

EFFECTIVENESS OF BOILED SAPODILLA LEAVES (*Manilkara zapota*) IN REDUCING THE NUMBER OF BACTERIA AND ESCHERICHIA COLI GERMS ON EATING UTENSILS

Laili Agustina, Rahmawati, Erminawati, Abdul Haris

Banjarmasin Ministry of Health Polytechnic, Department of Environmental Health

Jl. H. Mistar Cokrokusumo No. 1A Banjarbaru South Kalimantan 70714

E-mail: laili4gustina@gmail.com

Article Info

Article history:

Received July 30, 2023

Revised June 30, 2024

Accepted July 01, 2024

Keywords:

Sapodilla leaf boiled water

Escherichia coli

Germ count

Sanitizing

ABSTRACT

Effectiveness of Boiled Sapodilla Leaves (*Manilkara zapota*) in Reducing the Number of Bacteria and Escherichia coli Germs on Eating Utensils. Several studies have shown that the high number of germs in cutlery is due to the inability to carry out the sanitizing process. The sanitizing process can be done physically and chemically, using natural ingredients that contain active compounds such as flavonoids, tannins, and saponins, which function as antibacterials, such as sapodilla leaves. Sapodilla leaf boiled water can be a simple disinfectant for society to improve the quality of cutlery hygiene. The study aimed to determine the concentration of sapodilla leaf boiled water, which effectively reduces *E. coli* and the number of germs. The statistical test used was Kruskal Wallis, followed by a nonparametric post hoc test. A total of 24 swab samples were used with eight treatments and three repetitions. The results showed that the examination for the presence of *E. coli* on cutlery was negative. Examination of the germ count showed that the highest germ rate was in the control and the lowest was 10%. Statistical test results showed that a concentration of 10% is effective in reducing the germ number. This study concludes that *E. coli* in the samples were all negative, and the effective concentration in reducing the number of germs on cutlery is 10%, reducing from 60,768.11 colonies/cm² to 12 colonies/cm². Suggestions for future researchers are to conduct trials using other extraction methods.

This is an open access article under the [CC BY-SA](#) license.



INTRODUCTION

Toxin-producing microbes can cause food poisoning, an undesirable event that arises from the food production process due to biological, chemical, and foreign matter contamination, potentially endangering human health [1]. According to the 2021 BPOM report, extraordinary incidents of food poisoning occurred in 22 provinces in 2021. Food is the cause Food from household cooking, catering services, snacks, and restaurants can cause poisoning [2]. Microbial contamination is contamination in food that comes from microbes, which can be detrimental to human health [1].

One source Unclean eating utensils cause food contamination. The process of washing cutlery with the right washing method is important to reduce the number of germs, especially on cutlery. [3]. The table of tableware provisions outlines the method of washing, drying, and storing equipment to ensure it is always clean before use, in good condition, and not damaged. Eating and drinking utensils must not have germ levels that exceed the existing threshold values; the surfaces of utensils that come into direct contact with food must be smooth and

free of dead corners; utensils in direct contact with food must not contain any dangerous substances [4].

The Republic of Indonesia Minister of Health Regulation Number 1096 of 2011 requires the storage of cleaned equipment and food in a location free from animal contamination. [5]. According to Budon, it is necessary to carry out the washing stages of scraping, flushing, washing, rinsing, disinfection, and toweling to ensure that tableware meets sanitation requirements. Disinfection is the process of eradicating pathogenic microbes on objects' surfaces. You can physically sterilize cutlery by immersing it in hot water, hot air (oven), UV rays from sunlight, and hot steam (steam), or chemically by immersing it in a chlorine solution. [6]

According to research by Merceline et al., the bacteriological quality of cutlery in sub-district restaurants (Mahakeret West and East) does not meet the requirements because the number of germs exceeds health standards, and 2 out of 6 restaurants, or 33.3%, are positive for *E. coli* [7]. According to RI Minister of Health Regulation No. 14 of 2021, the biological parameter under examination is *E. coli*, a sign of fecal contamination in food and eating utensils [8]. According to Amaliyah, factors that influence the number of germs on cutlery include the cutlery's basic material, its initial condition, water, the tub, the washing power, and the scrubber [9]. The neglect of the disinfection process results in the cutlery not meeting the required cleanliness standards.

