Contents lists available at iocspublisher

Science Midwifery

journal homepage: www.midwifery.iocspublisher.org

The Inhibitory Activity Test of Green Tea Ethanol Extract (Camellia sinensis) Sidamanik Against Escherichia coli

[1]Aliyah Fahmi, [2]Sumaryati Syukur, [3]Zulkarnain Khaidir

[1]Health Faculty, Efarina University, Pematang Siantar, North Sumatera, Indonesia
[2]Chemistry Department, Andalas University, West Sumatera, Indonesia

A R T I C L E I N F O

Keywords:

E. coli, Sidamanik,
Green Tea,
Disc Method,
Inhibitory

ABSTRACT

A study was done in analyzing the inhibitory activity of green tea ethanol extracts against Escherichia coli. The green tea is sourced from a tea plantation in Sidamanik, North Sumatra. The green tea was macerated with ethanol 96 % for 24 hours. After that, the filtrate was taken and then concentrated to give a concentrated extract and evaporated ethanol content until run out and obtained thick extract. The thick extract was then diluted with distilled water to extract 5%, 10%, and 15% concentration variations. The test method for inhibitory activity was the disc method using distilled water as a negative blank. The results obtained with blank, 5%, 10% and 15% green tea were 0. 5.7; 7.10, and 8.05 mm The conclusion of this study is that the concentration of the extract is directly proportional to its inhibitory power, and can be used as an antibacterial agent against E. coli.

E-mail
Sumaryatisyukur_unand@yahoo.co.id

1. Introduction

Escherichia coli (E. coli) is a coliform bacterium that belongs to the Enterobacteriaceae family. Enterobacteriaceae are enteric bacteria or bacteria that can live and survive in the digestive tract. E. coli is a rod-shaped bacterium that is Gram-negative, facultative anaerobic, does not form spores, and is a natural flora in the mammalian intestine. [1]

Some of these bacterial strains provide benefits to humans, for example preventing the colonization of pathogenic bacteria in the human digestive tract. However, there are several other groups that can cause disease in humans, known as pathogenic E. coli. Pathogenic E. coli was first identified in 1935 as a cause of diarrhea. [2]

E. coli pathogens that cause diarrhea or also known as diarrheagenic E. coli (DEC) consist of six types, namely entero-toxigenic E. coli (ETEC), entero-pathogenic E. coli (EPEC), entero-hemorrhagic E. coli (EHEC), entero-invasive E. coli (EIEC), entero-aggregative E. coli (EAEC), and diffusely adherent E. coli (DAEC). [3]

Four types of E. coli, namely ETEC, EPEC, EHEC, and EIEC, are known to cause foodborne illness . [4]. Several research results also show that EAEC is a bacterium that contaminates food and causes diarrhea. [5]

E. coli is divided into 3 major groups based on their interactions with the host (humans), namely non-pathogenic (commensal), gastrointestinal pathogens, and pathogens outside the digestive tract (extra-intestinal). This classification is mainly based on the presence or absence of DNA regions that are often associated with a particular pathogen. E. coli bacteria are also known as sanitation and hygiene indicator bacteria, namely bacteria whose presence in a food product indicates a low level of sanitation applied. The presence of these bacteria is often associated with contamination from feces, because E. coli in general are bacteria that live in the intestines of humans (and animals) so that the presence of these bacteria in water or food indicates a processing process that has been in contact with feces. . With regard to food safety, it is known that E. coli

accounts for a number of cases of enteric disease in children in several developing countries. E. coli is the main etiologic cause of diarrhea. [6]

In some cases it can cause symptoms of hemolytic uremic syndrome which can result in kidney failure. These infections can even lead to death. [4]

Infants and children are the population most susceptible to exposure to E. coli bacteria. This is reinforced by reports of poisoning or infection by these bacteria being found in children. Examples of food contaminated with pathogenic E. coli are meat, milk, vegetables, drinking water, minimally processed ready-to-eat food, as well as street snacks that are very popular with children. [2;7;8;9;10;11]

Green tea is a beverage which can be used as a healing agent in many researches especially for antimicrobial activities. The article review about the inhibition power of green tea extract to E. coli was investigated. [12]

The study had been learnt in Korea about the antimicrobial activity of ethanol extract of green tea against E. coli with different solvent fractionated ethanol extracts (hexane, ethyl-acetate, chloroform and water) and got the significant inhibition activity. [13]

The study from Iran about the minimum inhibitory concentration (MIC) of Camellia sinensis against E. coli which isolated from patient's urine were shown that the highest MIC of Camellia sinensis against E. coli was 10 mg/ml. [14]

Today, The authors try to determine the inhibitory activity of green tea ethanol extract from Sidamanik Plantation, North Sumatera against E. coli using distilled water as blank negative with disk method..

