Journal of Midwifery and Nursing, 5 (1) (2023) pp. 1-6

Published by: IOCSCIENCE

Journal of Midwifery and Nursing

Journal homepage: www.iocscience.org/ejournal/index.php/JMN

Activity Testing Of Dahlia (Dahlia Variabilis) Tubers Against Escherichia Coli And Salmonella Typhi Bacteria In Vitro

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ARTICLE INFO

Article history:

Received Jan 02, 2023
Revised Jan, 16, 2023
Accepted Jan 30, 2023

Keywords:

Dahlia Bulbs;
Escherichia coli;
Inulin;
Salmonella typhi;

ABSTRACT

Dahlia tubers are a source of carbohydrates in the form of inulin. Dried dahlia tubers are thought to contain inulin as much as 65-76% of the total carbohydrates contained. Inulin or fructoolgosaccharide is a food component that can act as a prebiotic and also as a soluble dietary fiber in humans. Inulin is thought to be able to maintain the balance of the other normal flora of the large intestine. The balance of microflora in the intestine is very necessary because these microbes can inhibit the growth of pathogenic bacteria. Escherichia coli and Salmonella typhi are among the choliform bacteria belonging to the Enterobacteriaceae family. Enterobacteriaceae are enteric bacteria or bacteria that live and can survive in the digestive tract. This study aims to compare the activity of dahlia tuber starch against Escherichia coli and Salmonella typhi bacteria by determining the activity of dahlia tuber starch. The results showed that dahlia tuber starch had antibacterial activity against Escherichia coli and Salmonella typh. Effective concentration in inhibiting Escherichia coli and Salmonella typhi bacteria at a concentration of 80% with an average LDH of 22.71 mm for Escherichia coli bacteria, while for Salmonella typhi the average LDH of 15.08 mm.

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1. Introduction

Indonesia is a developing country that cannot be separated from various health problems, one of which is a disease related to sanitation, namely diarrhea. This disease is one of the main causes of death in children under five, with an incidence rate of 3.5% and a prevalence of 7% (Kemenkes RI, 2011). The main cause of diarrhea and typhoid fever is pathogenic bacteria. One type of bacteria that causes diarrhea is Escherichia coli and the cause of typhoid fever is Salmonella typhi (Wila et al., 2018). One type of plant that can be used for medicine is the dahlia plant (Dahlia variabilis). Dahlia tubers with red flowers showed antimicrobial activity against Escherichia coli, Basillus subtilis, Staphylococcus aureus, Candida utilis, and also the bacteria Penicillium sp (Fitriyah et al., 2013).

Dahlia plants are known to contain secondary metabulites, namely compounds (Purba et al, 2012). Dahlia tubers are a source of carbohydrates in the form of inulin. Inulin also has several biological properties that are beneficial to health such as lowering blood serum cholesterol and triglyceride levels,

reducing the risk of colon cancer, maintaining stable blood sugar levels and maintaining the population of intestinal microflora. (Iskandar, Yetti Mulyati , Sri Pudjiraharti, 2014).

Dahlia tubers may contain inulin and are able to maintain intestinal microflora, so the authors wanted to know the effect of dahlia tubers on bacterial growth by calculating the area of inhibition zone of dahlia tuber starch and at what concentration of starch from dahlia tubers can inhibit the growth of bacteria that cause diarrhea and typhoid fever.

2. Research Method

2.1 Tools and materials

The tools used in this study were aluminum foil, autoclave, oven, stirring rod, Bunsen, beaker glass, blender, petri dish, porcelain dish, scissors, hotplet, incubator, oven, dropper pipette, bath, test tube rack, caliper, spatula, test tube, analytical balance.

