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Testing the Effectiveness of Mangosized Skin Extract (*Garcinia mangostana*) as an Antimicrobial Against Bacteria Causing GI Tract Infections

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ABSTRACT

The mangosteen fruit, which in Latin is called Garcinia mangostana Linn, is a plant whose entire parts can be used, including the skin of the fruit. Mangosteen fruit has bioactive compounds such as xanthones, especially α, β, and γ-mangostin where the level of antimicrobial action of α-mangosteen has been explored by many researchers and it has been observed that it is not only effective against bacteria but also against other microbes such as fungi and mycobacteria. Gastrointestinal system infections are a serious health problem and have not been completely resolved until now because cases are increasing every year. Gastrointestinal tract infections are a health problem in the world, including in Indonesia. Some infectious diseases of the gastrointestinal system that are often found in the wider community are typhoid fever, paratyphoid fever, diarrhea, and gastroenteritis. The bacteria that cause typhoid fever and paratyphoid fever are caused by the bacteria Salmonella Typhi & Salmonella Paratyphi. While diarrhea and gastroenteritis are caused by food poisoning and drinks contaminated by E. coli. This research is an experimental laboratory research using the disc diffusion/Kirby Bauer method by measuring the diameter of the clear zone which is a sign that there is an obstacle to the growth of Salmonella typhi and E. Coli bacteria by the antimicrobial compounds contained in the mangosteen fruit extract. The calculation results of this research showed that mangosteen peel extract (Garcinia Mangostana L) against E. Coli bacteria with the antibiotic Chloramphenicol as control showed an average diameter of the inhibition zone of 30.5 mm, whereas Salmonella bacteria were not found or no inhibition zone was formed at the smallest concentration up to greatest concentration

Keywords: Mangosteen Peel, garcinia mangostana l., Gastrointestinal Infections, Effectiveness of Mangosteen Peel, Antibiotics

INTRODUCTION

Gastrointestinal infections are inflammation of the stomach wall caused by microorganisms such as bacteria, viruses and parasites, usually called gastrointestinal infections. Gastrointestinal infections are a global problem that commonly attacks the stomach/intestine. Gastrointestinal infections are often not taken seriously because they can be cured without treatment, but in certain people, diarrhea has a high morbidity and mortality rate, especially in elderly people, young children, or in people who suffer from chronic diseases or whose immune systems are compromised. Low levels can cause acute dehydration requiring medical

attention.[1]. Gastrointestinal system infections are a serious health problem and have not been completely resolved until now because cases are increasing every year. Gastrointestinal tract infections are a health problem in the world, including in Indonesia. According to WHO and UNICEF, there are around 2 billion cases of diarrhea and 1.9 million children under five die from diarrhea throughout the world every year. Of all the deaths, 78% occurred in developing countries, especially in Africa and Southeast Asia. Based on Riskesdas, the prevalence of diarrhea for all age groups is 8%, the prevalence rate for toddlers is 12.3%, and for infants, the prevalence rate for diarrhea is 10.6%. Data from Komdatkesmas during January – November 2021, the death rate due to postneonatal diarrhea was 14% [2]. Symptoms of digestive tract infections are generally diarrhea with symptoms of defecation with liquid or soft stools, a frequency of three or more times per day, or defecation more often than usual for a person [3].

Some infectious diseases of the gastrointestinal system that are often found in the wider community are typhoid fever, paratyphoid fever, diarrhea, and gastroenteritis. The bacteria that cause typhoid fever and paratyphoid fever are caused by the bacteria Salmonella Typhi & Salmonella Paratyphi [3]. while diarrhea and gastroenteritis are caused by food poisoning and drinks contaminated by E. coli [4]. Acute diarrhea occurs due to the entry of viruses (Rotavirus, Adenovirus enteris, and others), bacteria (Campylobacter, Salmonella SP, Escherichia coli, and others), and parasites (Giardia lamblia, Cryptosporidium). Some of these pathogenic microorganisms cause infections in cells by producing enterotoxins or cytotoxins that damage cells or attach to the intestinal wall. Diarrhea can be transmitted via fecal to oral route from one sufferer to another, in some cases diarrhea can be transmitted through food and drink contaminated with pathogenic microorganisms [5,6]. The mechanism of gastrointestinal infection is in two ways, namely damage to the intestinal villi which results in malabsorption and diarrhea due to the release of toxins that bind to enteric receptors resulting in the release of chloride ions into the interstinal lumen causing secretory diarrhea.[6]. Some bacteria produce several adhesion factors (adhesins) and proteins that function to help adhere to the intestinal wall, for example, vibrio cholerae which uses surface adhesins to attach or attach to the intestine. [7].