Disinfection can be done by immersing in 100°C hot water for 2 minutes, soaking in active chlorine solution for 2 minutes, using hot air, using ultraviolet light (in the morning from 9 to 11), or using electrical equipment that produces ultraviolet and hot steam, which is usually found in dishwashers [10]. Islamiyah's research results show that the juice of kaffir lime leaves can reduce the number of germs on dishes [11]. Useful plants, such as leaves, stems, roots, fruit, and flowers, can serve as alternative medicines. Sawo manil is one such plant [12]. Various countries, including Indonesia, have preserved Sapodilla, a tropical plant that adapts easily. Sapodilla is widely planted in home gardens and is easily available on the market [13]. According to Dr. CGGJ Van Steenis [14], the Manila sapodilla plant occupies a specific taxonomic position.

Kingdom	: Plantae
Division	: Magnoliophyta
Class	: Magnliopsida
Nation	: Ebenales
Ethnic group	: Sapotaceae
Clan	: Manilkara
Type	:Manilkarazapota

The sapodilla part You can use the leaves as an alternative medicine to relieve fever, bleeding, wounds, or boils [15]. According to Yunika, tannin and flavonoid compounds are found in many young fruits, skin, stems, and sapodilla leaves [15]. According to Mustary, people use fruit, bark, and leaves as a traditional medicine for diarrhea. The tannin compounds contained in it can inhibit and even kill bacteria, one of which is *E. coli* [16]. *E. coli* bacteria are also indicators of drinking water quality because their presence in water indicates that the water is contaminated by feces [17]. Tannin acts as an antibacterial by in plants the formation of the bacterial cell wall, which causes cell chromosomes,[18]. Antibacterials are substances that inhibit growth and even kill the DNA. Bacteria a annoyingly metabolism microbes detrimental [19]. The bioactivity of Sapodilla leaves is anti-inflammatory, antibacterial, antidiarrheal, and so on [20].

Pratiwi et al. conducted an experiment on sapodilla root extract to determine its antibacterial activity against *E. coli* and *S. aureus*. They used a maceration extraction method with 5 ppm methanol and chloroform and compared it with the negative control [21]. Apart from maceration, boiling is the way to remove the active compounds in sapodilla leaves. The boiling method is an easy way that people can use to make disinfectants because it only requires

water as a solvent. Research by Anggarini et al. stated that boiled sapodilla leaf water has an inhibitory effect on *E. coli* of 13.3 mm at a temperature of 60 °C for 30 minutes. [22].

MATERIALS AND RESEARCH METHODS

We conducted experiments using varying weights of sapodilla leaves, specifically 40, 50, 60, 70, 80, 90, and 100 grams, boiling them for 30 minutes in 1 liter of water to achieve concentrations of 4%, 5%, 6%, 7%, 8%, 9%, and 10%. We carried out each treatment, including the control, three times. The ingredients used are water and old sapodilla leaves. We collected data by examining swab samples of cutlery on 24 randomly selected plates. The Environmental Health Microbiology Laboratory at Banjarmasin Health Polytechnic conducted the examination. We collected the cutlery from one of the stalls in Pekauman Ulu Village, East Martapura. We used the Kruskal-Wallis statistical test, followed by a nonparametric post hoc test [23].

RESEARCH RESULTS AND DISCUSSION

The results of *E. coli* examination on cutlery swabs, before and after disinfection treatment using sapodilla leaf boiled water are presented in table 1.

Table 1. Results of *E. Coli* examination on plate cutlery swabs

test	Treatment/Concentration							
	Control 0%	A 4%	B 5%	C 6%	D 7%	E 8%	F 9%	G 10%
1	negative	negative	negative	negative	negative	negative	negative	negative
2	negative	negative	negative	negative	negative	negative	negative	negative
3	negative	negative	negative	negative	negative	negative	negative	negative

Table 1 shows that no *E. coli* bacteria were found on the plates.

Table 1 presents the results of the *E. coli* examination on cutlery before and after disinfection using boiled water from sapodilla leaves.

Table 2 presents the results of examining germ numbers on cutlery swabs before and after disinfection treatment using sapodilla leaf-boiled water.

Table 2. Results of checking germ numbers on plate cutlery swabs

Test	Number of bacteria (colony/cm ²)							
	Control 0%	A 4%	B 5%	C 6%	D 7%	E 8%	F 9%	G 10%
1	2,238	167	99,417	11,915	525	10	50	15
2	26,570	122,500	2	130	9	17	1	9
3	153,496	84	380	8,985	19	63	6	11
Average	60,768	40,917	33,266	7,010	185	30	19	12

According to table 2, the number of germs in the control sample is higher than the number of germs in the treated swab samples. The highest concentration of germs is 4%, and the lowest is 10%.

