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Phytochemicals and Antibacterial Activities of Soursop Leaf (*Annona muricata*) against *Edwardsiella tarda* (In Vitro)

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ABSTRACT: *Edwardsiella tarda* is bacteria in fish caused of Edwardsiellosis disease and it was include to plague bacteria that can harm. *Edwardsiella tarda* infection controlled using a natural antibacterial that is *Annona muricata*. The aim of this research was to know the content of secondary metabolites in leaves of the soursop (*Annona muricata*) as well as an effective solvent to extract *Annona muricata* to be an antibacterial against on *Edwardsiella tarda* by disc diffusion method. Experimental method used in this research is descriptive and it is based on 2 types of leaves (fresh and dried), 3 different treatment solvent (ethanol, ethyl acetate and chloroform) and three replications. Data obtained from the study was statistically analyzed used statistical applications, SPSS version 21.0. The results of the crude extract of dried *Annona muricata* is in accordance with the results of phytochemical contained phenols, saponins, tannins and flavonoids as well as the best use of ethyl acetate solvent to the extent of inhibition with 15:41 mm.
Key words: Antibacterial, *Edwardsiella tarda*, *Annona muricata*, Phytochemicals, Solvent

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INTRODUCTION

Some obstacles often encountered in aquaculture as disease. *Edwardsiella tarda* is bacteria in fish farming and caused of Edwardsiellosis disease. Edwardsiellosis is a bacterial disease that is a very serious systematic cultivation of eels (Japanese eel) in Japan and Taiwan, Japanase flounder fish and other farmed fish. In the USA, *Edwardsiella tarda* caused of septicemia infectious diseases in fish channel catfish (*Ictalurus punctatus*), host and geographic distribution. These bacteria include in fish pests and diseases of quarantine (HPIK) class II which require high vigilance to prevent of entry and spread of this disease in Indonesia because these diseases relatively can infect and harming quick time [1].

Infection control efforts by fish health management through control measures [2]. *Edwardsiella tarda* bacteria by using a natural antibacterial with environmentally friendly, one of them can use the leaves of soursop *Annona muricata*. In general, according to Octavia [3], soursop leaves is known to contain active compounds that are toxic, this situation allows the soursop can be used as an antibacterial compound. Upendra and Khandenwal [4] stated that extracts of *Annona muricata* has shown antibacterial activity against in eight species of pathogenic bacteria. The investigation clearly revealed the antibacterial properties of this plant can be used in the prevention of diseases caused by pathogenic bacteria.

The purpose of this research is to know the content of secondary metabolites in leaves of the soursop (*Annona muricata*) by phytochemical test and get the kind of effective solvent to extract *Annona muricata* as an antibacterial against *Edwardsiella tarda* by disc diffusion method.

MATERIAL AND METHODS

This study was conducted in November 2014 - January 2015 in the Laboratory of Parasitic Diseases Fish and Fishery Product Technology Laboratory, Faculty of Fisheries and Marine Science, University of Brawijaya, Malang. *Edwardsiella tarda* bacterial identification was done in Fish Quarantine (Station I), Perak, Surabaya.

Extraction of the leaves of *Annona muricata* (fresh and dried) with multilevel maceration method (2x24 hours) using the appropriate solvent polarity in a row, chloroform, ethyl acetate, and ethanol. The method used in this research was descriptive based on the experimental methods. This study consisted of 3 different treatment solvent (ethanol, ethyl acetate and chloroform) and 3 replications, also used as a comparator antibiotic ampicillin as positive control. The parameters observed in this study were the extent of inhibition (mm).

Data obtained from the results of the research statistically analyzed using statistical applications, SPSS version 21.0. *Annona muricata* analyzed descriptively to phytochemical test of secondary metabolites.

RESULTS

Yield of *Annona muricata* extract

The results obtained from the extraction process of soursop leaf *Annona muricata* extract for fresh and dried leaves are different. The extraction of soursop leaves *Annona muricata* as % yield of extract can be seen in Table 1. Based on Table 1, yield of the resulting extract solvent chloroform was the smallest (4.98%), while the solvent ethyl acetate was 7.85% and the ethanol was 8:43%.

Table 1. Yield of *Annona muricata* extract

Solvent	Yield of extract (%)	
	Fresh	Dried
Chloroform	3.37	4.98
Ethyl acetate	5.98	7.85
Ethanol	6.20	8.43

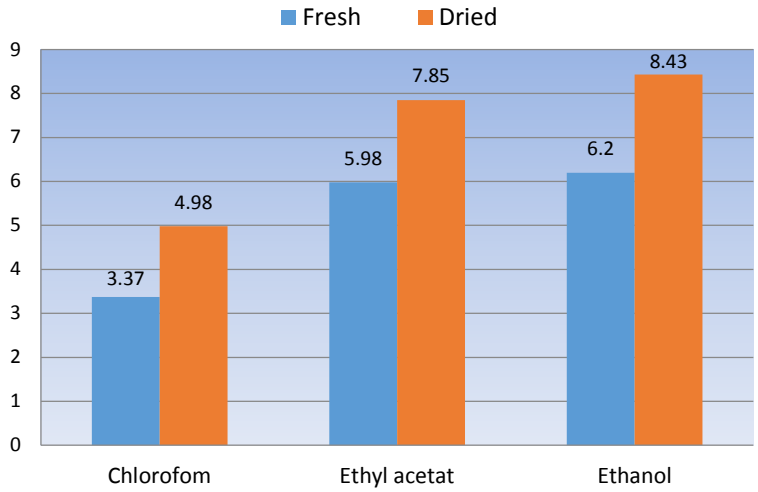

Figure 1. Yield of *Annona muricata* Extract

Yield of extract seen from the chart above that the extract produced from either fresh or dried leaves of the soursop showed that soursop leaf extract with ethanol and ethyl acetate to produce yield of extract was more than chloroform. To fresh extract produce yield of extract relatively smaller because there are many components of water it so difficult to the solvent to enter and extract the compounds contained. This suggests that the bioactive compounds of *Annona muricata* more soluble in ethanol and ethyl acetate as compared with chloroform. Components carried on the extraction process is a component in accordance with the polarity of the solvent, therefore the type of solvent used can affect the amount of yield extract produced [5]. Obi and Onuoha [6] reported that alcohol is the best solvent for extracting the active component of plants.

