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Effect of Black Garlic Extract in Feminine Liquid Soap as Antibacterial (*Escherichia coli*)

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Abstract: One of the causes of infection is pathological vaginal discharge caused by *Escherichia coli*. Handling of this infectious disease is still limited to giving antibiotics. So, an alternative therapy is needed using herbs. Black garlic is a fermented product that has stronger antibacterial properties. Based on this, it is necessary to test the potential of liquid soap with black garlic extract as antibacterial as scientific evidence. Research Objectives: To analyze the effect of black garlic extract on women's liquid soap on the growth of *Escherichia coli*. This research is quantitative research with an experimental research design. In this study, the intervention given was giving black garlic extract in feminism hygiene liquid soap preparations with various doses for bacteria *Escherichia coli* with disc diffusion technique. Black garlic extract was tested for phytochemicals to determine the content of active compounds. The results showed that black garlic extract in feminine hygiene liquid soap inhibited the growth of *Escherichia coli* bacteria; at a concentration of 40%, black garlic had the highest antibacterial activity with an average inhibition zone of 12.7mm. Therefore, it is proven that black garlic extract is effective as an antibacterial *Escherichia coli* in the formulation of feminine hygiene liquid soap.
Keywords: Black garlic; *Escherichia coli*; feminim hygine; liquid soap.

INTRODUCTION

The causes of maternal death in Indonesia are bleeding (30%), hypertension in pregnancy (25%), infection (6%), and others (39%)[1]. Even though the infection has a small percentage, prolonged infection can harm the mother and fetus. One of the causes of infection that needs attention is a pathological vaginal discharge caused by bacteria: *E. Coli, S. auerus, Enterococci*. The normal vaginal flora is replaced by microorganisms which may originate from the gut and most commonly are *E. coli* bacteria[1,2]. Aerobic bacteria increase three to five times in the vagina of patients with vaginal discharge compared to normal vaginal microflora[3]. A recent Indian study found that of patients presenting with abnormal vaginal discharge, 15.0% were diagnosed with AV (Aerobic Vaginitis) by culture, including infections with *Escherichia coli* (7.5%), *Staphylococcus aureus* (4.5%), *Klebsiella* (2.0%), and *Enterococcus* species (1.0%)[4]. *Escherichia coli* associated with AV is extraintestinal *E. coli*. *E. coli* infection can cause abortion, PROM, preterm labor, and IUFD[5]. AV (Aerobic Vaginitis) has several characteristics, such as a

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reduced number of colonies or even absence of *Lactobacillus* colonies, foul odor in the vagina, and increased pH; the vagina of women with AV often appears red and edematous and may even show small erosions or ulcerations. Meanwhile, vaginal discharge in patients with AV is yellow to green and slightly thick and slimy[6]. AV is a vaginal infection often found in women of reproductive age, which is 19-24%[7]. AV is associated with a six-fold increase in HIV transmission. Leucorrhoea in pregnancy can increase the risk of abortion, premature labor, low birth weight, premature rupture of membranes, and postpartum endometritis[8].The results of a study report that women with AV have a risk of experiencing preterm labor 3-8 times higher than women who have flora normal. Likewise, the incidence of premature rupture of membranes was experienced more by women with AV (46%) than by women who did not suffer from AV (4%)[9].

Until recently, the most common treatment for AV was using antibiotics, metronidazole, or clindamycin. These antibiotics can be administered orally or intravaginally. However, these two drugs have been unable to prevent AV events' recurrence. As many as 30% of women experience relapse within a month after therapy, especially patients who use intravaginal therapy[6]. Therefore, there is a need for alternative therapies to assist in the treatment and prevention of AV recurrence[4]. According to Halimah (2016), it is necessary to find new agents and treatments that are more effective in inhibiting the activity of vaginal aerobic bacteria and have lower side effects, one of which is using traditional ingredients[10]. Black garlic is a fermented product from garlic that is heated at a certain temperature with a humidity of 70 – 80% of room temperature for one month[1]. Black garlic contains phenolic antioxidants, Flavonoids, S-Allylcysteine, and the DPPH-radical scavenging activity strong[11]. Black garlic has stronger antibacterial properties and two times higher antioxidants than regular garlic because it contains S-allycysteine[12].