Figures 1, 2, and 3 show graphs of germ numbers with sapodilla leaf-boiled water disinfection treatment.

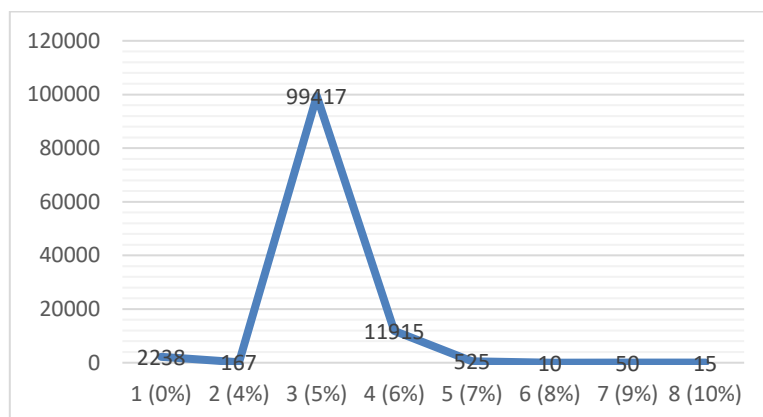


Figure 1 Graph of Germ Numbers in Repetition 1

In repetition 1, the number of germs at 5% concentration increased because there was a possibility of contamination during sampling. Germ numbers decrease at concentrations of 7% to 10%.

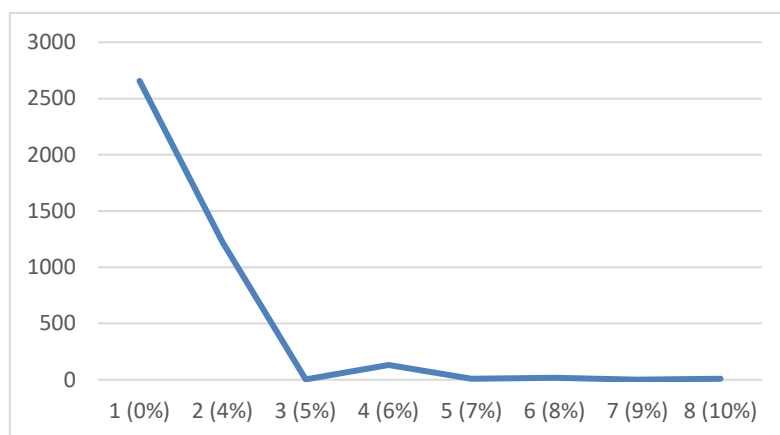


Figure 2 graph of repetition germ number 2

In repetition 2, the germ number at a concentration of 0% (control) was high, then decreased at a concentration of 5%. There was an increase at a concentration of 6%, but the number was below 500. Then, at a concentration of 7% to 10%, the germ number fell again.

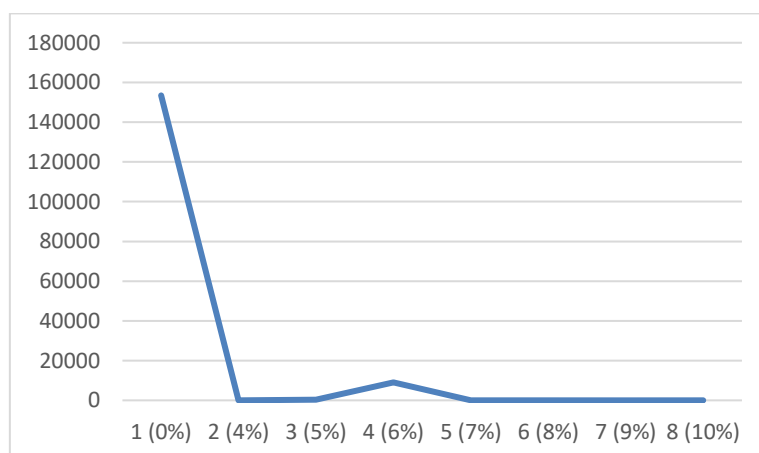


Figure 3 graph of repetition germ number 3

In repetition 3, the number of germs was high at a concentration of 0% and decreased at a concentration of 2%. At a concentration of 6%, there was an increase, followed by a decrease again at a concentration of 7% to 10%.

