by Hasnawati and Yamin showed that methanol extract of the root of akar kuning also inhibits the growth of *S. dysenteriae* and *S. thypi*.[9] Maryani *et al* found that methanol extract of akar kuning contains alkaloid, flavanoid, saponin, and terpenoid. These active compounds are known to have antibacterial properties.[10]

Both water and methanol extracts of akar kuning's root have been identified to have antibacterial properties against *S. dysenteriae* and *S. thypi*. However, we found limited studies analyzing antibacterial potency of this plant in infusion form.[8,9,11,12] Furthermore, we found no studies assessing differences in antibacterial potency of the infusion of akar kuning's root against these bacteria. Hence, we aimed to analyze the difference in antibacterial potency of the infusion of akar kuning's root against *S. dysenteria* and *S. typhi* in vitro.

RESEARCH METHOD

This was an experimental study using post-test only with control group design. The intervention groups were the infusion of akar kuning's root in 3 concentrations (16%, 32%, and 64%), ciprofloxacin as a positive control, and aquadest as a negative control with 3 replications for each treatment based on calculation by using the Federer's law.[13] These concentration were determined based on our preliminary study. The results demonstrated that the minimum inhibitory concentration (MIC) for the infusion to inhibit the growth of both bacteria was 16%. This research protocol has been approved by The Ethic Committee of Health Research, Faculty of Medicine, Universitas Lambung Mangkurat, Banjarmasin under the number of No 403/KEPK-FK UNLAM/EC/X/2020.

The main materials for this study were akar kuning's root and pure bacterial isolates, i.e. *S. dysenteriae* ATCC 13313 and *S. typhi* ATCC 13311. Each bacteria was standardized with a standard solution (McFarland 1). These bacteria were provided by the Laboratory of Microbiology, Faculty of Medicine, Universitas Lambung Mangkurat, Banjarmasin. Our study was also used some medias, i.e., Muller Hilton Agar (MHA), Brain Heart Infusion (BHI), Nutrient Agar (NA), sterile aquadest, antibiotic ciprofloxacin 5 µg, sterile paper disks, McFarland 1 standard solution, NaOH, Pb-acetate 10%, Dragendorff reagent, Mayer reagent, gelatin 1%, $FeCl_3$ 3%, benzene, chloroform, anhydrous acetic acid, and concentrated sulfuric acid. Ciprofloxacin is a broad spectrum antibiotic. This antibiotic

inhibits DNA gyrase enzyme (topoisomaerase II and topoisomerase IV). Hence, the release of this bacterial enzyme is also inhibited.[14,15] We used glasses tools, ose wires, a bunsen lamp and matches, sterile cotton rods, a caliper ruler (mm), an analytical scale, rolls of aluminium foil, an autoclave, an aerobic incubator, a water bath, a blender, an oven, a laminary air flow, flannel materials, filter papers, clips, a stove, a knife, and an infusion pot.

Akar kuning plant was collected from Tamiyang Layang city, East Barito regency, Central Kalimantan province. The plant was then identified at the Basic Laboratory, Faculty of Math and Natural Sciences, Universitas Lambung Mangkurat, Banjarbaru, South Kalimantan under the certificate number No 069/LB.LABDASAR/III/ 2020.

Akar kuning's root was washed and dried in room temperature. They were cut into small pieces and dried in an oven (60^0C) until their weight was stable. They were then blended until becoming powder. 100 g of simplicia were mixed with 100 ml of aquadest to make a 100% concentration of the infusion. This mixture was then heated in an infusion pot on a stove for 15 minutes from the water temperature reaches 90^0C. Occasionally, this mixture was stirred. The infusion was then filtered. If the remain volume was <100 ml, hot water was added on top of the residue. Finally, a dilution process was done by adding hot water to make 16%, 32% and 64% concentration of the infusion.

