

Testing of Andaliman Extract (*Zanthoxylum Acanthopodium* Dc) With 4 Types of Solutions (Ethyl Acetate, Aquades, Methanol, And Hexane) on Growth of Bacteria *Escherichia Coli*

Al Muzafri, Ria Karno

Agrotechnology Study Program, Biology Education Study Program, Pasir Pengaraian University, Indonesia

amuzafri@gmail.com, ria_karno@ymail.com

Article Information

Submitted: 03 January 2022

Accepted: 10 January 2022

Online Publish: 20 January 2022

Abstract

In general, this study aims to see whether Andaliman extract is able to inhibit the growth of Escherichia coli microorganisms. the first step in this research is the extraction of Andaliman with 4 types of solvents (equates solvents, methanol, ethyl acetate, n-hexane). In the second stage, phytochemical tests were carried out on the andaliman extract to see the content of the active compounds in the andaliman extract, in the andaliman extract the active compounds were found to be alkaloids, steroids, and free-form triterpenes, saponins, tannins, flavonoids, and glycosides. The third stage of this study was the zone activity test. inhibit andaliman extract on Escherichia Coli assay on bay leaf extract using blank disc paper. The extract solution that has been diluted with a concentration of 25%, 50%, 75%, 100%, Andaliman extract with an ethyl-acetate solvent with a concentration of 100% has the greatest inhibition on the growth of Escherichia coli with dimensions of 19.15 mm, while the has the smallest inhibition of hexane solvent with a concentration of 25% at 5.80 mm.

Keywords: *Andaliman; Escherichia Coli; Ethyl Acetate; Aquades; Methanol; Hexane;*

Testing Of Andaliman Extract (*Zanthoxylum Acanthopodium* Dc) With 4 Types of Solutions (Ethyl Acetate, Aquades, Methanol, And Hexane) On Growth of Bacteria *Escherichia Coli*

Introduction

The bacteria *Escherichia coli* (*E. coli*) is a bacterium that normally lives in the human gut that is beneficial in maintaining digestive health, in general, *E. coli* bacteria are harmless to human health. *Escherichia coli* whose presence in humans is avoided is bacteria *Escherichia Coli* which are pathogenic, namely *Escherichia Coli* bacteria O157:H7. Infection by this bacterium can be caused by direct contact with infective animals or by consuming food such as fish, shrimp, meat, fruit, vegetables, contaminated water, and unpasteurized milk (Anggraini, Salim, and Mardiah 2013).

There are two groups of *Escherichia coli* that cause disease in humans. The first group is called Enteron Toxigenic *Escherichia coli* (ETEC) which is able to produce enterotoxins in the small intestine and cause diseases such as cholera, incubation time of 8-24 hours with symptoms of diarrhea, vomiting and dehydration similar to cholera. The second group is called Enter invasive *Escherichia coli* (EIEC), where cells are *Escherichia coli* able to penetrate the intestinal wall and cause colitis (inflammation of the large intestine) or symptoms such as dysentery (Nurwantoro and Djarijah 1997).

Andaliman is one of the famous plant species in North Sumatra, Indonesia. *Andaliman* is used as a food additive for the typical *Bataknese*, *andaliman* contains aromatic compounds with a bitter taste that produces a vibrating sensation on the tongue that eats it, and sometimes causes the tongue to numb (Muzafri, Julianti, and Rusmarilin 2018). According to (Anggraeni 2020), *Andaliman* has several names in various countries, yan-jiao (China), mouh laht faa jiu (Cantonese Chinese), mao la hua jiao (Mandarin Chinese), Indonesian lemon pepper (English), Indonesian zitronenpfeffer (Germany), tambhul (India), sansho (Japan), and emmay/yerma (Tibet).

Andaliman contains flavonoid compounds that have antioxidant activity which is very beneficial for health and plays an important role in maintaining the quality of food products from various damages such as rancidity, changes in nutritional value and changes in food color and aroma. In addition, the flavonoid compounds in *andaliman* can also be used as antimicrobials. This provides an opportunity for *andaliman* as a raw material for antioxidant and antimicrobial compounds for the food and pharmaceutical industries (Wijaya 1999).