Table 3 Kruskal Wallis Statistical Test Results

Sig.	Conclusion	Information
0.035	H0 is rejected	There is a significant difference between variations in the concentration of sapodilla leaf boiled water and the reduction in the number of germs on eating utensils

Based on table 3, it can be seen that the p value of 0.035 is smaller than α (0.05), so H0 is rejected, and Ha is accepted. The results of the nonparametric post hoc test showed that only the treatment with a concentration of 10% was different from the treatment with a concentration of 0% or the control [24].

p value the obtained result, 0.028, falls below the threshold. The table's p value shows different concentrations between treatments. The p-value matrix table in the Kruskal-Walli's test results is as follows:

Table 4 Sig Value Matrix. Kruskal wallis Advanced Test

	0	4	5	6	7	8	9	10
0								
4	0.488							
5	0.225	0.603						
6	0.563	0.908	0.525					
7	0.184	0.525	0.453	0.453				
8	0.078	0.165	0.386	0.133	0.453			
9	0.009	0.057	0.165	0.043	0.204	0.603		
10	0.001	0.009	0.038	0.007	0.049	0.225	0.489	

The matrix above demonstrates that a concentration of 9% differs from a concentration of 0%, and a concentration of 10% differs from a concentration of 0% due to a smaller p value, indicating the rejection of H0 and acceptance of Ha. Concentrations of 9% and 10% are effective in reducing the number of germs on eating utensils.

Table 5 shows the percentage reduction in germ numbers by using sapodilla leaf-boiled water on eating utensils.

Table 5 Percentage reduction in germ numbers

Concentration	Average TPC (colonies/cm2) Control 0%	Decline (N)	Percentage (%)
4%	40,917	19,869.13	33%
5%	33,266	27,501.87	46%
6%	7,010	53,758.31	88.4%
7%	60,768.11	185	99.696%
8%	30	60,738.21	99.950%
9%	19	60,748.91	99.968%
10%	12	60,756.31	99.980%

Table 5 demonstrates that boiling sapodilla leaf water can significantly reduce the number of germs on cutlery, with a reduction percentage ranging from 88.4% to 99.980% at concentrations of 6% to 10%. According to Yunika, the chemical compounds contained in sapodilla plants are tannins and flavonoids found in young fruit, skin, stems, and leaves. Tannins play a role in damaging bacterial cell walls, while flavonoids inhibit bacterial energy metabolism, so that sapodilla leaf-boiled water can reduce the number of germs on cutlery. [15].

You can use leaf sapodilla as an antibacterial on cutlery. According to Hasyim et al.'s research, manila sapodilla leaves have antibacterial activity against *E. coli* growth [25]. Statistically, the concentration that effectively reduces the number of germs on eating utensils is between 9% and 10%.

CONCLUSIONS AND RECOMMENDATIONS

Inspection *E. coli* on cutlery was negative, and there was a difference in the decrease in the number of germs on cutlery based on variations in the concentration of sapodilla leaf decoction. The highest percentage reduction in germ numbers is 99.980%, and the lowest is 33%. Amount At 0% concentration, the germ number was 60,768.11 colonies/cm². The lowest concentration in reducing germ numbers is 4%, namely 40,917 colonies/cm², and the highest concentration in reducing germ numbers is 10%, namely 12 colonies/cm².

The concentration of sapodilla leaf-boiled water is effective in reducing the number of germs on eating utensils by 8%. This concentration is able to reduce the number of germs by 99.950%, namely from 60,768.11 colonies/cm² to 30 colonies/cm². The statistical analysis reveals a significant difference between the 10% and 0% concentrations, with an adj.sig value of 0.028. Thus, treatment with a concentration of 10% is effective in reducing the number of germs on eating utensils.