Phytochemical screening[16,17]

Phytochemical tests were qualitatively done for akar kuning's root to identify its secondary active metabolites. This was conducted at the Laboratory of Pharmacology, Faculty of Medicine, Universitas Lambung Mangkurat, Banjarbaru.

a. Alkaloid test (Dragendroff test and Mayer test)
   1 ml of the infusion was added with 1 ml of Dragendroff reagent. Alkaloid test is considered as positive if red sediment is found. For Mayer test, 1 ml of the infusion was added with 1 ml of Mayer test. Mayer test is positive if yellow sediment is formed.
b. Flavonoid test (Alkaline reagent test and acetic-Pb test)
   1 ml of the infusion was added with a few drops of NaOH solution. Flavonoid is detected if yellowish solution is formed and gradually disappeared when weak acetic acid solution is added. For acetic-Pb test, 1 ml of the infusion was added with 1 ml of acetic-Pb 10%. The mixture was then shaken. Flavonoid test is positive if the mixture becomes yellowish brown.
c. Saponin test (Foam method)
   2 ml of the infusion was shaken with 2 ml of water. Saponin test is positive if foam is formed and lasts for about 10 minutes.
d. Terpenoid test (Salkowski's test)
   The infusion was added with chloroform and then filtered. The mixed solution was added with concentrated sulfuric acid. Terpenoid test is positive if goldish yellow solution is formed.
e. Phenol test (Ferri chlorida test)
   1 ml of the infusion was added with 1 ml of $FeCl_3$ 3%. Phenol test is positive if blackish green sediment is formed.
f. Steroid test (Libermann Burchard's test)
   The infusion was added with chloroform and then filtered. The mixed solution was added anhydrous acetic acid, and then heated and chilled. Concentrated sulfuric acid solution was slowly placed on the glasses wall. Steroid test is positive if brownish ring is formed.
g. Tanin test (Gelatin test)
   2 ml of the infusion was added with 2

ml of gelatin 1% containing NaCl. Tanin test is positive if white sediment is formed.

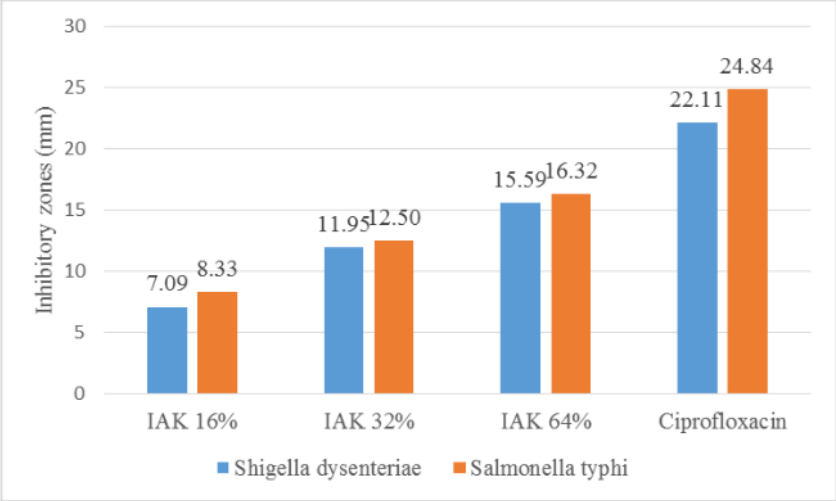
h. Antraquinone test (Brontrager test)
2 ml of the infusion was added with 5 ml of benzene. This mixed solution was added ammonia and then shaken. Antraquinone test is positive if red color is formed.