To maintain the female organ system, women usually use cleansing liquid to clean it. According to Marhaeni (2016), the way to prevent leucorrhoea is to maintain the cleanliness of the genitals using vaginal washing products that are by the normal pH of the vagina, namely 3.8-4.2[13]. There has been research on the effectiveness of black garlic ethanol extract in inhibiting the growth of Streptococcus pneumoniae and Klebsiella pneumoniae[10], but other research is needed to further expand scientific data, especially on processed feminine hygiene soap products from black garlic in its function as an antibacterial, especially against *Escherichia coli*, so this study aims to analyze the effect of black garlic extract on women's liquid soap on the growth of *Escherichia coli*.

MATERIALS AND METHODS

In this study, the treatment/intervention was giving black garlic extract in feminism hygiene liquid soap preparations with various doses for bacteria *Escherichia coli* with disc diffusion technique. Black garlic extract was tested for phytochemicals to determine the content of active compounds.

Materials and Tools Used in Research

Black garlic uni cloves, methanol, hexane, dichloromethane, distilled water, ethanol 80%, Mg powder, 0.5 M HCl, 5% NaNO2,10% AlCl3•6H2O and 1 M NaOH, NH3 vapor, DPPH (1,1-diphenyl-2-picrylhydrazyl), and quercetin standard. Equipment used in this study, namely glassware, knives, rotary evaporator B-ONE modern RE 1000VN,

blender, vortex, magnetic stirrers, ovens, analytical balances, filter cloths, andUV-VIS spectrophotometer instrument (Ultraviolet-Visible) Genesys.

Black Garlic Sample Collection

Black garlic is processed from garlic (*Allium sativum*), which is obtained from Berkat Karunia Processing Center for Medicinal Plants located in the city of Denpasar, Bali Province, Indonesia. Garlic 100 grams is heated in an oven at 70^{o}C for 28 days. The result of heating black garlic is obtained. Once it's done, extraction by maceration method. Results Black garlic extract was tested for phytochemicals to see the content of active compounds and antibacterial activity.

Black Garlic Extraction

Black garlic as much as 100 grams. Grind until you get onion powder black. Black garlic powder is extracted by maceration and 1,000 ml methanol as the solvent. Extraction results were then filtered. The filtered residue is re-macerated again with the aim that all active compounds can be extracted into the solvent. Filtrate is collected and placed in the rotary evaporator at room temperature for 24 hours to evaporate the solvent so that the black garlic extract is obtained.

Figure 1. Black Garlic

Black Garlic Extract Phytochemical Test

Phytochemical tests were carried out to identify the active compounds in black garlic qualitatively. The active compounds tested were alkaloids, tannins, saponins, sterols, and flavonoids.

The alkaloid test was done using 1 ml of 2N hydrochloric acid, and 9 ml of water was added, heated over the bath for 2 minutes, cooled, and filtered. The phytate obtained is used for the following experiment: Take three drops of filtrate, then add two drops of Mayer's arcsin to yield a white/yellow precipitate. Take three drops of filtrate, then add two drops of Boacardad reagent to produce a dark brown precipitate. Take three drops of the filtrate, then add two drops of reagent Dragendorf, producing a brick-red precipitate. If there is a white precipitate at least with 2 or 3 of the tests above, the simplicia is declared to contain alkaloids[14].

The tannin test was carried out by taking as much as 3 mL of black garlic extract, then extracted with hot distilled water, and then cooled. After cold, five drops of 10% NaCl were added and later filtered. The resulting filtrate is added FeCl3 5%. The presence of tannins is indicated by a dark brown precipitate formed[15].

