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Phytochemical contents and diuretic activity of ethanolic extract of the red leaf lettuce (*Lactuca sativa* L.)

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ABSTRACT

This study was conducted to evaluate of phytochemical content and diuretic activity of the red leaf lettuce. Red leaf lettuce was extracted by cold maceration method using 70% ethanol for the 3 days and remaceration for 1 day. The phytocemical content in ethanolic extract was evaluated by the qualitative method. A total of 25 male rats were divided into 5 groups with CMC Na, a standard drug (furosemide 10 mg/kg), and three different doses (200, 300, and 400 mg/kg) of ethanol extract. Parameters used to determine diuresis activity include first urine latency, urine pH and cumulative urine volume. The ethanolic extract induced diuresis in a dose dependent manner as compared to the negative control. Extracts at doses of 200, 300, and 400 mg/kg produced significant diuresis effects ($p<0.05$) compared to negative controls with values of diuretic action 1.35; 1.43; and 1.53, respectively. In addition, there was a slight change in the pH of urine samples of the extract-treated group compared with the negative control. Phytochemical analysis revealed the presence of alkaloids, flavonoids, phenolics, and tannins.

1. Introduction

Plants have many benefits for life, because besides being a source of food can also be as medicine. Indonesia for generations has used various types of plants for traditional medicinal materials. The use of traditional medicinal plants is indeed being promoted by most people as one of the treatment efforts in the context of back to nature, because it is considered relatively cheaper, efficient and safer from side effects compared to synthetic drugs [1].

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One of the potential medicinal plants that need to be explored and can be utilized for treatment is its efficacy as a diuretic agent. Diuretics are compounds that can increase the speed of urine formation. Diuretics are used in situations where more urine is desired, namely in edema, hypertension, and kidney stones. Most diuretics work by reducing sodium reabsorption, thereby increasing urinary output.[2] Synthetic drugs that are efficacious as diuretics generally have side effects such as hypokalemia, hyperuricemia, hyperlipidemia and hyperglycemia [3]. These things cause traditional medicine to be more considered because it is believed to have minimal side effects [4].

Red lettuce (Lactuca sativa L.) is one type of plant that is thought to have properties as a diuretic agent. Red lettuce is rich in fiber and nutrients that are beneficial to the body and also contains secondary metabolite compounds, including: flavonoids, saponins, tannins, phenolics, steroids, triterpenoids, and alkaloids [5]. Flavonoid compounds have an effect as diuretics by inhibiting the reabsorption of sodium, potassium and chloride. Alkaloids act directly on the tubules by increasing the excretion of sodium and chloride. Saponins decrease surface tension, stimulate the kidneys to work more actively, and increase the excretion of sodium and water. Thus, the content of alkaloids, flavonoids, and triterpenoid saponins is thought to work synergistically to cause diuretic effects [6]. Based on these descriptions, the authors are interested in further testing the potential of red lettuce leaf extract as a diuretic agent.

2. Methods

Chemical

Folin–Ciocalteu reagent (Sigma-Aldrich, Germany), the standard drug furosemide (Sanbe Farma, Indonesia), NaCl 0,9%, ethanol, ether, aquadest, acetic acid anhydride, H_2SO_4, $MgSO_4$ and $FeCl_3$were from Merck. All chemicals and reagents used were of analytical grade.

Sources of plant material

The leaves of red lettuce were collected from Ceper, Klaten, Central Java, Indonesia. The plant specimen was cultivated with hydroponic system. Lettuce leaves are sorted manually to remove damaged leaves and the bottom 3 leaves and then washed with tap water to remove foreign matter. After that, lettuce leaves are dried with sunlight covered with a black cloth, and finally dried in an oven at 40^0C for 12 hours. Then, dried leaves were pulverized using grinder and sieved using a mesh number 40.

Preparation of red leaf lettuce extract

The leaves of red lettuce were extracted using the cold maceration technique. Four hundred and fifty grams of the red leaf lettuce powder was soaked with 70% ethanol in a glass bottle and placed for 3 days at room temperature. The filtrate obtained is filtered using Whatman® filter paper and the pulp is re-extracted by adding another fresh solvent for 1 day. The fluid extract was dried in a rotary evaporator and water bath at 50^0C until an extract with a constant weight was obtained.

