Antibacterial Activity of Humped Bladderwort (Utricularia Gibba) Extracts Resulting From Multistage Extraction Method

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Research Article

ANTIBACTERIAL ACTIVITY OF HUMPED BLADDERWORT *(UTRICULARIA GIBBA)* EXTRACTS RESULTING FROM MULTISTAGE EXTRACTION METHOD

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ABSTRACT

The purpose of this research was to identify antibacterial activity of humped bladderwort *(Utricularia gibba)* with multistage extraction utilizing swamp plants as a natural antibacterial. The parameters observed were tannins and phenol by quantitative analysis. The yield of extracts with solvent n-hexane, ethyl acetate and ethanol were 0.50%, 1.66%, 3.27%, respectively. Quantitative testing of tannins from humped bladderwort *(Utricularia gibba)* extract were 215.12 ppm from the extract with n-hexane solvent, 221.86 ppm from the extract with ethyl acetate solvent and 225.70 ppm from the extract with ethanol solvent and testing of phenol levels were 108 ppm from the extract with n-hexane solvent, 122 ppm from the extract with etyl acetate solvent and 152 ppm from the extract with ethanol solvent. The antibacterial activity indicate that the extract of humped bladderwort *(Utricularia gibba)* can inhibit the bacterial of *Bacillus subtilis, Salmonella thypimurium* and *Escerichia coli*. Inhibitory zone was highest at concentration of 2.000 ppm using an ethyl acetate solvent.

Keywords: antibacterial, humped bledderwort, phytochemicals

INTRODUCTION

Research on various types of aquatic plants, especially swamp, has been carried out to determine their potential as bioactive compounds. Previous research shows plants have bioactive compounds that have the potential as antibacterial. Multilevel extraction is carried out using solvents with different polarity levels. The level of polarity can affect the yield made by the extraction process. Extraction using a single method for humped bladderwort (*Utricularia gibba*), yielded a low extract yield of 3.59%[1]. Water clover (*Marsilea crenata*) using a multilevel method using n-hexane, ethyl acetate and methanol as a solvent extract of 11.98%[2]. Therefore, a combination of solvents is needed to optimize the extraction results from the extraction process of humped bladderwort (*Utricularia gibba*) to be used as a natural antibacterial alternative.

The presence of natural antibacterial on humped bladderwort *(Utricularia gibba)* has the potential to be used as an antibacterial against *Bacillus subtilis, Salmonella typhimurium* and *Escherichia coli* bacteria which can later be used as a natural antibacterial alternative. In this study, phytochemical compounds were extracted using different solvents which had different polarity levels, namely, n-hexane, ethyl acetate and ethanol which were carried out in stages. Extraction obtained was tested for antibacterial activity of pathogenic bacteria and food spoilage bacteria.

MATERIAL AND METHODS

Humped bladderwort *(Utricularia gibba)* was collected from Indralaya swampand immediately brought to the laboratory in sterile plastic bags containing water to prevent evaporation. Humped bladderwort *(Utricularia gibba)* were washed thoroughly with distilled water to remove extraneous materials and shade-dried for 10 days at room temperature until constant weight obtained. The dried of humped bladderwort *(Utricularia gibba)* was powdered and stored in refrigerator for future use.

Preparation of humped bladderwort *(Utricularia gibba)* extract

The extraction method that is carried out is maceration level. This extraction was carried out using 3 types of solvents with different polarity levels, namely n-hexane (non-polar), ethyl acetate (semi-polar) and ethanol (polar) solvents by humped bladderwort (*Utricularia gibba*) soaking for these solvents for 48 hours. Humped bladderwort (*Utricularia gibba*) extraction stages are as follows: Stage I, 750 grams of humped bladderwort (*Utricularia gibba*) powder and put into Erlenmeyer soaked with n-hexane with a ratio of 1: 5 for 48 hours at room temperature then filtered using Whatman 42 paper which produces n-hexane and residual filtrate. Stage II, the residue is then soaked with a 1: 5 ethyl acetate solvent for 48 hours then filtered using Whatman paper which produces ethyl acetate and residual filtrate. Stage III, the residue is then soaked again with ethanol as a ratio of 1: 5 for 48 hours then filtered using Whatman paper which produces ethanol and residual filtrate. Then evaporation of each filtrate obtained using Vacuum rotary evaporator at 45 °C for 3 hours. After evaporation the extract was weighed to find out the extract yield.

Yield of extraction

The yield of extraction is a comparison between the weights of the material used. Yield calculations done to measure the effectiveness of the solvent to extract the bioactive components.