The Saponin test was carried out using Fort's method. Extract sample 2 mL of black garlic is put in the tube reaction added 10 mL of distilled water and shaken for 30 seconds. If there is the foam that does not disappear for 30 seconds, it indicates the

presence of saponins[14]. The sterol test was carried out by the method Salkowski. Black garlic extract dissolved in chloroform was then filtered, and The filtrate was used for testing. Solution: Add a few drops of concentrated sulfuric acid and observe the color change. If the red color formed on the bottom layer contains sterols, a golden yellow indicates triterpenoids[16].

The Flavonoid test was carried out by the method of Bate Smith-Metchalf. Black garlic extract of 3 mL was evaporated and then washed with hexane until clear. The residue was dissolved in 20 mL of ethanol and further filtered. The filtrate was added with 0.5 mL HCl concentrated, then heated with heated water if the red color changes show positive results[15].

Preparation of Liquid Soap Base

Liquid soap preparation containing black garlic extract is made in 3 formulations with different concentration variations, namely 10%, 20%, and 40% by extracting black garlic in the preparation of female liquid soap weighed as much as 1 gram, 2gr, 4gr then each dissolved with 10ml aquadest.

Table 1. Black Garlic Feminim Hygine Liquid Soap Formulation

Materials	Amount			**Unit**
	10%	**20%**	**40%**	
Ekstrak Black Garlic	1	2	4	Gram
Sodium Lauril Sulfat (SLS)	180	180	180	Gram
Nacl	65	65	65	Gram
Propilenglikol	26	26	26	ml.
Carbocylic	1,3	1,3	1,3	Gram
EDTA	0,5	0,5	0,5	Gram
Propil Paraben	2,9	2,9	2,9	ml.
Fragrance	1	1	1	%
Aquadest *add*	1	1	1	L

Preparation of Filter Paper Disc

Antibacterial activity test was carried out by testing the inhibition power of black garlic extract in the preparation of female liquid soap using the diffusion method using a paper disk with a diameter of 6 mm immersed in various concentrations of black garlic extract in different female liquid soap preparations, in negative control using sterile distilled water and positive controls using antibiotics amoxicillin hcl 25 ug for 5 minutes.

Antibacterial Activity Test

Testing the antibacterial activity of black garlic extract in feminine hygiene liquid soap was carried out using the paper disc diffusion method. *Escherichia coli* bacteria

grown on McConkey agar (BD), Muller Hinton Agar (BD) as a test medium for bacterial inhibition, and 0.5% Mc Farland standard (1% sulfuric acid 99.5 mL and 1.175% barium chloride dihydrate 0.5 mL) were obtained from the Bali International University Biology laboratory. Sterilize all tools and materials to be used; put 1 ml of *Escherichia coli* bacteria suspension equivalent to 0.5% Mc Farland standard into a sterile petri dish, pour 15 ml of Mueller Hinton Sterile agar media, homogenize, and let stand until the media hardens, place the disc paper that has been soaked with various concentrations on the surface of the Mueller Hinton Agar medium using sterile tweezers, incubate for 18 hours at 370C, observe the results by measuring the inhibition zone in the form of an area not overgrown with bacteria with a caliper, record the results in millimeters (mm), the experiment was carried out in triple (3 Petri dishes). Approval for this study was obtained from the Ethics Committee of ITEKES Bali 03.0376/KEPITEKES-BALI/VIII/2023.

RESULTS AND DISCUSSION

Extraction Results Of Black Garlic Extract

Assay phytochemicals performed a qualitative test of the active compound content of black garlic extract. Based on the phytochemical test negative, black garlic extract content contains alkaloids and positively contains tannins, saponins, sterols, and flavonoids.