REFERENCES

1. BPOM. Pedoman Kriteria Cemarkan pada Pangan Siap Saji dan Pangan Industri Rumah Tangga [Internet]. 2012. Available from: https://standarpangan.pom.go.id/dokumen/pedoman/Buku_Pedoman_PJAS_tentang_Cemarkan.pdf
2. BPOM. Laporan tahunan 2021 Badan Pengawas Obat dan Makanan. WwwwPomGold 2022;
3. Marisdayana R, Harahap PS, Yosefin H. Teknik Pencucian Alat Makan, Personal Hygiene Terhadap Kontaminasi Bakteri Pada Alat Makan. *J Endur* 2017;2(3):376.
4. Tumelap HJ. Tikala Manado. Kondisi Bakteriologi Peralat Makan Di Rumah Makan Jombang Tikala Manad 2011;1(1):20–7.
5. PERMENKES No. 1096/MENKES/PER/VI/2011 Tentang Hygiene Sanitasi Jaskaboga. *Angew Chemie Int Ed* 6(11), 951–952 1967;2008(Mi):5–24.
6. Budon AS. Studi Kualitas Bakteriologi Air Pencucian Dan peralatan Makan Di Kantin UIN Makasar. آب و خاک 2013;(May):106.
7. Telew M, Joseph WBS, Pinontoan O, Masyarakat FK, Ratulangi US. Gambaran Angka Kuman Dan Keberadaan *Escherichia Coli* Pada Peralatan Makan Rumah Makan Di Kelurahan Mahakeret Barat Dan Mahakeret Timur Kecamatan Wenang Kota Manado. *Kesmas* 2019;7(5):5–8.
8. DepKes. Standar Kegiatan Usaha dan Produk Pada penyelenggaraan Perizinan Berusaha Berbasis Risiko Sektor Kesehatan. Permenkes No 14 2021;455.
9. Amaliyah N. No Title. Yogyakarta: Deepublish; 2017.
10. Amaliyah N. Penyehatan Makanan Minuman. Yogyakarta: Deepublish; 2017.
11. Islamiar AN, N, Rahayu U. Efektivitas Perasan Daun Jeruk Purut Terhadap Angka Kuman

- Pada Peralatan Makan Tahun 2017. Gema Lingkung Kesehat 2017;15(3):50–5.
12. Prihardini, Wiyono AS. Pengembangan Dan Uji Antibakteri Ekstrak Daun Sawo Manila (Manilkara Zapota) Sebagai Lotio Terhadap Staphylococcus aureus The Development And Antibacteria Test Of Manila Sapodilla Leaf (Manilkara Zapota) AS A LOTIO TO Staphylococcus aureus. J Wiyata, 2019;2(1):87–92.
 13. Puspaningtyas desty ervira. The Miracles of Fruits. PT AgroMedia Pustaka; 2013.
 14. Steenis CGG. Van. Flora. Kebon Raya Indonesia; 1953.
 15. Yunika N. Konsentrasi Hambat Minimum Ekstrak Daun Sawo (Achras Zapota L.) Terhadap Pertumbuhan Staphylococcus Aureus Secara In Vitro. Bioscience 2017;1(1):53.
 16. Mustary M, Djide MN, Mahmud I, Hasyim N. Uji Daya Hambat dan Analisis KLT-Bioautografi Perasan Buah Sawo Manila (Achras zapota Linn) Terhadap Bakteri Uji Salmonella Thyposa. Angew Chemie Int Ed 6(11), 951–952 [Internet] 2018;3(1):10–27. Available from: <https://medium.com/@arifwicaksanaa/pengertian-use-case-a7e576e1b6bf>
 17. Rahayu WP, Nurjanah S, Komalasari E. Escherichia coli: Patogenitas, Analisis, dan Kajian Risiko. J Chem Inf Model 2018;1(5):1–151.
 18. Mufti N, Bahar E, Arisanti D. Uji Daya Hambat Ekstrak Daun Sawo terhadap Bakteri Escherichia coli secara In Vitro. J Kesehat Andalas 2017;6(2):289.
 19. M M. Brock Biology of Microorganisme. london: PrenticeHall; 2005.
 20. Islam MR, Parvin MS, Banu MR, Jahan N, Das N, Islam ME. Antibacterial and phytochemical screening of ethanol extracts of Manilkara zapota leaves and bark. Int J Pharma Sci [Internet] 2013;3(6):394–7. Available from: <http://ijps.aizeonpublishers.net/content/2013/6/ijps394-397.pdf>
 21. Pratiwi N, Retnosari R, Prabaningtyas S. Preliminary Study on Antibacterial Activity of Sawo Kecik (Manilkara kauki (L.) Dubard) Roots Extract. J Biodjati 2021;6(1):146–52.
 22. Dewi Anggraini IAK, Trisna Darmayanti LP, Sugitha IM. Pengaruh Lama Perebusan Pada Pembuatan Minuman Herbal Daun Sawo (Manilkara zapota) terhadap karakteristik dan daya hambat pertumbuhan Escherichia coli. J Ilmu dan Teknol Pangan 2020;9(3):272.
 23. Indah SJ. Uji alternatif kruskal wallis & post hoc. 2023.
 24. Purwanto. Statistika untuk Penelitian. Yogyakarta: Pustaka belajar; 2001.
 25. Hasyim, Muh F, Patandung G, Irfiana. Uji Aktivitas Antibakteri Infusa Daun Sawo (Manilkara zapota L) Terhadap Escherichia coli. Pap Know Towar a Media Hist Doc 2014;16–9.