Phytochemical compounds of humped bladderwort *(Utricularia gibba)* extract

Total tannins were determined according to the method of Sun *et al*[3] and total phenols were assayed according to Dewanto *et al*[4].

Antibacterial test

In the antibacterial testing refers to the standard procedure Kirby-bauer method according to the Atlas[5] as follows: 1. Take each 1 ml of bacteria *Bacillus subtillis, Escherichia coli* and *Salmonella typhimurium* in liquid nutrient in the test tube and inoculated into selective media medium test bacteria on a petri dish using a pour plate technique. 2. Medium bacteria that selectively poured into a petri dish with a minimum thickness of 5 mm by 10 ml after being inoculated with a test microorganism. 3. After a selective medium to harden on the surface of the medium, 6 mm diameter disc paper has been dipped into the sample with a concentration of 500 ppm, 1000 ppm, 1500 ppm, and 2000 ppm, respectively, so that the entire surface of the paper disc touches the surface. medium. Incubation was carried out for 24 hours at 37 ° C. 4. Diameter barriers forming around the paper disc was measured by using a ruler[6].

Measurement is done by looking at the clear zone that will form around the disc paper at the end of the incubation. This visible zone is a zone that is not overgrown with bacteria because of the influence of the extract diffused into the medium. The diameter of the obstacle area is measured after the incubation period ends.

RESULT AND DISCUSSION

Yield of extraction

The yield of extraction is a comparison between the weights of the material used. The yield of extract of humped bladderwort *(Utricularia gibba)* from each solvent can be seen in Figure 1.

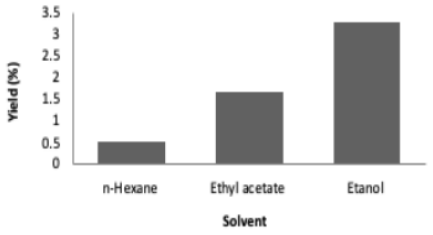

Figure 1: The yield of extraction of humped bladderwort *(Utricularia gibba)*

The yield of extracts with solvent n-hexane, ethyl acetate and ethanol were 0.50%, 1.66%, 3.27%, respectively. The difference in extract yield in this test was caused because each solvent used had a different level of polarity. This research is in line with the results of Baehaki *et al*[7], from water chestnut (*Eleocharis dulcis*)using n-hexane, ethyl acetate and ethanol extraction method indicating that ethanol extract produced a higher yield of extract than other solvents used, ethanol solvent had a higher level of polarity compared to other solvents. In addition, the high yield of ethanol extract is also due to the loss of non-polar and semi-polar compounds that have been carried out in the extraction process n-hexane and ethyl-acetate of the multilevel extraction method.

Phytochemical compound from extract of humped bladderwort *(Utricularia gibba)*

Phytochemical compound from extract of humped bladderwort *(Utricularia gibba)* can be seen Table 1

Table 1. Phytochemical compounds of humped bladderwort *(Utricularia gibba)*

Phytochemical compounds	(ppm)		
	N-Hexane	Ethyl acetate	Ethanol
Tannin	215.12	22.,86	225.70
Phenol	108.00	122.00	152.00

As seen as Figure 1 showed that the tannin content from extraction was higher than phenol content, it was suspected that n-hexane, ethyl acetate and ethanol dissolved tannins more than phenols in humped bladderwort *(Utricularia gibba)* extracts. When viewed from the structure, tannins are phenol compounds that have large molecular weights consisting of hydroxyl groups and several other groups such as carboxyl to form strong complexes that are effective with proteins and several other macromolecules.

Testing of phenol levels in this research was carried out using the folin-ciocalteumethod, this method was used based on the strength of reducing the phenolic hydroxyl group. All phenol compounds can react with folin-ciocalteu. The highest total phenol in extract of humped bladderwort *(Utricularia gibba)* was 225.70 ppm. The high level of phenol in ethanol extract was related to the ability of ethanol solvent to extract phenol compounds. Ethanol is a polar solvent that can extract phenols properly. In addition, the high total phenol content in the third stage of the multilevel extraction method due to phase I and II of other binding compounds was dissolved by n-hexane and ethyl acetate extract.

Tannins are active secondary metabolites which are known to have several properties as antigens, anti diarrhea and antibacterials. Tannin compounds can inhibit the growth of *Aeromonas hydrophila*[8]. The mechanism of tannin as an antibacterial is by damaging the bacterial cell membrane, tannin compounds can induce the formation of bonds of complex compounds to enzymes from microbial substrates. Phenol compounds can work as antibacterials. The mechanism of action of phenol in inhibiting bacteria by denaturing protein cells. Due to the denaturation of bacterial cell proteins, all bacterial cell metabolic activity is stopped, because all metabolic activities of bacterial cells are catalyzed by enzymes.