E. coli is a gram-negative rod-shaped bacterium, facultatively anaerobic, does not form spores, is a normal flora of the mammalian intestine (Yang and Wang, 2014). E.coli belongs to the Enterobacteriaceae family. Some strains of E.coli are pathogenic in humans known as pathogenic Escherichia which can cause diarrhea. Escherichia Pathogens that cause diarrhea are also called diarrheagenic E.coli (DEC), which consists of Enterotoxigenic E.coli (EEC), Enteropathogenic E.coli (EPEC), Enterohemorrhagic E.coli (EHEC), Enteroinvasive E.coli (EIEC), Enteroaggregative E.coli (EAEC) and Diffusely Adherent E.coli (DAEC) (Kaper et al. 2004). Symptoms of diarrhea caused by E. coli occur as a result of invasion and destruction of the small intestine. Another virulence factor that plays a role in gastrointestinal infections due to bacteria is the production of toxins including enterotoxins. This enterotoxin causes diarrhea with a watery stool consistency due to its secretory effect on the small intestinal mucosa [7]. Salmonella is a bacterium that can cause infections in the digestive tract, the disease caused by Salmonella bacteria is usually called salmonellosis. Salmonella is a gram-negative bacterium which is in the Enterobacteriaceae family. Salmonella is an enteric pathogen that causes foodborne disease. Salmonella has three antigens, namely: the O antigen which is found on the surface of the germ body, the H antigen on the flagellum, and the Vi (virulence) antigen on the capsule [8].

Mangosteen fruit or Garcinia mangostana Linn. (commonly known as mangostin),

belongs to the Guttiferae family, the delicious and aromatic fruit is native to China, India, Indonesia, Malaysia, Myanmar, the Philippines, Thailand, and other regions in Southeast Asia and this fruit is synonymous with good health and is labeled as 'fruit super'[9].

Mangosteen fruit is included in the kingdom Plantae, division spermatophyte, sub divisiono Angiosparmae, class dicotyledoneae, order guttiferanales, family guttiferae, genus Garcinia and species Garcinia mangostana L. Apart from the fruit, the skin of the mangosteen fruit is very useful. Because it contains xanthones (mangostin), especially α, β, γ-mangostin. Meanwhile, mangosteen rind extract contains tannins, terpenes, anthocyanins, benzophenone, depsidone, phloroglucinol, polyphenols, and flavonoids. In the mangosteen plant (Garcinia Mangostana L), the fruit, skin, roots, and bark contain a lot of mangostin. [9]. The roots, bark, and skin of the mangosteen fruit contain saponins, the roots and stems contain plavanoids and polyphenols, while the skin of the mangosteen fruit contains tannins, flavonoids, steroids/triterpenoids and compounds, as well as the elements sodium, potassium, magnesium, calcium, iron, zinc, and copper. The bark, rind and dry latex of mangosteen contain yellow dye which comes from the metabolate of mangostin and beta mangostin [10]. Mangostin (Xanthon) is the main component although the levels of beta mangostin are smaller than other phytochemicals found in mangosteen fruit. Xanthone compounds have biological activity as antibacterial, anti-inflammatory, antioxidant and can also inhibit the growth of colon cancer cells [10].

Treatment of digestive tract infections caused by bacteria is by administering antimicrobials, but this must be recommended by a doctor. The use of herbal medicines for bacterial infections has been around for thousands of years, long before the discovery of synthetic chemical medicines. Most use of herbal medicines can be caused by several factors, one of which is the belief that herbal medicines have fewer side effects than conventional medicines and are even thought to have no side effects because they are natural ingredients and herbal medicines are easy to obtain without requiring a doctor's prescription [13,14]. Many traditional medicines are used as antibacterials, one of which is the mangosteen plant which has antibacterial activity. Based on the background described above, the author is interested in conducting research with the title "Testing the effectiveness of mangosteen rind extract (Garcinia mangostana L.) as an antimicrobial against bacteria that cause gastrointestinal infections".