Another benefit of *andaliman* fruit based on research is as an insecticide to inhibit the growth of insects *Sitophilus zeamais*, the effect is to reduce insect appetite (Andayanie 2000). In Japan, *andaliman* leaves are used for flavoring food (Wijaya and Andarwulan n.d.). With the active compounds present in *andaliman* are expected to be able to become an antibacterial agent, will be conducted research on the ability of *andaliman* extract to inhibit or kill *Escherichia coli* bacteria.

Methods

Tools and Materials

The equipment used consisted of autoclave laminar flow, petri dish, test tube, measuring cup, incubator, Erlenmeyer, knife, label paper, camera, oven, hotplate, ose needle, spatula, desiccator, tweezers, Bunsen, knife, blender, scale, funnel, Rotary Evaporator, paper disc and

Testing Of Andaliman Extract (*Zanthoxylum Acanthopodium* Dc) With 4 Types of Solutions (Ethyl Acetate, Aquades, Methanol, And Hexane) On Growth of Bacteria *Escherichia Coli*

caliper. equipment Colony Counter, Chemicals and media, namely nutrient agar (NA), Mueller Hinton Agar (MHA), 0.9% physiological NaCl, ethyl acetate, *Escherichia Coli*, ethyl-acetate, methanol, aquades, and aquades.

Research Implementation

1. *Andaliman* Extraction.

Extraction of *Andaliman* using the maceration extraction method, *Andaliman* was first cleaned of dregs and washed, then dried in an oven at 40°C for 48 hours (Appendix 2). the dried samples were crushed using a blender until they were crushed into powder and then ready for extraction, the *simplicia* powder was put into a container and soaked (maceration) at room temperature with ethyl-acetate, methanol, *aquades* and *aquades* as solvents. The filtrate is separated and the dregs are soaked again with a new solution. Maceration was carried out 3 times. The filtrate obtained is then separated by solvent and extracted with a vacuum rotary evaporator at a temperature of 500C and evaporated so that the solvent is separated from the *andaliman* extract.

2. Phytochemical Test *Andaliman* Extract.

Phytochemical tests were carried out to see what active compounds were contained in the *andaliman* extract, including testing for free-form alkaloids, steroids, triterpenes, saponins, tannins, flavonoids, and glycosides. With the active compound content in *andaliman*, it is expected to be able to become an antibacterial agent in *E. coli*.

3. Test the activity of the microbial inhibition zone of bay leaf extract

Andaliman extract was tested using disc paper. Extracts that have been diluted with concentrations of 25%, 50%, 75%, 100%. The 100% concentration was obtained by adding 2 g of *andaliman* extract and adding 2 ml of DMSO, from the 100% concentration the extract concentrations were made at 75%, 50%, and 25%. Then the disc paper was immersed in the extract solution with various concentrations and was immersed for approximately one hour until the extract solution diffused well. A total of 10 ml of MHA media was poured into sterile Petri dishes and allowed to solidify. Discs containing *andaliman* extract with different concentrations were placed regularly on the surface of the test media that had been grown *E. coli* using sterile tweezers. 24 hours at 37 degrees Celsius.

Results and Discussion

The *andaliman* extraction process begins by drying 5 kg of *andaliman* fruit and then mashing it so that 2 kg of dry *andaliman* powder is obtained. The moisture content of dry *andaliman* powder is 6.6%. The resulting *andaliman* powder was then extracted with 4 types of solvents, namely water, methanol, ethyl-acetate and hexane. The yield of *andaliman* obtained can be seen in Table 1.