Qualitative phytochemical screening

Phytochemicals of red lettuce extract were qualitatively analyzed by following the standard protocol such as flavonoids by alkaline reagent, saponins by foam, alkaloids by Mayer, tannins by ferric chloride, and terpenoids by Lieberman-Burchard.

Experimental Animals

Male Sprague Dawley rats aged 5–10 weeks and with a weight range of 150–200 grams were used for the experiment. The animals were placed in plastic cages (5 rats per cage) under standard laboratory conditions (12 h/12 h light/dark cycle, 25 ± 2°C, and 55 ± 5% humidity). The animals were given free access to aqua dest and pellet and acclimatized to laboratory conditions for seven days before the experiment. All groups were dosed orally using oral gavage with an administration volume below 5 ml. Each rat was placed in an individual metabolic cage 24 hours before the experiment to avoid stress during testing that will affect the result of diuretic activity [7]. At the end of the experiment, all test animals were killed by the neck discoloration method that had previously been anesthetized with ether.

Diuretic Activity

The method used to determine the diuretic activity of plant extracts refers to research conducted by Meharie and Tunta [7]. All test animals used were fasted for 18 hours with free access to water. Rats were pretreated with normal saline thirty minutes before testing at an oral dose of 15 mL/kg to impose uniform salt load and water. Rats were randomly divided into five groups (positive control, negative control and three test groups) comprising 5 animals per group. Positive controls were treated with the standard drug, furosemide 10 mg/kg, negative control rats were treated with CMC Na and three test groups were treated with 200, 300, and 400 mg/kg of the ethanolic extract. Immediately after administration of the test substance according to its group (CMC Na, furosemide, 200,300, and 400 mg/kg of ethanolic extract), animals were placed in metabolic cages (a rat in a cage). Then, urine was collected and measured at the time of first urination, 1, 2, 3, 4, and 5 h after dosing and stored at –20°C for pH analysis. At the time of urine collection, the test animals did not have access to food or water [7].

The parameters recorded for each rat were time to the first urination, urine volume, and urine pH. To compare the effects of three different doses of the ethanolic extract with the positive and negative controls. Parameters such as diuretic action were also calculated using the following formulas.

$$\text{Diuretic action} = \frac{Mean\ urinary\ excretion\ of\ treathment\ group}{Mean\ urinary\ excretion\ of\ control\ group}$$

$$\text{Diuretic activity} = \frac{Diuretic\ action\ of\ test\ sample}{Diuretic\ action\ of\ standard\ drug}$$

Statistical Analysis

All results are expressed as mean ±standard error. The data was analyzed using one way of analysis of variance (ANOVA) followed by Tukey post hoc multiple comparison test. Significant differences were set at p values lower than 0.05.

Ethical Consideration

Rats are handled in accordance with animal care and welfare and experiment procedures have been approved by the health research ethics commission of Dr. Moewardi Hospital

3. Results and Discussion

3.1. Percent yield of ethanolic extract

The yield percentage of ethanolic extract of the red lettuce leaf is shown in Table 1. The percent yield produced is slightly different from previous research conducted by Utami and Dhamayanti 2023 which states that ethanolic extract of red lettuce leaf produces a yield of 19.39% [5]. The difference is likely due to several factors such as differences in sample drying methods, harvest times and extraction methods.

Table 1. Percent yield of ethanolic extract

Sample weight (g)	Extract weight (g)	Percent yield (%)
450	71,9	15,97

3.2. Phytochemical content of ethanolic extract

The phytochemical content of ethanolic extract of the red lettuce leaf (EERL) is shown in Table 2. The phytochemical evaluation showed the existence of many secondary metabolites such as flavonoids, tannins, alkaloids, terpenoids and saponins. The results of this study were similar to previous research conducted by Utami and Dhamayanti 2023 which stated that ethanolic extract of red lettuce leaves contains flavonoids, phenols, tannins, alkaloids, saponins, and terpenoids [5].