Antibacterial activity test for the infusion of akar kuning's root was performed by using disk diffusion method (Kirby-Baeur method). Bacterial isolates were incubated at 37^0C for 24 hours. An ose of isolate was placed into BHI media to make a growth suspension for bacteria. Bacteria was then incubated at 37^0C for 8 hours. Equalization process was done by adding aquadest until the turbidity was equal to a standard solution McFarland 1 (equal to $3x10^8$ cfu bacteria/ml). The equaled bacterial suspension was then swabbed with a sterile cotton rod on to MHA media. Disks were soaked in the infusion for 1 hour and then placed on to MHA media using a pair of tweezers. All media with the disks containing the infusion, the positive control, and the negative control were incubated at 37^0C for 24 hours. The inhibition of growth bacterial zone was determined from the diameters of limpid area formed around the disk (mm) by using a caliper. This test was conducted at The Laboratory of Microbiology, Faculty of Medicine, Universitas Lambung Mangkurat, Banjarmasin.

The data of the inhibition of bacterial growth for each treatment (the infusion, ciprofloxacin, and aquadest) were analyzed by using One-way ANOVA test followed by post-hoc test Least Significant Difference (LSD) and independent T-test at the confidence level of 95%. All the analyses were performed by using the statistical software IBM SPSS version 25.0 Phytochemical screening of akar kuning's root for their secondary active metabolites was described qualitatively.

RESULTS AND DISCUSSION

At the same MIC (16%), the infusion inhibited the growth of *S. dysenteriae* and *S. typhi* around the disks by 7.09 mm and 8.33 mm, respectively. Meanwhile at the highest concentration (64%), the inhibition zones for *S. dysenteriae* and *S. typhi* were 15.59 mm and 16.32 mm, respectively. Our study also demonstrated that the concentration is positively correlated with their inhibitory potency. The inhibitory of growth zone for each treatment was found in Figure 1.



IKA = the infusion of akar kuning's root

Figure1. Diameters of Inhibitory Zones for The Infusion of Akar Kuning's Root Against *Shigella dysenteriae* and *Salmonella typhi*

Our findings support previous studies done by Sukandar *et al* and Pratiwi. They found that the higher a concentration of extracts is given to bacteria, the bigger an inhibitory zone is formed.[18,19]

The statistical analysis using One-Way ANOVA demonstrated that the p-value was 0.000 ($p<0.05$) at 95% confidence interval. It indicates that there was a significance difference in the inhibitory zones among the treatments. In LSD post-hoc test, we then figured out that all treatment groups, were statistically difference including towards ciprofloxacin. Thus, all concentration of the infusion were not able to optimally inhibit the growth of both *S. dysenteriae* and *S. typhi* in comparison to ciprofloxacin.

Previous study conducted by Hasnawati and Yamin also demonstrated that akar kuning could inhibit the growth of *S. dysenteriae* and *S. typhi*.[9] This result is supported by the results of phytochemical screening of akar kuning's root as shown by Table 1.

Table 1. Phytochemical Screening on the Infusion of Akar Kuning's Root (*Fibraurea tinctoria* Lour.)

Metabolites	Tests	Results	Information
Flavonoid	Alkaline reagent test	+	Yellow solution disappear when weak acid added
	Pb-acetate test	+	Yellowish brown
Alkaloid	Dragendroff test	+	Red sediment
	Mayer test	+	Yellow sediment
Tanin	Gelatin test	-	No white sediment
Phenol	Ferri Chloride test	+	Blackish green sediment
Saponin	Foam method	+	Foam lasted for 10 minutes
Antraquinone	Antraquinone test	+	Red solution
Steroid	Leibermann Burchard's test	+	Brown ring
Terpenoid	Salkowski's test	+	Goldish yellow