Testing Of Andaliman Extract (*Zanthoxylum Acanthopodium* Dc) With 4 Types of Solutions (Ethyl Acetate, Aquades, Methanol, And Hexane) On Growth of Bacteria *Escherichia Coli*Table 1. Yield of *andaliman* extract

Type of Solvent	Yield Extract	
	Weight (g)	%
Aquades	33,3	3,33
Methanol	40,4	4,04
Ethyl-acetate	38,7	3,87
Hexane	31,2	3,12

I. Phytochemical Test**Phytochemical Test Results *Andaliman* Extract**

The results of phytochemical testing of *Andaliman* extract from various types of solvents can be seen in table 2.

Table 2. Phytochemical test results of *Andaliman* extract

Bioactive Compound	Solvent Extract <i>Andaliman</i>			
	Aquades	Methanol	Ethyl Acetate	Hexane
Alkaloids	-	+	+	+
Flavonoids	+	+	+	-
Glycosides	+	+	+	-
Saponins	-	+	+	-
Tannins	+	+	+	-
Triterpenes/steroids	-	+	-	+
Anthraquinone Glycosides	-	+	-	-

Description: (+) = Contains a group of compounds

(-) = Does not contain a group of compounds

The purpose of the phytochemical screening test for *andaliman* extract is to determine the class of secondary metabolites contained in the *andaliman* extract. After passing the phytochemical test, the *andaliman* extract contained alkaloids, flavonoids, glycosides, saponins, tannins, triterpenoids/steroids, and anthraquinone glycosides. Several compounds found in the *andaliman* extract are known to be antibacterial agents. Among them are alkaloids, flavonoids, saponins, and tannins (Rijayanti 2014).

Testing Of Andaliman Extract (*Zanthoxylum Acanthopodium* Dc) With 4 Types of Solutions (Ethyl Acetate, Aquades, Methanol, And Hexane) On Growth of Bacteria *Escherichia Coli*

II. Inhibitory zone test of Andaliman Extract against *E. coli* Bacteria

The antimicrobial activity of bay leaf extract in inhibiting the growth of *Escherichia Coli* can be seen in Figure 1. The results of the analysis of variance showed that the treatment between the type of solvent and the concentration of bay leaf extract significantly affected the inhibition of bacterial activity *Escherichia coli*.

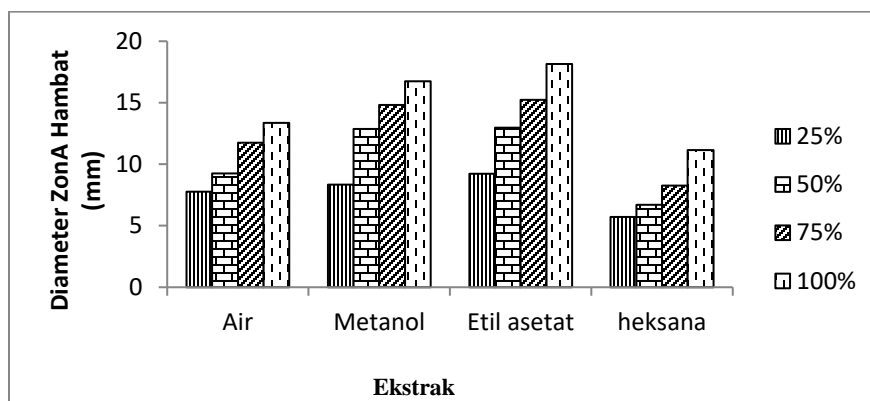


Figure 1. Effect of bay leaf extract from various solvents on the growth inhibition zone of bacteria *Escherichia Coli*.

The figure above shows that the ethyl-acetate solvent with a concentration of 100% had the greatest inhibition on the growth of *Escherichia coli*, which was 19.15 mm, while the one with the smallest inhibitory power was hexane solvent with a concentration of 25%, which was 5.80 mm. The difference in the inhibition zone was due to the ability of each solvent to attract the active compounds contained in the Andaliman extract. Andaliman extract with ethyl acetate and methanol as solvents attracted almost all the active compounds contained in bay leaves, while extracts with water and hexane solvents were less.