Inhibitory of *Annona muricata*

Antibacterial activity test used by the test method discs with a diameter of inhibition of bacteria. The amount of inhibition diameter of each extract can be seen in Table 2. Table 2 showed that the average of inhibition produced by extracts of dried leaves of *Annona muricata* on growth *Edwardsiella tarda* greater when compared to fresh leaves extract. This is because the dried leaves of *Annona muricata* more compounds are secondary metabolites that can easily extractable than fresh leaves of *Annona muricata* that there are still have some water [7].

Utilization of different types of solvents (chloroform, ethyl acetate, and ethanol) gives effect to the inhibition of bacterial growth resulting in *Edwardsiella tarda*. Type of solvent ethyl acetate has the greatest inhibitory 15:41 mm compared with solvent ethanol extracts with inhibition of 10.81 mm and chloroform 1:20 mm. This is because the solvent ethyl acetate that is semi-polar can partially dissolve polar and non-polar compounds contained in the sample. Active components that can be dissolved in ethyl acetate and are alkaloids, phenols, hydroquinone, flavonoids, terpenoids, saponins, steroids. Component content more antibacterial compounds contained in the ethyl acetate extract, where in the hydrophobic and hydrophilic components respectively contained in extracts and has a high antibacterial activity. As a result, the highest inhibition was

obtained in the ethyl acetate extract as component hydrophobic and hydrophilic able to enter the cell membranes and inhibit cell metabolism [8].

Table 2. Inhibition Test Results of *Annona muricata* Leaf Extract on *Edwardsiella tarda*

Solvent	Diameter of Inhibitory (mm) ($\bar{x}$ ± sd)	
	Fresh Leaves	Dried Leaves
Chloroform	0.67 ± 0.14	1.20 ± 0.21
Ethyl acetate	3.92 ± 0.13	15.41 ± 0.18
Ethanol	5.74 ± 0.28	10.81 ± 0.30

A compound having the optimum polarity will have a maximum antimicrobial activity, due to the interaction of a compound with the bacteria needed antibacterial hydrophilic-lipophilic balance [9]. Polarity is a compound physical properties important antimicrobial compound. Hydrophilic properties required ensuring the compound soluble in the water phase which is the place of microbial life, but compounds acting on cell membranes require also hydrophobic lipophilic properties, so that the antibacterial compound requires a hydrophilic-lipophilic balance to achieve optimal activity [10].

Phytochemicals of *Annona muricata*

Phytochemical test done to the best extract with ethyl acetate solvent in the leaves of soursop *Annona muricata* method [11] which consist of alkaloid test, steroids test, flavonoids test, tannins test / polyphenols, terpenoids test, and saponin test. Results of phytochemical test ethyl acetate extract of leaves of *Annona muricata* can be seen in Table 3. From the Table 3 it can be seen that the dry extract of *Annona muricata* contained saponins, tannins/polyphenols, flavonoids and steroids with positive results. Phytochemical test results to the ethyl acetate extract of *Annona muricata* according to study of Wisdom et al. [12], where in the phytochemical analysis of the leaves of the soursop their secondary metabolites such as Tanin/polyphenols, steroids, saponins and flavonoids. Flavonoid compounds contained in extracts of leaves of *Annona muricata* potential as an antibacterial for being able to inhibit the growth of bacteria by destroying the cell wall permeability, microsomes, lysosomes and bacterial cells as a result of interaction between flavonoids with DNA [13].

While the tannins/polyphenolic able to act as an antibacterial in the way it reacts with cell membranes, inactivate enzymes - essential enzymes, function and metabolism of the cell's genetic material so hampered and disrupted cell wall synthesis [14]. Saponins are surface active compounds resulting from steroid or triterpene group that binds to sugars, these compounds have a beneficial biological effect that is as hypocholesterolemic and anti-carcinogen and can boost the immune system [15]. Saponins inhibit the growth or kill microbes by interacting with membrane sterols. The main effect is the release of saponin tehadap bacterial proteins and enzymes of the cells [16].

Table 3. Result of Phytochemicals Test to Extract with Ethyl Acetate Solvent in the Leaves of *Annona muricata*

Compound	Result (+/-)
Saponin	+
Tannin/Poliphenols	+
Alkaloid	-
Terpenoids	-
Flavonoid	+
Steroid	+

CONCLUSION

Crude extract of *Annona muricata* in % yield of extract the highest of dry extract with ethanol (8.43%) and ethyl acetate (7.85%). For dry extract according to test results phytochemical classes of compounds containing phenol, saponins, tannins/polyphenols and flavonoids as well as the best solvent to extract *Annona muricata* using ethyl acetate with an area of inhibition that can inhibit the growth of *Edwardsiella tarda* (15.41 mm).

Competing interests

The authors declare that they have no competing interests.

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