MATERIALS & METHODS

Research design

This research is an experimental laboratory using the disc diffusion method/Kirby Bauer. This sensitivity test is carried out by measuring the diameter of the clear zone, which is a sign that there is inhibition of the growth of Salmonella typhi and E. Coli bacteria by the antimicrobial compounds contained in the mangosteen fruit extract.

Scope of Research

Research Time

This research was conducted from November 2023 to December 2023.

Research Place

This research was conducted at the Research Laboratory of the Faculty of Medicine, Indonesian Christian University

Research Subjects

The subjects in this study were E.coli and Salmonella typhi bacteria obtained from the collection of the microbiology department of the Faculty of Medicine, Indonesian Christian University, and Nutrient Broth (NB) and incubated for 24 hours at 37°C.

Inclusion Criteria

Inclusion criteria are the general characteristics of research subjects in the target population and accessible populations.

The inclusion criteria in this study are as follows:

1. Mangosteen fruit extract
2. Salmonella typhi and E. coli bacteria

Exclusion Criteria

Exclusion criteria are the exclusion of research subjects who do not meet the inclusion criteria and, for various reasons, cannot become research respondents. The exclusion criteria in this study are: Salmonella typhi and E.coli germs that have mutations.

Research procedure

1. Sterilization of Equipment

The tools and materials that will be used for research are sterilized first, except for mangosteen fruit extract and germ suspension to avoid contaminants.

2. Making Agar Media

a. Creation of EMB Media
b. NA Media Creation
c. Making MHA Media

3. Dilution of Mangosteen Fruit Extract

Mangosteen fruit extract is diluted using distilled water with concentration levels of 6.5%, 12.5%, 25%, 50%, and 100% as follows. 6.5% mangosteen fruit extract plus 50 µl of distilled water, 12.5% mangosteen fruit extract plus 25 µl of distilled water, 25% mangosteen fruit extract plus 25 µl of distilled water, 50% mangosteen fruit extract plus 6.25 µl of distilled water, and 100% pure mangosteen fruit extract.

4. Making McFarland

Making a turbidity standard of 0.5 Mc Farland units will be made from a mixture of 9.95 ml of 1% H2SO4 solution and 0.05 ml of 1% $BaCl_2$ solution.

5. Preparation of Bacterial Suspension

a. Take at one end of the loop a colony of E.coli and Salmonella typhi bacteria from pure culture.
b. Then pour it into a test tube filled with 5ml NaCl.
c. The suspension that has been made is compared until it is equal to a turbidity of 0.5 McFarland.
d. The germ suspension will be dripped onto the surface of the MHA and spread using a sterile cotton swab.

6. Creation of Concentration Variables

There were 7 variables used in this study, namely sterile distilled water as a negative control, varying concentrations of mangosteen fruit extract 6.5%, 12.5%, 25%, 50%, and 100% using prounalins ethanol solvent, and a positive control using antibiotics (C).

STATISTICAL ANALYSIS

Data Analysis and Data Processing

Processing and analysis of data originating from research results on the effect of mangosteen rind extract on the growth of Salmonella typhi and E.coli bacteria after being treated with mangosteen rind extract at various negative control and positive control concentrations. The data was analyzed using the Excel 2013 application which was presented in tabulated form containing details of the barrier zones formed due to mangosteen rind extract.

Data processing steps:

1. Editing Stage

This stage is carried out so that the data obtained is correct information. It is necessary to pay attention to whether the data is complete or not.

2. Coding

To abbreviate the data obtained so that it is easier to process and analyze by providing codes in the form of numbers.

3. Tabulation

The data that has been processed by the SPSS program is presented in tabular form. The test used for a sample size >30 in this study was the one-way ANOVA test. This analysis test aims to analyze 2 variables, namely the dependent variable and the independent variable to determine whether or not there is an effect of giving mangosteen peel extract (Garcinia Mangostana L) on Salmonella

typhi and E.coli bacteria. The interpretation of the analysis test is:

1. If the P value < α (0.05) then H_0 is accepted and H_1 is rejected. This shows that the sample data supports the effect of administering mangosteen rind extract on inhibiting the growth of Salmonella typhi and E.coli bacteria.
2. If the P value is > α (0.05), then H_0 is rejected and H_1 is accepted. This shows that the sample data does not support the effect of administering mangosteen rind extract on Salmonella typhi and E. coli bacteria.