Several active ingredients in akar kuning's root are potential as antibacteria to inhibit the growth of *S. dysenteriae* and *S. typhi*. These active ingredients are flavonoid, alkaloid, phenol, saponin, antraquinone, steroid, and terpenoid. Flavonoid denatures proteins of cell's walls and cytoplasmic membranes leading to the inhibition of bacterial growth.[20] Alkaloid inhibits topoisomerase enzyme. Thus, the walls of bacterial cells are not perfectly formed due to peptidoglycan forming components are destroyed by alkaloid.[21,22] Phenol interacts with bacterial cells through an absorption process involving hydrogen bonds and interfering internal milieu in bacterial cytoplasmic membranes.[23] Saponin is able to impair the permeability of cell's membranes leading to bacterial cell lysis.[24] Antraquinone is a phenolic group that inhibit bacterial synthesis by protein denaturation.[25] Steroid interacts with phospholipid membranes by decreasing and changing bacterial cell's walls integrity.[26] Terpenoid forms strong polymer bonds that destroy porins. It decreases bacterial cell's wall permeability. The lack of nutrition kills bacteria.[27]

Ciprofloxacin inhibited the growth of *S. dysenteriae* by 22.11 mm and *S. typhi* by 24.84 mm in average around the disks. According to Clinical and Laboratory Standards Institute (CLSI), ciprofloxacin has sensitive and intermediate activities against *S. dysenteriae* and *S. typhi,* respectively.[28] This antibiotic has similar mechanism of action with secondary active metabolites in akar kuning such as flavonoid and alkaloid.[20-22]

To compare the antibacterial efficacy of the infusion against *S. dysenteriae* and *S. typhi,* we then performed T-independent test. We found no significant antibacteria difference for the infusion at the same concentration (64%) for these bacteria (p=0.208). However, the infusion had bigger inhibitory zones against *S. typhi* than *S. dysenteriae* indicating that at the same concentration, the infusion has bigger antibacterial activity against *S. typhi* than *S. dysenteriae*. The differences in toxins and virulence factors for each bacteria are hypothesized to contribute. *dysenteriae* produces shiga toxin, an exotoxin. This bacteria also has virulence factors, i.e., polysaccharide as a smooth antigen that are able to invade erythrocytes and to proliferate, and generates toxins following penetrating the cells. *S. typhi* has virulence factors, i.e., lipopolysaccharide. This factor plays a role as an endotoxin dan is located at the outer layer of bacterial membrane. This bacteria has 3 major antigens, i.e., antigen O (somatic), antigen H (flagella), and antigen Vi (capsule).[2,13]

All inhibitory zones for the infusion were smaller than ciprofloxacin. The size of these zones might be influenced by bacterial structures. Gram negative bacteria, *S. dysenteriae* and *S. thypi* are enterobacteriaceae family. The wall structure of gram negative bacterial cells consist of thin peptidoglycan layers and contain many lipid components. Lipid and polisaccharide form lipopolisaccharide. This lipopolisaccharide has protein components ArcA, ArcB, and tolC that work as a pump to inhibit antimicrobials to invade into bacterial cells. The infusion might contain small quantity of secondary active metabolites, thus they are not able to optimally inhibit the growth of both *S. dysenteriae* and *S. typhi*.[2,13,29] Also, it might be caused by differences in extraction methods and solvents used.[30] Infusion method is used to extract thermolabile compounds in relatively short time, but a few of extracted compounds is gravitated once the solution is cold.[31] Furthermore, tanin as one of active compounds in akar kuning as antibacteria is not extracted by infusion, but easily by ethanol.[32]

CONCLUSION

Our study found that there was no statistically differences in antibacterial potency of the infusion of akar kuning's root against *S. dysenteriae* and *S. thypi* in vitro. However, the inhibitory growth zone for the infusion against *S. typhi* (16.32 mm) was bigger than against *S. dysenteriae* (15.59 mm) at the same consentration (64%).

For future studies we expect utilizing different extraction methods and solvents, increasing concentration of the infusion, testing specific active secondary metabolites for inhibiting the inhibitory growth zone from bacteria, increasing replication of treatment, and performing advanced tests such as in vivo research and toxicity tests prior to being used as phytopharmaca.