Conclusion

The content of flavonoids in *Andaliman* extract is very effective in inhibiting the growth of *Escherichia coli* bacteria, polar flavonoids so that it is easier to penetrate the microbial peptidoglycan layer which is also polar than non-polar lipid layers, cell walls *Escherichia coli* contain polysaccharides (terichoic acid) which are soluble polymers. In water that functions as a transport of positive ions in and out, disruption of the cell wall will cause cell lysis, another mechanism of flavonoids as antimicrobials is to inhibit cell membrane function by disrupting cell membrane permeability and inhibiting enzymes binding such as ATPase and phospholipase (Rijayanti 2014).

Besides the Content of flavonoids According (Rijayanti 2014) active substance being antibacterial agents in *andaliman* extracts are alkaloids, alkaloids antibacterial mechanism, namely by interfering components of the peptidoglycan in the bacterial cell, so that the lining se l is not

Testing Of Andaliman Extract (*Zanthoxylum Acanthopodium* Dc) With 4 Types of Solutions (Ethyl Acetate, Aquades, Methanol, And Hexane) On Growth of Bacteria *Escherichia Coli*

formed completely and causes the death of these cells. The mechanism of action of flavonoids as antimicrobials is by inhibiting the function of cell membranes by forming complex compounds with extracellular and dissolved proteins so that they can damage bacterial cell membranes and followed by the release of intracellular compounds, another mechanism is that flavonoids inhibit cell membrane function by disrupting cell membrane permeability and inhibiting binding. enzymes such as ATPase and phospholipase. In addition to flavonoids and alkaloids, saponins are also capable of being antibacterial.

References

- Andayanie, Like. 2000. "Kajian Daya Insektisida Alami Nabati Kulit Buah Manggis (*Garcinia Mangostana* L.), Buah Andaliman (*Zanthoxylum Acanthopodium* DC.), Getah Gambir (*Uncaria Gambir*, Roxb.) Dan Daun Teh (*Camellia Sinensis* L.) Terhadap Perkembangan Hama Gudang *Sitophilus Zeam*."
- Anggraeni, Ratih. 2020. "Uji Karakteristik Simplisia Buah Andaliman (*Zanthoxylum Acanthopodium* DC.)." *JIFI (Jurnal Ilmiah Farmasi Imelda)* 3(2): 32–38.
- Anggraini, R, Marniati Salim, and Elida Mardiah. 2013. "Uji Bakteri *Escherichia Coli* Yang Resisten Terhadap Antibiotik Pada Ikan Kapas-Kapas Di Sungai Batang Arau, Padang." *Jurnal Kimia Unand* 2(2): 17–21.
- Muzafri, A, E Julianti, and H Rusmarilin. 2018. "The Extraction of Antimicrobials Component of Andaliman (*Zanthoxylum Acanthopodium* DC.) and Its Application on Catfish (*Pangasius Sutchi*) Fillet." In *IOP Conference Series: Earth and Environmental Science*, IOP Publishing, 12089.
- Nurwantoro, Ir, and A S Djarijah. 1997. "Mikrobiologi Pangan Hewani-Nabati." *Yogyakarta: Kanisius*.
- Rijayanti, Rika Pratiwi. 2014. "Uji Aktivitas Antibakteri Ekstrak Etanol Daun Mangga Bacang (*Mangifera Foetida* L.) Terhadap *Staphylococcus Aureus* Secara in Vitro." *Jurnal Mahasiswa PSPD FK Universitas Tanjungpura* 1(1).
- Wijaya, C Hanny. 1999. "Andaliman, Rempah Tradisional Sumatera Utara Dengan Aktivitas Antioksidan Dan Antimikroba."
- Wijaya, C Hanny, and Nuri Andarwulan. "Aktivitas Antioksidan Ekstrak Buah Andaliman (*Zanthoxylum Acanthopodium* DC) Dalam Beberapa Sistem Pangan Dan Kestabilan Aktivitasnya Terhadap Kondisi Suhu Dan PH."

Copyright holder:

Al Muzafri, Ria Karno (2022)

First publication right:

KESANS: International Journal Health and Science