The materials used in this study were sulfuric acid, anhydrous acetic acid, hydrochloric acid, aquadest, pure cultures of Escherichia coli and Salmonella typhi bacteria obtained from the North Sumatra Laboratory, magnesium powder, raw dahlia tubers (which have not been dried) ± 8 kg obtained from the Brastagi area of North Sumatra, FeCl3, chloroform, negative control Aquadest, positive control Tetracylines, BaCl2-2H20 1.175%, Aquadest solution, Nutrient Agar (NA), disc paper, Dragendorff reagent, Lieberman-Bouchardat reagent.

2.2 Starch Making

The tubers are peeled off the skin, then washed using running water to remove the remaining dirt. The pieces of dahlia tubers are mashed using a grater to form a tuber pulp. Then the tuber pulp is filtered using a cloth to produce dregs and also the filtrate. Furthermore, the dregs must be filtered again with a ratio of (1: 2) until the filtrate is obtained. The filtrate is then deposited for a period of 3-5 hours. The precipitate that has been obtained is separated from the water and then dried at a temperature of 60 oC for a period of 5 hours. The dried starch is then crushed with a blender and sifted until smooth dahlia tuber starch granules are obtained (Suryani & Nisa, 2015).

2.3 Phytochemical Screening

1. Identification of Alkaloids

A total of 2 g of simplicia was weighed, then added with 1 ml of 2 N hydrochloric acid and 9 ml of distilled water, then heated in a water bath for about 2 minutes, then cooled and filtered. The filtrate will be used for the alkaloid test. Take 3 test tubes, then pour 0.5 ml of the filtrate into each tube.

a. Added 2-3 drops of Bouchardat reagent
b. Added 2-3 drops of Dragendorff's reagent
c. Added 2-3 drops of Meyer's reagent

Positive for alkaloids if precipitate or turbidity is formed in at least 2 test tubes of (Kumalasari & Andiarna, 2020).

2. Identification of Flavonoids

A total of 10 g of simplicia powder was added with 100 ml of hot water, then boiled for about 5 minutes and then filtered while still hot, the filtrate obtained was taken 5 ml and then added with 0.1 g of Mg powder and 1 ml of concentrated hydrochloric acid and 2 ml of amyl alcohol, then shaken, and allowed to separate. Positive flavonoids if there is a red, yellow, orange color change on the amyl alcohol layer (Isnania, 2014).

3. Steroid Identification

1 g of dahlia tuber simplicia powder was macerated with 20 n-hexane for approximately 2 hours, then filtered. The filtrate is evaporated in a vaporizer cup. The remainder in the cup is added with 2 drops of anhydrous acetic acid and also 1 drop of concentrated sulfuric acid. If there is a purple or red color change and then turns green, blue indicates a positive steroid (Marjoni & Ismail, 2016).

4. **Identification of Saponins**

0.5 g of dahlia tuber simplicia is put into a large test tube and then added with 10 ml of hot water, then cooled and shaken vigorously for about 10 seconds, foam will form as high as 1-10 cm. Then 1 drop of 2 N hydrochloric acid solution is added, if the foam still persists, it indicates the presence of saponins in the sample (Kumalasari & Andiarna, 2020).

2.4 Characteristic Test

1. Determination of Total Ash content

Determination of the total ash content is carried out by weighing 2 to 3 grams of the test material put into a porcelain crucible that has been ignited and tarred, ignited until the charcoal runs out, cooled and weighed. Then it is re-ignited to obtain a constant weight, then this step is repeated 3 times after which the total ash content is calculated (G. M. D. Putra*, D. A. Satriawati, N. K. W. Astuti, 2018).

2. **Determination of Water Content**

Determination of water content was carried out with a total of 5 grams of dahlia tuber simplicia powder weighed then put into a moisture analyzer, and wait for the pka results (G. M. D. Putra*, D. A. Satriawati, N. K. W. Astuti, 2018).

3. Determination of Ethanol Soluble Essence Levels

Sebanyak 5 g sampel dimaserasi kurang lebih 24 jam dengan 100 ml etanol 96% didalam labu sambil dikocok sesekali kurang lebih 6 jam pertama, lalu didiamkan selama 18 jam, selanjutnya disaring. Diambil 20 ml filtrat lalu diuapkan sampai sampel umbi dahlia kering dalam cawan penguap. Lalu kadar sari yang larut dalam etanol 96% dihitung (Depkes RI, 1995).