REFERENCES

1. Mutaqqin GME, Hartoyo E, Marisa D. Gambaran isolat bakteri aerob diare pada anak yang dirawat di RSUD Ulin Banjarmasin tahun 2015. *Berkala Kedokteran*. 2016; 12(1): 87-93.
2. Murray PR, Rosenthal KS, Pfaller MA. Medical microbiology. 8th ed. Philadelphia: Elsevier; 2016.
3. Riset Kesehatan Dasar (RISKESDAS). Badan Penelitian dan Pengembangan Kesehatan Departemen Kesehatan Republik Indonesia. 2018.
4. Sati HF, Bruinsma N, Galas M, et al. Characterizing *Shigella* species distribution and antimicrobial susceptibility to ciprofloxacin and nalidixic acid in Latin America between 2000-2015. *PLOS ONE*. 2019;14(8):1-15.
5. Puzari M, Sharma M, Chetia P. Emergence of Antibiotic resistant *Shigella* species: a matter of concern. *Journal of Infection and Public Health*. 2018; 11:451-454.
6. Rahman MA. Antimicrobial Resistance Patterns of *Salmonella typhi* Isolated from Stool Culture. *Chattagram Maa-O-Shishu Hospital Medical College Journal*. 2015; 14(1):26-30.
7. Noorcahyati. *Tumbuhan berkhasiat obat etnis asli Kalimantan*. Balikpapan: Balai Penelitian Teknologi Konservasi Sumber Daya Alam. 2012.
8. Heryani H, Nugroho A. Study of yellow root (*Arcangelisia flava* Merr.) as a natural food additive with antimicrobial and acidity –stabilizing effects in the production process of palm sugar. *Procedia Environmental Sciences*. 2015; 23: 346-50.
9. Hasnawati, Yamin. Potensi esktrak daun dan batang katola (*Arcangelisia flava* L. Merr) sebagai antimikroba. *Jurnal Farmasi, Sains, dan Kesehatan*. 2017; 3(2):23-7.
10. Maryani, Marsoedi, Nursyam H, dan Maftuch. The phytochemistry and the anti-bacterial activity of yellow root (*Arcangelisia flava* Merr.) against Aeromonas hydrophila. *Journal of Biology and Life Science*. 2013; 4(2):180-90.
11. Kaharap AD, Mambo C, Nangoy E. Uji efek antibakteri ekstrak batang akar kuning (*Arcangelisia flava* Merr.) terhadap bakteri *Staphylococcus aureus* dan *Escherichia coli*. *Jurnal e-Biomedik*. 2016; 4(1): 1-4.
12. Maryani, Rosita, Monalisa SS, dan Rozik M. In vitro test of natural antibacterial activity of yellow-fruit moonseed *Arcangelisia flava Merr*. leaf on bacterium *Pseudomonas* fluorocens under different doses. *AACLBioflux*. 2018; 11(1): 288-94.
13. Brooks GF, Karen CC, Janet SB, Stephen AM, Timothy AM. Jawetz, Melnick & Adelberg's *Medical microbiology. 26th ed*. New York: Mcgraw Hill Medical. 2013.