Table 2. Phytochemical content of ethanolic extract

Phytochemical compound	Reagent	Test result	Description
Flavonoid	$MgSO_4$ concentrated HCl	Red orange formed	+
Tanin	FeCl3	Green and sediment formed	+
Saponin	Aquadest and HCl	Foam formed	+
Alkaloid	Dragendrof	Sediment formed	+
Terpenoid	Acetic acid anhidrat and H_2SO_4	Green formed	+

+ (positive) = There were indications of bioactive compounds

Previous studies revealed that these phytochemicals had diuretic and natriuretic activities via several mechanisms. For example, alkaloids and flavonoids inhibit carbonic anhydrase in the renal tubule, stimulate renal blood flow through vasodilation of renal afferent arteries, and possibly inhibit tubular reabsorption of water and electrolytes [8], [9]. On the other hand flavonoids also inhibit angiotensin-converting enzyme (ACE), increase the bioavailability of prostacyclin, nitric oxide and bradykinin, or exert an inhibitory effect on Na+/K+-ATPase [10]. Flavonoids are one phytochemical that act as adenosine A1 receptor antagonists that can induce diuresis and Na+ excretion by direct inhibition of Na+ reabsorption in proximal tubules, or indirectly by promoting afferent arteriole dilation [11]. It is reasonable to suggest that these secondary metabolites may act individually or

synergistically to produce the observed diuretic activities of ethanolic extract of red lettuce.

3.3. Diuretic activity of ethanolic extract

3.2.1. Effect on the first urinary latency

The effect of ethanolic extract of red lettuce leaf on the first urinary latency is presented in Figure 1. Rats treated with EERL 200mg/kg did not show fast onset in their urination as compared to the negative control. EERL 300mg/kg and EERL 400mg/kg showed a minimal delay in the onset of urination, 47,4 ± 8.79, and 46.2 ± 10.91 min., respectively, ($p>0.05$) as compared to the positive control (33,2 ± 5.72 min). The absence of differences in the latency of the first urine at a dose of 200 mg/kg may be due to low levels of ethanol extract which results in slow therapeutic effects appearing.