Table 2. Black Garlic Extract Phytochemical Test

Active compound	Phytochemical Test	Result	Eksplanation	Meaning
Alkaloid	HCL 2M + Wagner	-	No precipitate	Does Not Contain Active Compounds
Tanin	Nacl 10% + $FeCL_3$ 5%	+	Dark brown	Contains active compounds
Saponin	Aquadest	+	A little foamy	Contain active compounds
Sterol	Kloroform+ H_2SO_4 Pekat	+	Golden yellow (triterpenoid)	Contain active compounds
Flavonoid	Etanol +HCL pekat + mg powder	+	Yellow	Contains active compounds

The results of the phytochemical screening examination of black garlic extract showed that it contained a group of chemical compounds in the form of tannin, saponin, sterol, and flavonoid. Identifying the presence of alkaloids in the extract plants is done by reagent test Phytochemicals, namely Wagner reagents. The reactant Wagner is characterized by forming a precipitate brown to yellow. Black garlic extract does not contain alkaloid compounds because the results of the phytochemical test showed no formation of a brown-to-yellow precipitate in Wagner's reagent[17]. Antibacterials can interfere with the growth or even kill bacteria by interfering with harmful microbial metabolism[6]. Flavonoids are phenol derivatives that can cause denaturation and coagulation of bacterial cell proteins whereby flavonoid compounds in damaging bacterial

cells take advantage of the difference in polarity between the lipids that make up bacterial cells and the alcohol groups in flavonoid compounds[14]. Tannins have antibacterial activity. Tannin toxicity can damage bacterial cell membranes; the mechanism of action of tannin compounds in inhibiting bacterial cells is by denaturing bacterial cell proteins, inhibiting cell membrane function (transport of substances from one cell to another), and inhibiting nucleic acid synthesis so that bacterial growth can be inhibited[14]. Saponins are a class of combined chemical compounds, one of the secondary metabolites found in natural sources and from various plant species. Specifically, saponins are amphipathic glycosides with a soap-suds-like structure that is produced when shaken in an aqueous solution and whose structure consists of one or more hydrophilic glycosides combined with a lipophilic triterpene derivative[10].

Black garlic is obtained in this way. Heat the garlic at 70^{o}C for 28 days. Onion heating black between 70–80^{o}C can increase reducing sugar content. Warm temperatures of 70^{o}C cause some damage to the structure of reducing sugars present in black onions, whereas when using temperatures below 60^{o}C, the onion manufacturing process black takes a very long time[18]. Onion black has a fragrance that is not very intense compared to fresh white onions. This is due to changes in compounds aline into derivative compounds such as S allylcysteine (SAC). Alline compounds are compounds that cause pungent odors on garlic. With a change of aline compounds into SAC aroma from onions, white will disappear. The SAC content of black garlic is 4–8 times that of white onions. The smell of garlic starts to fade after a heating time of 15 days and changes to the distinctive fresh aroma of black garlic on the 25th day. In black garlic, the level of water content experienced a decrease of 34.3%, content protein increased by 0.7%, fat increased by 0.4%, carbohydrates increased by 18.3%, levels of total sugar increased by 34.6%, sugar increased by 24%, pH decreased by 3.3%, brightness color decreased by 64.1%; redness increased 6.5%, decreased 30.5% yellow color, calories increased 89.1% compared to garlic[1].

The heating process in manufacturing black garlic can also affect discoloration. The onion color changes from white to black garlic, causing a browning process to non-enzymatic. The Maillard reaction causes the non-enzymatic browning process that occurs in the heating process of Garlic. Maillard reactions involve change reactions reducing sugars and several amino acids[19].

Results of the Antibacterial Activity Test

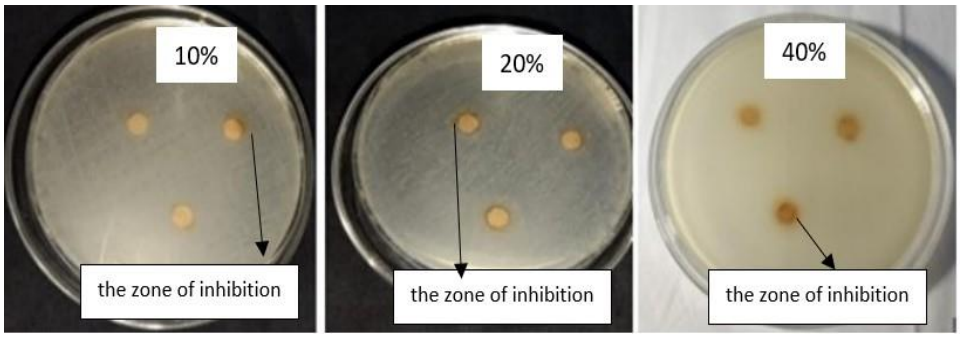

Figure 2. Zone of Inhibition of Extracts with Various Concentrations Against *Escherichia coli*