14. Gunawan SG. *Farmakologi dan terapi*. Jakarta: Balai Penerbit FK UI; 2011.
15. Katzung BG, Masters SB, Trevor AJ. *Farmakologi dasar dan klinik. Edisi XII*. Jakarta: Salemba Medika. 2013.
16. Marliana SD, Suryanti V, Suryono. Skrining fitokimia dan analisis kromatografi lapis tipis komponen kimia labu siam (*Sechium edule* Jacq. Swartz.) dalam ekstrak etanol. *Biofarmasi*. 2005;3(1):27.
17. Sholihah MA, Wan Rosli WI, Nurhanan AR. Phytochemicals screening and total phenolic content of Malaysian *Zea mays* hair extract. *International Food Reviews Journal*. 2012; 19(4): 1534.
18. Sukandar D, Radiastuti N, Jayanegara I, Hudaya A. Karakterisasi Senyawa Aktif Antibakteri Ekstrak Air Bunga Kecombrang (*Etlingera elatior*) Sebagai Bahan Pangan Fungsional. *Valensi*. 2010; 2(1): 333-339.
19. Pratiwi S. Uji efektivitas ekstrak daun cincau hijau rambat (*Cyclea barbata* Miers.) sebagai antibakteri terhadap *Bacillus cereus* dan *Shigella dysenteriae* secara *in vitro* dengan metode difusi.[skripsi]. Universitas Pembangunan Nasional Veteran Jakarta: 2016.
20. Farhadi F, Khameneh B, Iranshahi M, Iranshahy M. Antibacterial activity of flavonoids and their structure-activity relationship: an update review. *Phytotherapy Research*. 2018;1-28.
21. El-sakka MA. *Phytochemistry (3) alkaloids*. Al Azhar University. 2010.
22. Mariajancyrani J, Chandramohan G, Saravanan, Elayaraja A. Isolation and antibacterial activity of terpenoid from *Bougainvillea glabra* choicy leaves. *Pelagia Research Library*. 2013;3(3):70-73.
23. Putri DD. Nurmagustina DE, Chandra AA. Kandungan total fenol dan aktivitas antibakteri kelopak buah rosela merah dan ungu sebagai kandidat feed additive alami pada broiler. *Jurnal Penelitian Pertanian Terapan*. 2014; 14(3): 174-180.
24. Arabski M, Weglerek-Cluk A, Czerwonka G, Lankoff A, Kaca W. Effects of saponin againts clinical *E. coli* strains and eukaryotic cell line. *Journal of Biomedicine and Biotechnology*. 2012; 1-6.
25. Sindora G, Allimudin AH, Harlia. Identifikasi golongan senyawa antraquinon pada fraksi kloroform akar kayu mengkudu (*Morinda citrifolia* L). *Journal Kedokteran dan Kesehatan*. 2017; 6(1): 37-41.
26. Sudarmi K, Darmayasa IBG, Muksin IK. Uji fitokimia dan daya hambat ekstrak daun juwet (*Syzygium cumini*) terhadap pertumbuhan *Escherichia coli* dan *Staphylococcus aureus* ATCC. *Jurnal Simbiosis*. 2017; (2): 47-51.
27. Rahman FA, Haniastuti T, Utami TW. Skrining fitokimia dan aktivitas antibakteri esktrak etanol daun sirsak (*Annona muricata* L) pada *Streptococcus mutans* ATCC 35668. *Majalah Kedokteran Gigi Indonesia*. 2017; 3(1): 1-7.
28. Clinical and Laboratory Standards Institute. *Performance Standards for Antimicrobial Susceptibility Testing*. 28th Edition. 2018;35-36.
29. Kuete V, Ngameni B, Tangmouo JG, Bolla J. Efflux pumps are involved in the defense of gram negative bacteria against the natural product isobavachalcone and diospyrone. *Antimicrobial Agents for Chemothereapy*. 2010; 54(5): 1749-52.

30. Verdiana M, Widarta IWR, Permana IDGM. Pengaruh jenis pelarut pada ekstraksi menggunakan gelombang ultrasonik terhadap aktivitas antioksidan ekstrak kulit buah lemon (*Citrus limon* (Linn.) Burm. F). *Jurnal Ilmu dan Teknologi Pangan*. 2018; 7(4): 213-222.
31. Isnawati AP, Retnaningsih A. Perbandingan teknik ekstraksi maserasi dengan infusa pada pengujian aktivitas daya hambat daun sirih hijau (*Piper betle* L.) terhadap *Escherichia coli*. *Jurnal Farmasi Malahayati*. 2018; 1(1): 19-24.
32. Hartati, Syamsuddin B, Karim H. Pengaruh jenis pelarut terhadap kandungan senyawa metabolit sekunder klika kayu jawa (*Lannea coromendelica*). *Jurnal Sainsmat*. 2019;8(2):19-27.

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