RESULT

Mangosteen Peel Extract (Garcinia mangostana L.).

Researchers used mangosteen peel which had been extracted using 96% ethanol.

Results of the Inhibitory Power Test of Mangosteen Peel Extract

The inhibitory ability of mangosteen peel extract was assessed by measuring the diameter of the clear zone that appeared around the paper disc. In this study, the inhibitory power of mangosteen peel extract against E coli and Salmonella Typhi was tested using the Kirby-Bauer diffusion method in 5 concentrations (6.25%, 12 .5%, 25%, 50%, 100%) with gentamicin as positive control and DMSO as negative control. E.Coli and Salmonella Typhi bacteria were applied to the surface of the MHA agar then on the surface of the MHA agar a paper disc was attached which had been soaked with various concentrations of mangosteen peel extract, gentamicin, and DMSO for the Kirby-Bauer method. In this research, repetition was carried out 4 times using the Federer formula.

Table 1. Results of Measurement of the Inhibitory Zone of Mangosteen Peel Extract Against E. coli Bacteria

Mangosteen Peel Extract (*Garcinia mangostana L.*)							
Repetition	**Kirby-Bauer Treatment (mm)**						
	K. Positive	**6,25%**	**12,5%**	**25%**	**50%**	**100%**	**K. Negative**
I	31.67	2.12	3.13	4.24	4.30	6.36	-
II	29.48	2.25	3.36	4.30	3.24	7.36	-
III	30.00	2.10	3.50	5.00	5.10	6.20	-
IV	29.48	2.35	3.10	4.73	4.90	7.00	-
Average	30.15	2.20	3.27	4.56	4.38	6.73	-

Table 1, (against E.coli bacteria) based on the criteria for bacterial inhibitory strength by David and Stout, shows that from mangosteen peel extract (Garcinia Mangostana L) the smallest inhibition zone is formed at concentrations of 6.25%, 12.5%, 25%, 50% while the greatest barrier is at the highest concentration (100%) with an average zone barrier value of 6.73. In controls with the antibiotic Chloramphenicol, the average diameter of the inhibition zone was 30.5 mm. In the negative control with DMSO, no inhibition zone was found. This means that mangosteen peel extract can inhibit the growth of E.coli

Table 2. Results of Measurement of the Inhibition Zone of Mangosteen Peel Extract Against Salmonella Bacteria

Mangosteen Peel Extract (Garcinia mangostana L.)							
Repetition	**Kirby-Bauer Treatment (mm)**						
	K. Positive	**100%**	**50%**	**25%**	**12,5%**	**6,25%**	**K. Negative**
I	0	0	0	0	0	0	-
II	0	0	0	0	0	0	-
III	0	0	0	0	0	0	-
IV	0	0	0	0	0	0	-
Average	0	0	0	0	0	0	-

Table 2 (against Salmonella bacteria) was not found or no inhibition zone was formed at the smallest concentration to the largest concentration. The mangosteen rind extract was the same as the negative control for DMSO. This shows that mangosteen peel does not have effectiveness as an antimicrobial against Salmonella typhi bacteria.

DISCUSSION

This research aims to see whether extra mangosteen rind can inhibit the growth of E.coli germs. and Salmonella typhi as a cause of infection in the gastrointestinal tract and also knowing the minimum concentration that is effective for killing or inhibiting these bacteria. The occurrence of the barrier zone is due to the active substance contained in the skin of the mangosteen fruit which has antimicrobial properties. In research on e.coli bacteria, the inhibition zone was formed by each different concentration of mangosteen peel extract which increased according to the higher zone concentration. At greater concentrations, more antimicrobial compounds are found. This can be proven by the formation of an obstacle zone which becomes larger as the concentration of mangosteen peel extract increases.