2. Method

Equipment used is autoclave, stir bars, hot plates, incubators (Memmert), calipers, layered airflow cabinets, micropipettes, tweezers, eyelet needles, vortexes, and other glassware (beakers, petri dishes, reaction tubes, Erlenmeyer flasks, etc.). The materials used are nutrient agar medium (Oxoid), nutrient broth (Oxoid), blanks, distilled water, E. coli bacteria, green tea ethanol extract, black tea ethanol extract.

2.1. Preparation of green tea ethanol extract

The dried green tea was macerated with ethanol 96 % for 24 hours. After that, the filtrate was taken and then concentrated to give a concentrated extract and evaporated ethanol content until run out and obtained thick extract. The thick extract was then diluted with distilled water to extract 5%, 10%, and 15% concentration variations.

2.2. Preparation of bacterial culture

Bacterial colonies were removed with a round needle, sterilized then transplanted onto the surface of nutrient medium, scratched and tilted, and incubated at 37 °C. for 24 hours . [15]

2.3. Bacterial inoculation production

Bacterial colonies were removed from the culture stock using sterile eyelets, then suspended in tubes containing 10 ml of nutrient medium and incubated at 37 °C for 24 hours until turbidity was comparable to standard Mc. Farland. [15]

2.4. Antibacterial Activity Test

Place a total of 0.1 ml of bacterial inoculum in a sterile Petri dish, then pour the nutrient agar medium into a 15 ml Petri dish, then shake the Petri dish on the table surface to homogenize the medium and bacterial suspension, it can be integrated. Disc paper was given at the concentration of each test solution and then placed on the surface of solid medium. Sample was incubated at 37 °C for 18-24 hours. The diameter of the suppression zone formed around the paper disc was observed and measured with a caliper. [15]

3. Result And Discussion

The inhibit zone diameter between green tea and black tea ethanol extract had been formed and listed in table below.

Contents lists available at iocspublisher

Science Midwifery

journal homepage: www.midwifery.iocspublisher.org

TABLE1
COMPARISON OF INHIBITION DIAMETERS OF GREEN TEA ETHANOL EXTRACT

Concentration	Repetation	Inhibit Zone Diameter Green tea ethanol extract agains *E. Coli* (mm)
Blank	P1	6
	P2	6
Avarage ± ID		0
5 %	P1	11.3
	P2	12.1
Avarage ± ID		5.70
10 %	P1	12.0
	P2	14.2
Avarage ± ID		7.10
15 %	P1	13.4
	P2	14.7
Avarage ± ID		8.05

Note: ID is named as Inhibition Diameter (The calculation of ID is Total Diameter – Blank Diameter)

For detail, let us see the figures below. Figure 1 shows the inhibition diameter of green tea ethanol extract with concentration variations are 5%, 10 % and 15 % with negative blank uses distilled water. This work was done with two repetitions.

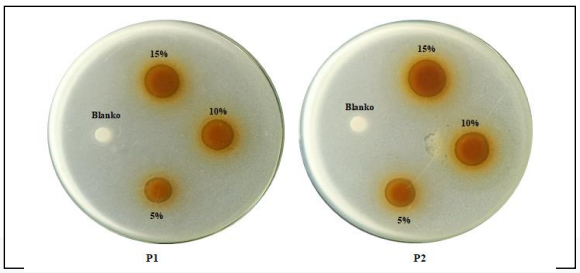

Figure 1.Green tea ethanol extract against *E. coli* (two repetitions)

For further detail, let us see the diagram below:

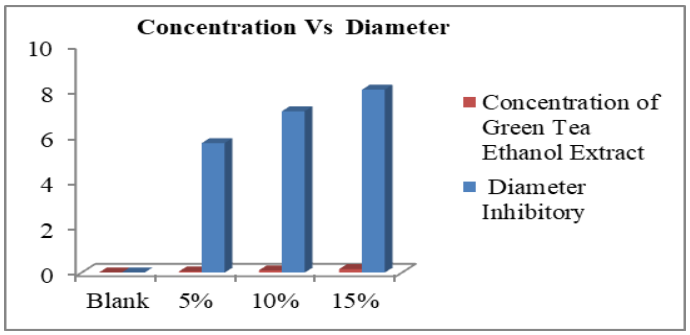

Figure 2. Antibacterial activity green tea ethanol extract against E. coli

3.1 Discussion

In the antibacterial activity test by [16], antibacterial activity was classified as weak if the suppression zone was less than 5 mm, moderate if the suppression zone was 5 until 10 mm, and it is classified as strong. The inhibition zone is 11 until 20 mm, and if the inhibition zone is 20 until 30 mm, it is classified as very strong. The formation of the inhibition zone was due to the antibacterial content contained in the green tea ethanol extract was higher as the percent concentration

increasing. Green tea has the active chemical content of EGCG. In the manufacturing process, in which the tea leaves are dried and wilted to make green tea without fermenting. [17] . The statement is referred to[18] , they studied the proportion of catechins between green tea and black tea is 30: 5%, and the proportion of oxidized phenol compounds between green tea and black tea is 0: 25%.