Table 3. Test Results Diameter of Inhibition

Sample	Variation Consetration	Repeat	Diameter (mm)	Meaning
Black Garlic Extract In Feminine Hygine Liquid Soap	10%	1	7,8	9,7
		2	9,6	
		3	11,7	
	20%	1	8,3	11,2
		2	11,8	
		3	13,7	
	40%	1	11,3	12,7
		2	12,8	
		3	14,2	
	Amoxicilin	1	14,8	14,8

The results of the antibacterial activity test of women's liquid soap with black garlic on *Escherichia coli* bacteria inhibited the growth of *Escherichia coli* bacteria. Black garlic extract in feminine hygiene liquid soap at a 10% concentration variation had an inhibition zone of 9.7 mm, black garlic at a 20% concentration variation of 11.2 mm, and black garlic at a 40% concentration variation of 12.7 mm. The antibiotic amoxicillin is an antibiotic that can reduce the development of bacteria in the vagina and can inhibit the growth of Gram-negative and Gram-positive bacteria. Another study stated the inhibition of black garlic at an extract concentration of 75% against streptococcus pneumonia with an inhibition zone of 11mm and klebsiella pneumonia with an inhibition zone of 13mm[10]. According to Haris (2021), the flavonoid content in black garlic can denature its cells. The presence of tannins has a mechanism that Inhibits bacterial growth by causing protein denaturation, thereby inhibiting the occurrence of plasma coagulation required by *Staphylococcus aureus*[20].

The results of this study prove that the higher the concentration of black garlic extract, the better it is to inhibit the growth of *Escherichia coli* bacteria. Proved by the greater the concentration of active substance dissolved, the more the size of the inhibition zone formed; this is also caused by the number of secondary metabolites such as tannins, saponins, steroids, and flavonoids contained in it and resulted in the activity of more antibacterial[21].

Our study has limitations. The highest concentration variation used in research is only 40%; concentrations above 40% can obtain a larger inhibition zone, but this was not done in research. Suggestions for further research are to provide treatment with a concentration of more than 40%.

CONCLUSION

Black garlic extract in feminine hygiene liquid soap can inhibit the growth of *Escherichia coli* bacteria; variations in the concentration of 40% black garlic have the highest antibacterial activity in inhibiting *Escherichia coli* bacteria. It is hoped that the results of this research can be developed back to the next test stage so that later this

product can be used safely by women in preventing vaginal discharge and reducing the use of chemical drugs.

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CONFLICT OF INTEREST

All authors state that there is no conflict or problem with any part of the writing of this journal publication.