4. Water Soluble Level

The sample is weighed 5 g, then dimeserated for approximately 24 hours with 2.5 ml of chloroform in 1 liter of distilled water, then shaken occasionally for about 6 hours, filtered, taken as much as 20 ml of filtrate, evaporated to dry, then calculated the percentage of water-soluble juice (Depkes RI, 1995)

2.5 Making Medium for Bacterial Growth

1. Sterilization of tools and materials

Tools to be used in research should be sterilized first. Non-glass tools were sterilized using an autoclave at 121°C for about 15 minutes. Glass utensils should be sterilized using an oven at 180°C for approximately 2 hours (Gerfan Patandung & Rosmiati Ibrahim, 2018).

2. Making Media for Leaning Bacterial Inoculum

Pour the heated Nutrient Agar (NA) into a test tube with a slope of 30° and cover it with gauze, then wait for the NA to harden. After solidifying inoculate the microorganisms in the test tube and incubate at 35°C for 18-48 hours (Yunus et al., 2014).

3. Preparation of Standard Solution Mc. Farland

9.95 ml of 1% H_2SO_4 solution was taken and added with 1.175% $BaCl_22H_2O$ solution of 0.5 ml in Erlenmeyer. Then shaken until a cloudy solution is formed. The turbid color formed is used for the turbidity standard of the test bacterial suspension (Toding et al., 2020).

4. Basic Media And Growth Media

Weighed Nutrient Agar as much as 3.6 g, then added with 180 mL of distilled water using an Erlenmeyer (Yunus, 2014).

5. Preparation of Test Bacterial Suspension

The bacteria in the media so that it was tilted were taken using a sterile wire loop and then put in a test tube which already contained 10 ml of 0.9% NaCl solution, shaken vigorously until the turbidity was the same as the standard color of the turbidity Mc solution. farland.

6. **Preparation of Dahlia Bulb Starch Test Solution Concentration**

Test solutions were made with concentrations of 20%, 40%, 60%, and 80% respectively by weighing 0.2 g, 0.4, 0.6 g, 0.8 dahlia tuber starch and then dissolved in 1 ml of aquadest solution.

7. **Antibacterial Effectiveness Test**

The antibacterial activity of dahlia tubers (Dahlia variabilis) was tested using the disc diffusion method. The disc paper to be used has a size of 6 mm. The boiled NA media is poured into a petri dish, wait until it hardens. Then the suspension was taken using a cotton swab and smeared on the NA medium evenly. In a petri dish, a paper disc that has been treated with a negative control was given aquadest solvent, a paper disc was given tetracycline antibiotics as a positive control, and a paper disc was given a treatment which was tested with dahlia tuber starch, by dipping the disc in each concentration of the test starch and then allowed to stand. approximately 30 minutes so that the solvent absorbs the paper disc to be used, then it is placed on the media that has been spotted with suspension. Then incubated at a temperature of 36-37°C for 24 hours. Furthermore, the measurement of the bacterial inhibition zone was carried out by measuring the clear area formed around the paper disc (Wila et al., 2018).

3. Research Results And Discussion

3.1 Phytochemical Screening Results

Dahlia tubers showed the presence of a class of alkaloid compounds, flavonoids, steroids, saponins, and tannins. Phytochemical screening aims to determine the presence of groups of secondary metabolites in dahlia tubers and indicate the presence of groups of alkaloids, saponins, steroids, flavonoids, and tannins. The results of phytochemical screening can be seen in the table below:

Table 1.
Phytochemical Screening Results of Dahlia Bulbs Simplicia

Group of compounds	Results
Alkaloids	-
Flavonoids	+
Steroid	-
Saponins	+
Tannins	-