Inhibition tests of ethanol extracts from green tea leaves (Camellia Sinensis) against E. coli was done. The results obtained with blank, 5%, 10% and 15% green tea were 0. 5.7; 7.1 and 8.05 mm and black tea ethanol extract were 0.2.50; 3.20 and 5.30 mm. The conclusion of this study is that the concentration of the green tea ethanol extract is directly proportional to its inhibitory power, so it can be used as antibacterial agents against E. coli.

4. Conclusion

Based on the results of research with respondents related to feelings of depression with the conditions experienced, it can be concluded Before giving lavender aromatherapy, the patient's depression was confirmed positive Most (32.9%) 18 respondents experienced feelings of sad depression, 9 respondents (16.3%) lost interest in doing any activity, 10 respondents (18.1%) decreased pleasure in hobbies, 12 respondents (21.8%) experienced early morning awakening and 6 respondents (10.9%) experienced fluctuating feelings throughout the day. After giving lavender aromatherapy, there was a decrease in feelings of depression where the difference in the decrease in points of loss of interest was 9.1%, reduced pleasure in hobbies as much as 9.1%, reduced feelings of sadness as much as 16.6%, decreased early morning wake up as much as 12.8 % and a 5.5% reduction in mood swings throughout the day.

References

[1] YANG, X.; WANG, H. pathogenic E. coli. Lacombe Research Centre, Lacombe. Canada. 2014.

[2] MANNING, Shannon D. Escherichia coli infections. Infobase Publishing, 2010..

[3] KAPER, James B.; NATARO, James P.; MOBLEY, Harry LT. Pathogenic escherichia coli. Nature reviews microbiology, 2004, 2.2: 123-140.

[4] FDA, Bad Bug Book. Handbook of foodborne pathogenic microorganisms and natural toxins. Food and Drug Administration, Silver Spring, MD, USA,, 2012.

[5] KAGAMBEGA, Asseta, et al. Diarrheagenic Escherichia coli detected by 16-plex PCR in raw meat and beef intestines sold at local markets in Ouagadougou, Burkina Faso. International journal of food microbiology, 2012, 153.1-2: 154-158.

[6] PARASHAR, Umesh D., et al. Global illness and deaths caused by rotavirus disease in children. Emerging infectious diseases, 2003, 9.5: 565.

[7] RAM, Siya, et al. Culture-free detection and enumeration of STEC in water. Ecotoxicology and environmental safety, 2011, 74.4: 551-557.

[8] SEN, Keya, et al. Development of a sensitive detection method for stressed E. coli O157: H7 in source and finished drinking water by culture-qPCR. Environmental science & technology, 2011, 45.6: 2250-2256.

[9] MOHAMMED, Mahmoud Ahmed Mahmoud. Molecular characterization of diarrheagenic Escherichia coli isolated from meat products sold at Mansoura city, Egypt. Food Control, 2012, 25.1: 159-164.

[10] RUSSO, Pasquale, et al. A fast, reliable, and sensitive method for detection and quantification of Listeria monocytogenes and Escherichia coli O157: H7 in ready-to-eat fresh-cut products by MPN-qPCR. BioMed research international, 2014, 2014.

[11] LAY, Bibiana Widyati, et al. Identification of class 1 integron of Escherichia coli from street foods in Jakarta. Microbiology Indonesia, 2007, 1.1: 4-4.

[12] NOORMANDI, Afsaneh; DABAGHZADEH, Fatemeh. Effects of green tea on Escherichia coli as a uropathogen. Journal of traditional and complementary medicine, 2015, 5.1: 15-20.

[13] CHO, Sun-Young, et al. Antimicrobial activities of green tea extract and fractions on the E. coli O157: H7. Journal of Food Hygiene and Safety, 2005, 20.1: 48-52.

[14] SEPEHRI, Zahra, et al. Antibacterial effect of ethanol extract of Camellia sinensis l against Escherichia coli. Asian Pacific Journal Microbiology Research, 2014, 2.1: 6-8.

[15] DEPKES, R. I. Farmakope indonesia edisi IV. Jakarta: Departemen Kesehatan Republik Indonesia, 1995, 45.

[16] DAVID, W. W.; STOUT, T. R. Disc plate method of microbiological antibiotic assay. Microbiology, 1971, 22.4: 659-665.

[17] XU, Ning; CHEN, Zong-mao. Green tea, black tea and semi-fermented tea. In: Tea. CRC Press, 2002. p. 47-68.

[18] SONG, Jae Min; SEONG, Baik Lin. Tea catechins as a potential alternative anti-infectious agent. Expert Review of Anti-infective therapy, 2007, 5.3: 497-506.