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The Effect Of Administration Of Beluntas *(Pluchea indica L)* Leaf Extract On Albumin Levels And Total White Rat Serum Protein *(Rattus novergius)* Which Is Induced With *E.coli* Bacteria

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ABSTRACT

This study aims to determine whether giving beluntas leaf extract has an effect on albumin levels and total serum protein in white mice induced with E.coli bacteria. RAL was used consisting of 3 treatments and 5 replications. White mice were induced and then given 0.5 ml of extract at a dose of 0.00 mg/kgBW; 300 mg/kgBW; and 600 mg/kgBB. The results showed that administration of the extract had a very significant effect on albumin levels and total serum protein in white mice. Giving the extract at a dose of 600 mg/kgBW gave the highest serum albumin levels but gave the lowest total protein levels. Giving the extract at a dose of 0.00 mg/kgBB; 300 mg/kgBW; and 600mg/kgBW in white mice were induced to give average albumin levels of 2,706 g/dL respectively; 2.844 g/dL; 4.454 g/dL and the mean total serum protein levels were 8.51 g/dL respectively; 8.86 g/dL; 8.8 g/dL.

Keywords: Beluntas leaves, albumin, total protein

1. INTRODUCTION

Beluntas (*Pluchea indica L)* is a plant that has long been known and grown in Indonesia. Beluntas are included in the Asteraceae family which has been widely used by the community as food and traditional medicine[1]. Plant parts such as roots, stems, leaves, flowers, bark and fruit have been used as medicine.[1.2] Beluntas leaves *(Pluchea indica L)* contain alkaloids, flavonoids, tannins, essential oils, sodium, potassium, aluminum, calcium, magnesium and phosphorus. The phytochemical content of beluntas leaves is flavonoids (4.18%), tannins (2.351%), essential oils (1.88%), and alkaloids (0.316%).[1.3]

Flavonoids are a group of phenols that are currently receiving a lot of attention because they have various pharmacological activities. The flavonoids in beluntas leaves have antibacterial activity against

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Staphylococcus sp. Propiono-bacterium and sp. Coryne-bacterium. The growth of Escherichia coli bacteria can be disrupted by the presence of phenolic compounds contained in beluntas leaf extract. The mechanism of action of phenol in this case is by increasing the permeability of the cytoplasmic membrane, causing leakage of intracellular components and cytoplasmic coagulation. resulting in cell lysis. Furthermore, tannin is an active compound contained in plants that is phenolic in nature, has an astringent taste and has the ability to tan the skin. Tannin compounds act as an astringent, the mechanism of tannin as an astringent is that it shrinks the intestinal surface or is a substance that is protective of the intestinal mucosa and can coagulate proteins. Therefore tannin compounds can help stop diarrhea. Beluntas leaves also have pharmacological and antiseptic activity against bacteria that cause diarrhea, namely Staphylococcus aureus, Escherichia coli, and Salmonella typimurium.[2.2]

Diarrhea is an infectious disease that is still a major problem in developing countries, including Indonesia. Diarrhea is generally caused by the quality of environmental hygiene and sanitation which still does not meet the requirements. In North American countries, diarrhea causes approximately 15–34% of all deaths. Which amounts to approximately 300 deaths per year. In developing countries, the main cause of diarrhea is the *Echerichia coli (E coli)* bacteria which is usually found in the intestines of humans and warm-blooded animals.[3] Most strains of *E.coli* are harmless, but there are some types that are dangerous, such as shiga toxin-producing *E.coli* (STEC) which is similar to the toxin produced by Shigella dysenteriae. STEC bacteria cause bloody diarrhea (hemorrhagic colitis), fever and vomiting can also occur, and can even cause life-threatening diseases such as hemolytic uraemic syndrome (HUS). HUS is characterized by acute kidney failure, hemolytic anemia, and thrombocytopenia (low blood platelets). *E.coli* 0157:H7 is the most important STEC serotype of public health relevance. *E.coli* 0157:H7 is transmitted to humans primarily through consumption of contaminated food, such as raw or undercooked meat products, raw milk, vegetables and raw sprouts.

2. EXPERIMENTAL

2.1. Chemicals, Equipment and Instrumentation

The tools used in this research were a set of distillation equipment, dark bottles, blender, 60 mesh sieve, maceration bottle, Buchner funnel, vacuum, filter paper, Erlenmeyer, chemical beaker, rotary evapotator, hot plate, aluminum foil, analytical balance, dropper pipette. , measuring cup, glass funnel, watch glass, test tube, test tube rack, measuring flask, spatula, autoclave, petri dish, tube needle, laminar flow, white rat cage, oral probe, syringe, bent scissors, 3cc EDTA tube, and UV-VIS spectrophotometer.