3.2 Results of Simplicia Characterization Examination

The characterizations carried out in this study were determination of ethanol soluble extract content, determination of water content, determination of water soluble extract content, and determination of total ash content. The results of the research on the simplicia characteristics of dahlia tubers have met the requirements of Indonesian Medical Materials (MMI). The results of the characterization can be seen in the table below:

Table 2.
Results of Characterization of Dahlia Tubers Simplicia

Parameter	Results
Total Ash Content	7,47%
Water content	9,61%
Ethanol Soluble Extract Level	18,5%
Water Soluble Level	19,5%

3.3 Antibacterial Effectiveness Test Results

The results of measuring the diameter of the inhibition zone of dahlia tuber starch against Escherichia coli bacteria can be seen in table 3.

Table 3.
The results of the measurement of the diameter of the inhibition zone of dahlia tuber starch against Escherichia coli bacteria

Treatment	I	II	III	Average
20%	11,37	11,37	11,42	11,39
40%	15,12	15,97	16,11	15,73
60%	17,14	17,66	18,22	17,6
80%	22,27	22,90	22,97	22,71
+ control	26,38	26,40	26,44	26,40
- control	0	0	0	0

The results of measuring the diameter of the inhibition zone of dahlia tuber starch against Salmonella typhi bacteria can be seen in table 4.

Table 4
The results of the diameter of the inhibition zone of dahlia tuber starch against Salmonella typhi bacteria

Treatment	I	II	III	Average
20%	11,12	11,15	11,19	11,15
40%	11,69	11,73	11,80	11,74
60%	12,33	12,36	12,44	12,3
80%	14,98	15,10	15,16	15,08
+ control	25,27	25,35	25,30	25,30
- control	0	0	0	0

Antibacterial activity test was carried out using disc paper dipped in each concentration of dahlia tuber starch. Antibacterial activity was determined by measuring the inhibition zone formed around the hard disk. The negative control in this study was aquadest. The negative control was the solvent used as a diluent for dahlia tuber starch. While the positive control used was Tetracycline because it is broad spectrum.

Dahlia tuber starch is able to inhibit the growth of antibacterial Escherichia coli and Salmonella typhi because it contains active compounds such as flavonoids and saponins. Flavonoid compounds and saponins have antibacterial properties because of the mechanism of action of flavonoids by damaging cell membranes, so that protein activity in cell membranes dies. Saponins themselves have a mechanism of action by interfering with the work of enzymes so that they inhibit nutrient transport so that bacteria die.

The antibacterial power of dahlia tuber starch is effective against gram-negative bacteria (Escherichia coli and Salmonella typhi), especially Escherichia coli because the average obtained is 22.71 mm at a concentration of 80% greater than the average for Salmonella typhi bacteria. namely 15.08 at 80% concentration. Dahlia tubers have a high inulin content which causes a decrease in pH and inhibits the growth of Escherichia coli bacteria. Dahlia tuber starch with a concentration of 80% has antibacterial power with the largest average diameter of the inhibition zone is 22.71 mm for Escherichia coli bacteria and 15.08 mm for Salmonella typhi.

4. Conclusion

Based on the results of the study, it can be concluded that the average obtained from dahlia tuber starch on Eschericia coli bacteria is, the concentration of 20% is 11.39 mm, the concentration of 40% is 15.73 mm, the concentration of 60% is 17.6 mm, the concentration is 80% 22 ,71mm. Meanwhile, the average Salmonella typhi bacteria obtained were, 20% concentration average dahlia tuber starch 11.15 mm, 40% concentration 11.74 mm, 60% concentration 12.3 mm, 80% concentration 15.08 mm. Dahlia tuber starch (Dahlia variabilis) has antibacterial activity against the growth of Escherichia coli and Salmonella typhi bacteria characterized by a clear inhibition zone around the paper disc. There is a comparison of the diameter of the inhibition zone on Escherichia coli and Salmonella typhi bacteria,

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