REFERENCES

1. Kim K-H, Park JK, Choi Y-W, Kim Y-H, Lee EN, Lee J-R. Hexane extract of aged black garlic reduces cell proliferation and attenuates the expression of ICAM-1 and VCAM-1 in TNF-α-activated human endometrial stromal cells. Int J Mol Med. 2013;32(1):67–78.
2. Kędzia A. Antimicrobial activity of garlic (*Allium sativum* L.). Postępy Fitoter. 2010; (1):46–52.
3. Venugopal S, Gopalan K, Devi A, Kavitha A. Epidemiology and clinico-investigative study of organisms causing vaginal discharge. Indian J Sex Transm Dis AIDS. 2017;38(1):69.
4. Donders GGG, Bellen G, Rezeberga D. Aerobic vaginitis in pregnancy. BJOG An Int J Obstet Gynaecol. 2011;118(10):1163–70.
5. Han C, Li H, Han L, Wang C, Yan Y, Qi W. Aerobic vaginitis in late pregnancy and outcomes of pregnancy. Eur J Clin Microbiol Infect Dis. 2019;38(2):233–9.
6. Tao Z, Zhang L, Zhang Q, Lv T, Chen R, Wang L. The pathogenesis of Streptococcus anginosus in aerobic vaginitis. Infect Drug Resist. 2019;(7) 45–54.
7. Sonthalia S, Aggarwal P, Das S, Sharma P, Sharma R, Singh S. Aerobic vaginitis–An underdiagnosed cause of vaginal discharge–Narrative review. Int J STD AIDS. 2020;31(11):(10) 18–27.
8. Homayouni A, Bastani P, Ziyadi S, Mohammad-Alizadeh-Charandabi S, Ghalibaf M, Mortazavian AM. Effects of probiotics on the recurrence of bacterial vaginosis: a review. J Low Genit Tract Dis. 2014;18(1):79–86.
9. Prasad D, Parween S, Kumari K, Singh N. Prevalence, etiology, and associated symptoms of vaginal discharge during pregnancy in women seen in a tertiary care Hospital in Bihar. Cureus. 2021;13(1). 1-11
10. Halimah LS, Hasan K. Differences of bio (chemical) characterization of garlic and black garlic on antibacterial and antioxidant activities. In: Journal of Physics:

Conference Series. IOP Publishing; 2021. (17) 64-75
11. Wang D, Feng Y, Liu J, Yan J, Wang M, Sasaki J. Black garlic (*Allium sativum*) extracts enhance the immune system. Med Aromat Plant Sci Biotechnol. 2010;4(1):37–40.
12. Tran G-B, Pham T-V, Trinh N-N. Black garlic and its therapeutic benefits. Med Plants-Use Prev Treat Dis. 2019; (3) 37-63
13. Marhaeni GA. Keputihan pada wanita. J Skala Husada J Heal. 2016;13(1) 66-78.
14. Yuan H, Sun L, Chen M, Wang J. The comparison of the contents of sugar, Amadori, and Heyns compounds in fresh and black garlic. J Food Sci. 2016;81(7):(6) 62–68.
15. Liu R, Yang G, Guo J, Wu T, Sui W, Zhang M. Effects of incorporation of black garlic on rheological, textural and sensory properties of rye (Secale Cereale l.) flour noodles. CYTA-Journal Food. 2018;16(1):1102–8.
16. Saifudin A. Natural Compounds Secondary Metabolites Theory. Concept, and Purification Tech, Deep Yogyakarta. 2014; (3) 45-52
17. Kusuma EW, Anggraini DI. Uji Antipiretik Ekstrak Etanol Bawang Hitam (Black Garlic) Pada Tikus Putih Jantan. Humantech J Ilm Multidisiplin Indones. 2022;1(7):968–79.
18. Zeng L, Zhang L, Shi J, Gu L, Grogan W, Gargano MM. The primary microbial pathogens associated with premature rupture of the membranes in China: a systematic review. Taiwan J Obstet Gynecol. 2014;53(4):443–51.
19. Wardhani GAPK, Azizah M, Hastuti LT. Value of Total Flavonoids in Black Garlic (*Allium sativum* L.) Based on The Solvent Fraction and Antioxidant Activity. J Agroindustri Halal. 2020;6(1):20–7.
20. Haris, R. S. W., Mujnisa, A. and Jamilah. Uji Daya Hambat Antibakteri Black Garlic Sebagai Alternatif Feed Additive Pada Pakan Unggas, Buletin Nutrisi dan Makanan Ternak. 2021; 15(1). doi: 10.20956/bnmt.v15i1.14463.
21. Widhowati D, Mudji EH, Prakoso YA, Aulia Q. Sensitivitas black garlic terhadap pertumbuhan *Salmonella* Sp. VITEK Bid Kedokt Hewan. 2022;12(2):16–22.