Secondary metabolites that show positive results in phytochemical screening tests can support the antibacterial activity of humped bladderwort *(Utricularia gibba)* extracts. These compounds that can have potential as natural antibacterials that can inhibit bacterial growth are by denaturing bacterial cell proteins.

Table 2: Inhibition zone of humped bladderwort *(Utricularia gibba)* extracts

Bacteria	Solvent	Inhibition zone(mm)			
		500	1000	1500	2000
Bacillus subtilis	*N*-hexane	8.0	9.5	10.5	11.0
	Ethyl acetate	9.0	11.5	11.5	14.5
	Ethanol	11.5	10.5	11.5	13.5
Salmonella thypimurium	*N*-hexane	8.0	8.0	9.0	11.0
	Ethyl acetate	9.5	9.0	13.5	13.0
	Ethanol	8.0	9.0	10.5	12.0
Escerichia coli	*N*-hexane	8.0	8.5	7.5	8.5
	Ethyl acetate	8.5	8.0	8.5	11.5
	Ethanol	7.5	9.0	8.5	10.0

Table 2, antibacterial activity of humped bladderwort *(Utricularia gibba)* plants showed the diameter of the inhibition zone in stage I (n-hexane solvent), stage II (ethyl acetate solvent) and stage III (ethanol solvent) using concentrations of 500 ppm, 1000 ppm, 1500 ppm and 2000 ppm. The average diameter of the highest inhibitory zone of *B. subtilis* was 14.5 mm at a concentration of 2000 ppm, the results of this humped bladderwort *(Utricularia gibba)* was higher, compared to the Baehaki *et al*[7] using hydrilla extract (*Hydrilla verticullata*) against *B. subtilis*. Inhibitory zone of *B. subtilis*was 11.6 mm at the same extract concentration.

Different responses of the two Gram bacterial groups to humped bladderwort *(Utricularia gibba)* extracts were caused by different sensitivities between gram negative bacteria and Gram positive bacteria. Pelczar and Chan stated that Gram negative bacterial cells have multiple layers and relatively higher fat content (11-12%), so they are more resistant to environmental changes caused by chemicals. While the type of Gram positive bacteria generally has a simpler cell wall structure of 90% where the cell wall consists of peptidoglycan layer while the other layer isteichoic acids[10].

According to Fitrial *et al*[11] generally cell walls of gram-negative bacteria contain an outer membrane that can block the passage of large molecules including antibacterial molecules. This is thought to result in Gram-positive bacterial cell walls easily damaged by antibacterial compounds from bubble grass extracts from Gram-negative bacteria. The difference in inhibitory activity between *B. subtilis, E. coli* and *S. typhimurium* can be caused by the use of various types of solvents. Ethyl acetate is a semi-polar organic compound, so filtered bioactive compounds can be polar and non-polar in accordance with the properties of ethyl acetate compounds which can dissolve polar and non-polar compounds. The inhibition zone produced by humped bladderwort *(Utricularia gibba)* with this multilevel extraction method was higher, compared with extraction using methanol solvent against *Bacillus subtilis, vibrio cholera* and *Listeria monocytogenesis* with single extraction method[1].

The second stage of humped bladderwort *(Utricularia gibba)* extract with ethyl acetate (semi polar) produced phenol and tannin. This phytochemical component dissolved in semi-polar solvents causes test bacteria to have different inhibitory effects and greater effect on *E. coli, B. subtilis* and *S.typhi*. This is also supported by the opinion of Ningtyas[12], stating that semi-polar compounds are difficult to pass through Gram-negative cell walls because the cell wall content of Gram-negative bacteria consists of more lipid content than Gram-positive bacterial cells whose cell wall content is peptidoglycan.

CONCLUSION

Quantitative testing of tannins from the extract of humped bladderwort *(Utricularia gibba)* was 215.12 ppm of the extract n-hexane, 221.86 ppm of the extract ethyl acetate and 225.70 ppm of the extract ethanol and testing of phenol levels was 108 ppm of the extract n-hexane, 122 ppm of the extract etyl acetate and phenol levels 152 ppm of the extract ethanol. The antibacterial activity indicate that the extract humped bladderwort *(Utricularia gibba)* can inhibit the bacterial of *B. subtilis, S.typhimurium*, and *E. coli.* Inhibitory zone was highest at 2.000 ppm using an ethyl acetate solvent.

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