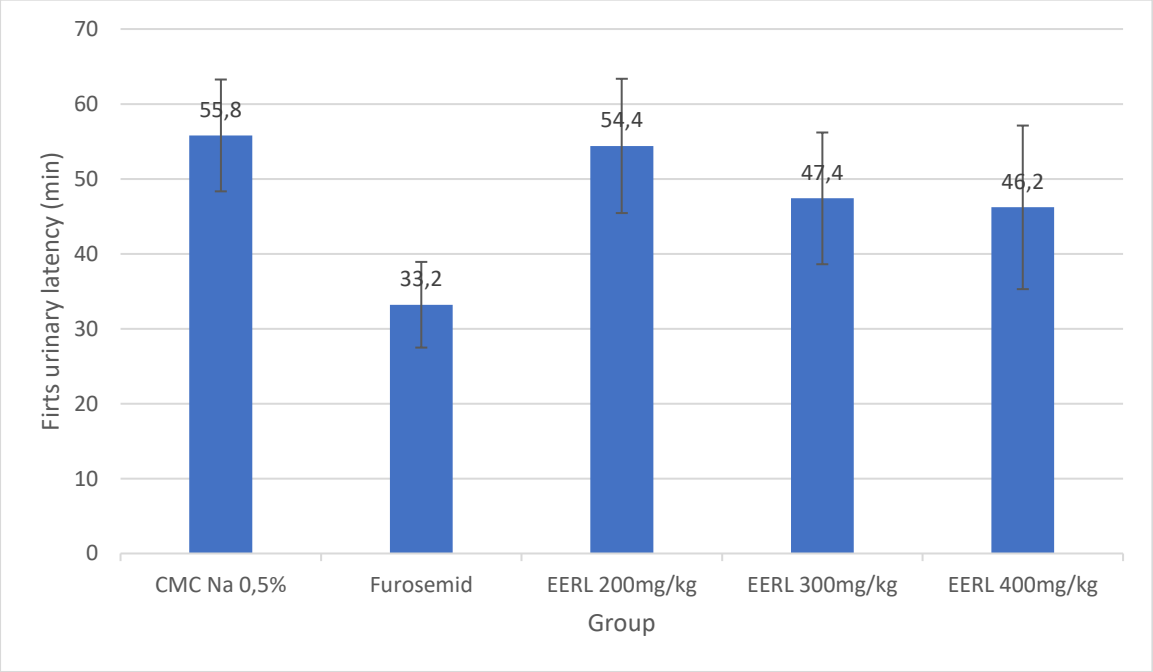

Figure 1. Effect on the first urinary latency

3.2.2. Effect on urine volume

The effect of ethanolic extract of red lettuce leaf on urinary output is shown in Table 1. The ethanolic extract produced diuresis effect which is associated with dosage level ($p<0.05$). EERL 200 mg/kg, EERL300 mg/kg and EERL400 mg/kg had a comparable diuretic effect with furosemide as positive control. Among the three doses of the ethanolic extract, EERL 400 mg/kg produced the highest diuresis effect compared to EERL 200 mg/kg and EERL 300 mg/kg. EERL 200 mg/kg, 300 mg/kg and EERL 400 mg/kg exhibited a diuretic action of 1.35; 1.43; and 1.53, respectively (Table 3). Besides, the diuretic actions of three doses of ethanolic extract which are nearly similar to the diuretic action of standard drug. All three doses of ethanolic extract of red lettuce had mild category diuresis activity as their values were 0.83, 0.88 and 0.94 respectively for EERL 200 mg/kg, EERL 300 mg/kg and EERL 400 mg/kg (Table 3). Diuretic activity is good if it is more than 1.5, moderate if it is between 1–1.5, mild if it is between 0.72–0.99, and nil if it is less than 0.72 [12].

Table 3. Effect of ethanolic extract of red lettuce on urine volume in rats

Group	Volume of urine (mL)					Diuretic action	Diuretic activity
	1 h	2 h	3 h	4 h	5 h		
CMC Na 0,5%	0,34±0,46	1,38±1,22	0,52±0,79	0,74±0,96	0,84±1,06	1	
Furosemid	3,82±1,75	0,92±0,61	0,5±0,61	0,8±0,81	0,08±0,18	1,62	1
EERL 200 mg/kg	1,34±1,55	2,18±1,68	0,52±0,72	1±0,47	0,1±0,22	1,35	0,83
EERL 300 mg/kg	0,92±1,01	2±1,36	0,9±0,57	1,26±0,99	0,4±0,55	1,43	0,88
EERL 400 mg/kg	2,9±1,98	1,66±1,97	0,44±0,54	0,76±0,38	0,08±0,18	1,53	0,94

3.2.3. Effect on cumulative urine output

The cumulative urine output of rats treated with ethanolic extract of red lettuce leaf is presented in Figure 2. EERL 200 mg/kg, EERL 300 mg/kg and EERL 400 mg/kg have produced significantly different urine output with negative control groups. As shown in Figure 2, the cumulative urine output produced after administration of EERL 400 mg/kg is similar to the standard drug.