The materials used in this research were 15 adult male white rats aged 2-3 months with a body weight of 130-170 grams, standard pellets and water, beluntas leaf extract, ethanol (C2H5OH) 96%, hydrochloric acid (HCL) 2N, distilled water (H2O), Mayer's reagent, Wagner's reagent, anhydrous acetic acid (CH3CO)2O, concentrated sulfuric acid (H2SO4), magenisum powder (Mg), hydrochloric acid (HCl) 2%, iron (III) chloride reagent ($FeCl_3$) 1 %, hot water, Mueller Hinton Agar (MHA), NaCl 0.9%, 70% alcohol, E. coli bacteria, reagent 1 total protein, reagent 2 total protein, and reagent albumin.

2.2. Research Procedure

This research procedure consists of six stages which include, (1) Preparation of cages and white mice, (2) Preparation of beluntas leaf extract, (3) Phytochemical screening, (4) Preparation of Escherichia coli bacteria, (5) Treatment of white mice, and (6) Analysis of albumin and total protein levels in white mice.

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Cage Preparation and White Rats, Three white rat cages measuring 900 cm^2 are provided, into each cage 5 adult white rats aged 2-3 months with relatively uniform body weight will be placed, which will be obtained from the Faculty of Pharmacy, University of North Sumatra.

Preparation of Beluntas Leaf Extract, The beluntas leaves that have been collected are washed thoroughly to separate dirt from the sample, cut into small pieces, then air-dried until dry. After the leaves are dry, they are ground using a blender and sifted using a 60 mesh sieve until a fine powder is obtained.

Phytochemical Screening, Phytochemical screening of beluntas leaves (Pluchea indica L) includes examination of alkaloid, steroid/triterpenoid, flavonoid, saponin and tannin compounds.

Preparation of E.coli Bacteria, Making Selective Media Agar is made by dissolving 19.0 grams of Mueller Hinton Agar (MHA) selective media in 500 mL of distilled water. The solution was put into an autoclave at a pressure of 1.5 atm, temperature 121ºC for 15 minutes, with the aim of dissolving the media and sterilizing the media.

Providing treatment, In the study, 15 male white rats were used which were divided into 3 treatment groups. All experimental mice were adapted for 7 days. The next stage, the mice were fasted for 60 minutes and then induced orally with Escherichia coli ATCC 25922 0.5 mL/head. Observations of white mice were carried out when diarrhea began. To find out how long diarrhea occurs after an injection of Escherichia coli bacteria until the stool returns to normal, the time period for diarrhea is determined. The time period for diarrhea is calculated by subtracting the time when feces return to normal from the time when diarrhea begins.

Analysis of Albumin and Total Protein Levels in Rat Blood Serum, Analysis of albumin levels was carried out using the Bromcesol Green (BCG) method.

3. RESULTS AND DISCUSSION

3.1. Analysis of Characterization Results

From the results of the drying, blender and sifting process of beluntas leaves, 750 grams of fine powder was obtained. Next, the fine powder was extracted using the maceration method with 96% ethanol solvent, resulting in an extract of 95 grams. Thus, the percentage of extract produced from 750 grams of beluntas leaf simplicia in this study was 12.7%.

Phytochemical Screening Test Results, The beluntas leaf extract in this study was tested using a phytochemical test to qualitatively analyze the secondary metabolite compounds contained in the ethanol extract of beluntas leaves. Based on phytochemical screening of the ethanol extract of beluntas leaves, secondary metabolite compounds were obtained, including alkaloids, flavonoids, saponins, steroids and tannins.

Alkaloid Identification, The alkaloid test was carried out using the Mayer, Wagner and Dragendorff methods. A 3 mL sample was placed in a test tube, then 5 mL of 2 N HCl and 5 mL of distilled water were

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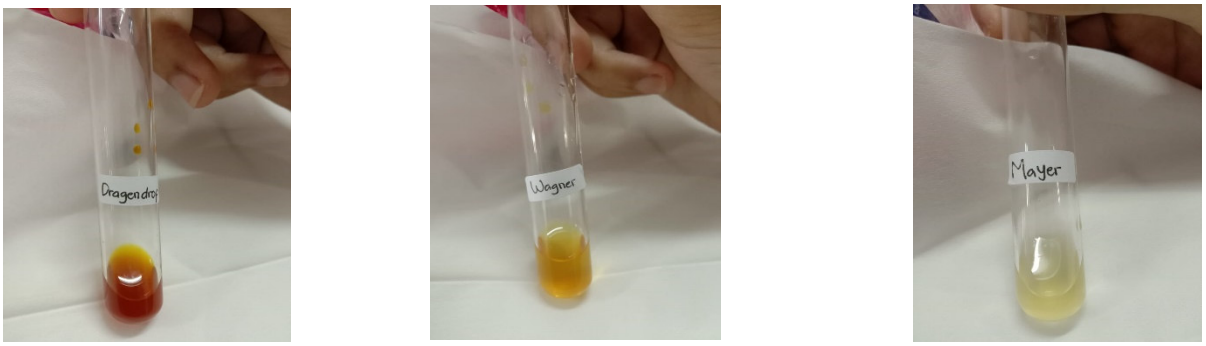
added, then heated over a water bath for 2 minutes. Cool the sample to room temperature and filter. The filtrate obtained was divided into 3 parts A, B, and C. Filtrate A plus Dragendorff reagent was characterized by the formation of a brick red precipitate if it was positive for alkaloids, filtrate B with Mayer's reagent was characterized by the formation of a white or yellow lumpy precipitate. Meanwhile, filtrate C was added with Wagner's reagent, the results were positive for alkaloids, indicated by the formation of a brown precipitate.