These results show similarities to Ryandini and Hemawan's 2019 research, which proves that mangosteen peel contains alkaloids, flavonoids, tannins, phenols, terpenoids. Alkanoid compounds, phenols, flavonoids, saponins, and tannins have antimicrobial activity [15]. According to Ovalle-Magallanes et al. (2017), the main phytochemical compound that is most commonly found in mangosteen rind extract is xanthone which is a class of polyphenolic compounds or simple aromatic compounds that have a dibenzo gamma-pyrone skeleton core [16]. Based on research by Tatiya-Aphiradee et all, xanthones have bacteriostatic and bactericidal mechanisms. The antibacterial mechanism of flavonoids is to inhibit nucleic acid synthesis, damage cell membrane function, damage bacterial permeability, suppress energy metabolism reduce cell adhesion, and form biofilms [17]. Saponin functions as an antibacterial by increasing cell membrane permeability. Aglicon is a saponin-forming structure that works as an antimicrobial by entering the lipid layer of bacteria. The saponins contained in mangosteen peel extract can damage the phospholipid structure of cell membranes by changing protein function [18]. Tannins inhibit bacterial growth by binding to bacterial cell walls and inhibiting protease activity [19]. Terpenoids can inhibit the microbial growth process by absorbing oxygen and oxidative phosphorylation and affecting lipid membrane activity [20]. The results of this study show that the antibacterial activity of mangosteen peel extract (Garcinia Mangostana L) can inhibit the growth of E.coli bacteria, however, in this study for Salmonella typhi bacteria the inhibition zone was not formed by each different mangosteen peel extract. This result is the same as research conducted by Ermi Sukasih et al, which stated that mangosteen peel extract did not have antimicrobial activity against Salmonella typhi bacteria [21].

E.coli and Salmonella typhi are Gram-negative. In this study, there were differences in results where Salmonella typhi bacteria did not have effectiveness as an antimicrobial because each bacterium had sensitivity to antimicrobials. Prescott, et all (2005) and Yayang (2013) stated that Gram-negative bacteria have a selection system against foreign substances in their cells. lipopolysaccharide layer. Apart from that, bacterial sensitivity can be caused by the plasmids that the bacteria have. Plasmid is a genetic material that contains various genes such as antibacterial resistance genes. This research is not in line with research by Nurul Hidayahti (2015) who said that boiled mangosteen rind can inhibit the growth of Salmonella typhi bacteria. Several other studies regarding, among others:

1. Mangosteen peel extract can be made as an anti-cancer tablet formulation. This was proven in research by Salahuddin Al

Madury, Farida Fakhruninisa, and Azizah Amin in 2012 regarding the benefits of mangosteen peel (Garcinia Mangostana L) as a practical and economical anti-cancer tablet formulation.

2. Antioxidant extract from mangosteen peel by Y.I.P. Arry Miryanti et al (2011) explained that mangosteen peel extract can act as an antioxidant, proven by the mangosteen peel extract reacting to the flavonoid, polyphenol, and saponin tests.
3. Anti-bacterial power of ethanol extract of mangosteen peel (Garcinia Mangostana L) against Porphyromonas Gingivalis bacteria by Rexsy Ajie Nuperdanna Sriyono and Ika Andriana (2013) explained that mangosteen peel extract has antibacterial power against porphyromonas.

CONCLUSION

Based on the results of research that has been carried out, it can be concluded that: Mangosteen rind extract (Garcinia Mangostana L) has the potential to act as an antimicrobial against Escherichia coli bacteria; The diameter of the bacterial inhibition zone starting from the lowest concentration of 6.25%, 12.5%, 25%, 50% and 100% shows that the average inhibition zone is 2.20 – 6.73; Mangosteen fruit does not have the potential to inhibit the growth of Salmonella typhi bacteria. This can be seen from the fact that there is no barrier zone formed from the lowest to the highest concentration.

Suggestion

1. It is necessary to think about conducting clinical research (In vivo) on mangosteen fruit.
2. It is also necessary to carry out further research on processed products made from mangosteen fruit to examine whether there is antibacterial potential for mangosteen fruit in processed form.
3. It is necessary to carry out further research such as qualitative and quantitative biochemical screening to determine the activity of specific compounds contained in mangosteen fruit as antibacterials.
4. It is necessary to carry out other methods of testing the antibacterial power of mangosteen peel abstracts, for example other extraction methods. And choosing a solvent for the extraction process to get more accurate results.

Declaration by Authors

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Conflict of Interest: The authors declare no conflict of interest.

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