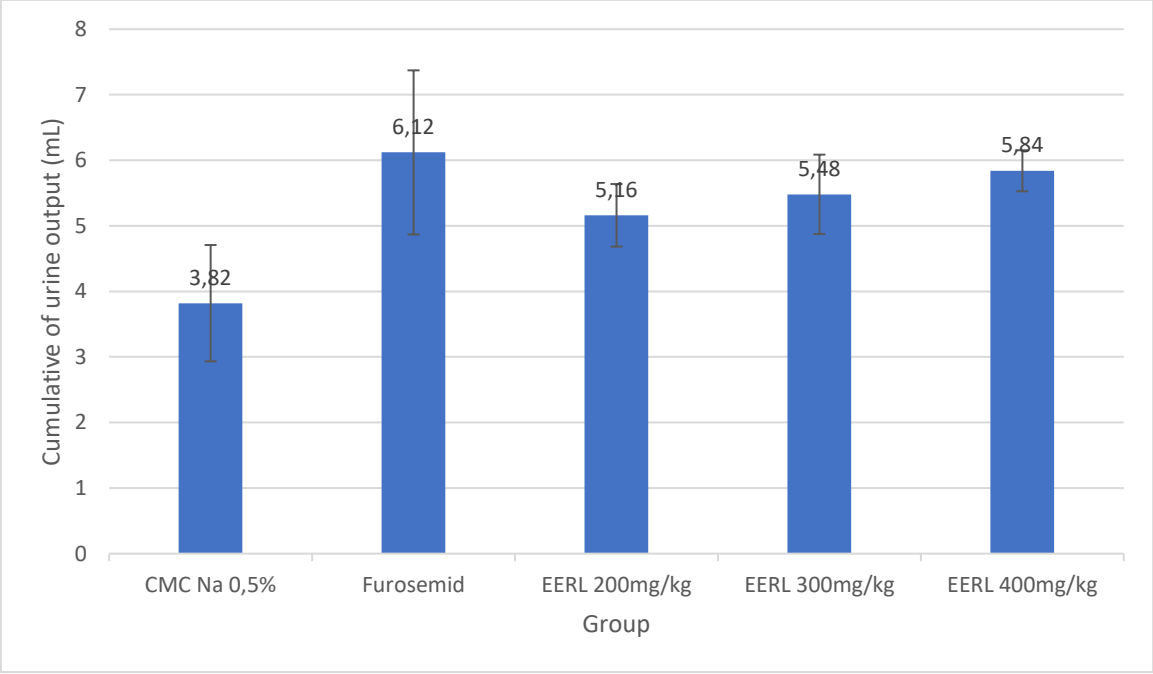

Figure 2. Effect on cumulative urine output

3.2.4. Effect on urine pH

The urinary pH was measured and different treatment groups had resulted in different urine pH Figure 3. EERL 200 mg/kg, EERL 300 mg/kg and EERL 400 mg/kg produced a relatively alkaline urine than negative control (pH = 7,78 ± 0.58, 7.6 ± 0.43, and 7,26 ± 0.54), respectively. The standard drug had also produced slightly alkaline urine than negative control (pH = 7.86 ± 0.33).

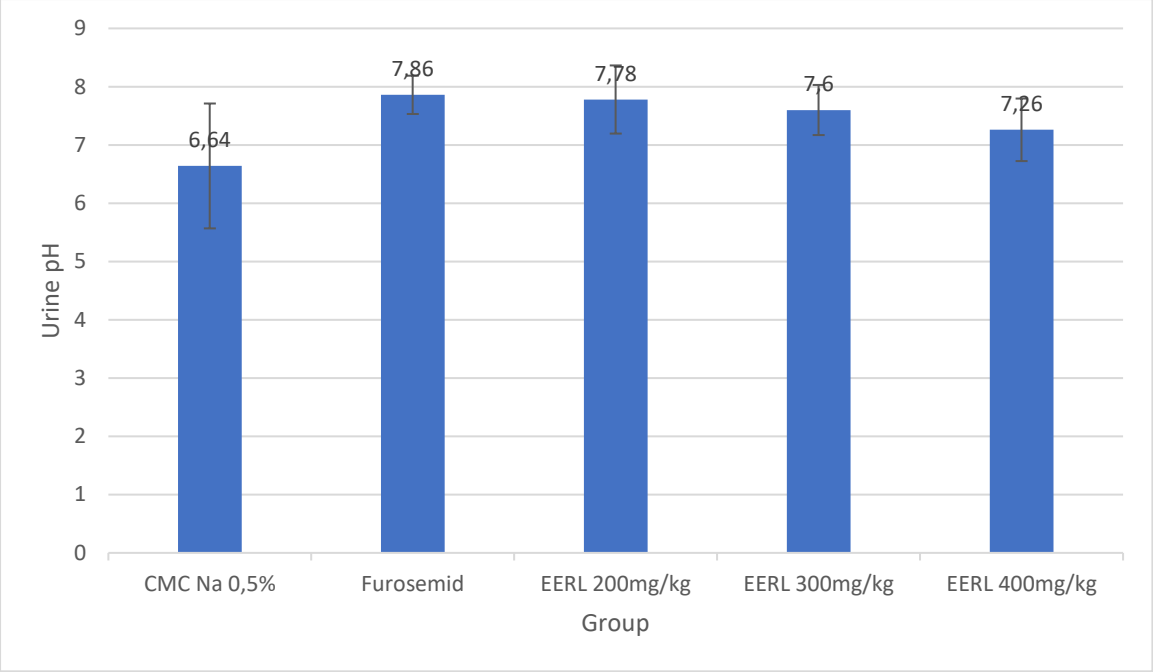

Figure 3. Effect on urine Ph

4. Conclusion

This study provides scientific evidence for the use of red lettuce leaves as a diuretic agent through increased excretion of sodium and water. Flavonoids, saponins, alkaloids, terpenoids and tannins act individually or in synergy via multiple mechanisms to produce the observed effect, though the specific compound is not yet determined. Ethanolic extract of red lettuce leaves at a dose of 400 mg/kg produced a diuresis effect comparable to standard drug (furosemide 10 mg/kg).

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