(a) (b) (c)

Figure 1. Alkaloid Identification Results (a) Dragendorff's Reagent (b) Wagner's Reagent (c) Mayer's Reagent

Flavonoid Identification, Identification of flavonoid compounds was carried out by adding two drops ($FeCl_3$ 5%) to five drops of ethanol extract. The results of this experiment were that the color changed to greenish or black and blue, which indicated positive flavonoids in the extract.

Figure 2. Flavonoid Test Results with Reagent ($FeCl_3$ 5%)

Saponin Identification, Saponin identification was carried out by adding 10 mL of distilled water to 1 mL of sample then shaking for 10 minutes to produce constant foam and when 3-5 drops of 2N HCl were added the foam did not disappear, indicating the presence of saponin compounds. This experiment showed positive results by still producing foam when 2N HCl was added.

Identify Steroids and Terpenoids, Identification of steroids was carried out by adding 10 drops of acetic anhydrous to 0.5 grams of beluntas leaf extract, then adding 2 drops of concentrated sulfuric acid,

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shaking and leaving for several minutes and the appearance of green and blue colors showed that it was positive for steroids and negative for terpenoids.

Identify Tannins, Tannin identification was carried out by adding 0.5 grams of beluntas leaf ethanol extract with drops of 1% $FeCl_3$ so that a blackish green color appeared which indicated it was positive for containing tannin. The results of this experiment showed a color change to blackish green which indicates positive tannin.

Diarrhea Time Range

Table 1. Diarrhea Time Range for Each Treatment

Group	Treatment	Diarrhea time span (Minutes)
I	White mice were induced with Escherichia coli bacteria and after diarrhea were given Beluntas leaf extract at a dose of 0.0 mg/kgBW.	220 minutes
II	White mice were induced with Escherichia coli bacteria and after diarrhea were given Beluntas leaf extract at a dose of 300 mg/kgBW.	170 minutes
III	White mice were induced with Escherichia coli bacteria and after diarrhea were given Beluntas leaf extract at a dose of 600 mg/kgBW.	120 minutes

Based on the table above, it can be stated that the higher the dose of extract used, the faster the time span for diarrhea. The different doses in this study affected the strength of beluntas leaf extract in curing diarrhea in mice.

Analysis of Serum Albumin Levels of White Rats, Results of analysis of serum albumin levels of white rats induced by E.coli bacteria and given beluntas leaf extract at different doses. From the results of the analysis of serum albumin levels in the white mice studied, data was obtained that administration of beluntas leaf extract at a dose of 0.0 mg/kg BW; 300 mg/kg BW; 600 mg/kg BW, in white mice induced by E.coli bacteria, the average serum albumin levels of white mice were 2.70 g/dL, respectively; 2.84 grams/dL; 4.45 grams/dL. The following is a tabulation of the results of albumin levels in white mice induced by E.coli bacteria.

Analysis of Serum Total Protein Levels of White Rats, Results of analysis of total serum protein levels of white rats induced by E.coli bacteria and given beluntas leaf extract at different doses. From the results of the analysis of the total serum protein levels of the white mice studied, data was obtained that

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administration of beluntas leaf extract at a dose of 0.0 mg/kg BW; 300 mg/kg BW; 600 mg/kg BW, in white mice induced by E.coli bacteria the average total protein levels in white mice serum were 8.51 g/dL, respectively; 8.86 grams/dL; 8.8 grams/dL.

4. CONCLUSION

Administration of beluntas leaf extract had a very significant effect on albumin levels and total serum protein in white mice induced by E.coli bacteria. Administration of beluntas leaf extract at a dose of 600 mg/kgBW gave the highest serum albumin levels and the lowest total serum protein. Giving beluntas leaf extract at a dose of 0.00 mg/kgBB; 300 mg/kgBW; 600 mg/kgBW in white mice induced with E.coli bacteria gave an average serum albumin level of 2,706 g/dL; 2.844 g/dL; 4.454 g/dL and the mean total serum protein levels were 8.51 g/dL respectively; 8.86 g/dL; 8.8 g/dL.

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