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IAI SPECIAL EDITION

RESEARCH ARTICLE

Comparison of antipyretic activities of ethanol and ethyl acetate extracts of *Bandotan* herb (*Ageratum conyzoides L.*) in hyperpyrexia mice

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Keywords
Antipyretic
Bandotan
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Abstract

Background: Fever is one of the body's physiological responses to metabolic stress, characterised by increasing body temperature. Based on its phytochemical content, Bandotan (tropical whiteweed) is an alternative antipyretic therapeutic agent. Flavonoids are potential chemical contents that can be extracted with various solvents, including ethanol and ethyl acetate. So far, research on its antipyretic potential is limited. **Aim:** To compare the antipyretic activity of ethanol extract to ethyl acetate extract of Bandotan herb in hyperpyrexia mice. **Methods:** Bandotan herb extracts were prepared by maceration, followed by phytochemical screening and determining total flavonoid content. Hyperpyrexia mice were made by induction of baker's yeast. Four hours after injection, mice were treated with Bandotan herb extracts, a dose of 100, 200, and 400 mg/kg body weight (BW), then the rectal temperature was observed for four hours. **Results:** Both extracts contained flavonoid, alkaloid, saponin, tannin, and steroid, while terpenoid was only found in ethanol preparation. The total flavonoid content of ethyl acetate extract was higher than that of ethanol extract. The best antipyretic activity was a dose of 400 mg/kg BW, but there was no significant difference in the percentage of pyrexia inhibition. **Conclusion:** Both extracts have the same ability to be developed as an alternative antipyretic agent.

Introduction

Fever or pyrexia describes a state when the body temperature increases above 37.5°C (Quast & Kimberger, 2015). Infection from pathogens such as viruses, bacteria, and other pathogens is the primary cause of fever (Blatteis, 2010). This stimulation activates the leukocytes and releases pyrogenic cytokines, leading to the release of prostaglandin E2 (PGE2). PGE2 can trigger a fever response by increasing the thermoregulation of the setpoint (El-Radhi, 2019). Fever is a source of discomfort because it causes weakness, losing weight, chills, sweating, and chills (Anochie, 2013). Fever can also cause nerve damage and death when body temperature increases over 40.5°C (Silbernagl & Lang, 2016). Therefore, antipyretics are needed to reduce fever. Paracetamol is a commonly used antipyretic. However, prolonged and excessive use of paracetamol causes side effects of liver damage (Jozwiak-Bebenista & Nowak, 2014).

Another antipyretic alternative is the Bandotan herb (*Ageratum conyzoides* L.). *Bandotan* usually grows wild and is considered a weed (Dalimartha, 2008). Only one paper reported that *Bandotan* essential oil has antipyretic activity (Abena *et al.*, 1996). Other antipyretic potential contents are flavonoids due to their antioxidant and anti-inflammatory properties (Pal & Verma, 2013). However, research on antipyretic activity focusing on flavonoids as phytochemical contents in *Bandotan* herb has never been conducted.

The flavonoids in *Bandotan* herbs consist of various compounds with different polarity levels. Ethanol and ethyl acetate are commonly used as solvents for flavonoids extraction (Chaves *et al.*, 2020). However, solvent selection is the key to getting the optimal pharmacological activities in desired chemical

compounds. Therefore, this research aimed to compare the antipyretic activity of ethanol extract to ethyl acetate extract of *Bandotan* herb in fever mice.

Methods

Materials

Bandotan herb powder was obtained from UPT. Herbal Laboratory (Materia Medica Malang) had been determined with document number 074/681A//102.7/2018. The materials used in the study included ethanol, ethyl acetate, baker's yeast, normal saline, quercetin, paracetamol tablets, Sodium carboxymethyl cellulose (CMC Na), and reagents for phytochemical screening. The experimental animals used were male Balb-c mice, healthy, weight 20 - 30g, and about two to three months old.

Methods

Preparation of Bandotan herb extract

Bandotan herb extract was prepared with maceration. *Bandotan* herb powder (300g) immersed with 70% ethanol or ethyl acetate in an appropriate ratio (1:10) for three days and occasionally stirred. The resulting filtrate was concentrated using a rotary evaporator, then dried using an oven at a temperature of 50°C until a constant weight was obtained.

Phytochemical screening

Qualitative identification of flavonoid, tannin, alkaloid, saponin, and terpenoid content was carried out (Banu & Cathrine, 2015). Flavonoid was identified using HCl and magnesium, whereas a foam test determined saponin. Tannin was identified using $FeCl_3$, whereas alkaloid was determined using Wegner's reagent. Then, the terpenoid was identified using Liebermann Burchard's reagent.

Total Flavonoid content

0.5mL sample solution was pipetted into a 5mL volumetric flask. Then 1.5 mL methanol, 100 µL AlCl3, 100 µL of potassium acetate, were added with distilled water until the limit mark (Hassan *et al.,* 2012). The solution was left to stand for 35 minutes in a cuvette. The absorbance was measured at λ 428nm using a spectrophotometer. Then, total flavonoid concentration was expressed in mg QE/g extract using the quercetin standard calibration equation.

Antipyretic activity assay

The whole procedure for the care and treatment of experimental animals had obtained the approval of the ethics committee of the Medical Research, Faculty of Dentistry, Universitas Jember with certificate number 945/UN25.8/KEPK/DL/2020. Mice were induced sub-cutaneously with 20% (b/v) baker's yeast. After four hours of induction, hyperpyrexia mice were divided into negative control (CMC Na 1%), positive control (Paracetamol 100mg/kg BW), and three *Bandotan* extracts treatment groups (dose 100, 200, and 400mg/kg BW). After being treated, rectal temperature was obtained every hour for four hours. The percentage of pyrexia inhibition was calculated using the following formula (Muhammad *et al*., 2012):

$$\frac{B - Cn}{B - A} x\ 100\%$$

B: the rectal temperature four hours after yeast induction
A: the baseline temperature
Cn: the rectal temperature after being treated at a specific hour.

Results

A comparison of both extract yields can be shown in Figure 1 (A). From the picture, it is stated that the yield of ethanol extract (14.4%) is almost three times higher than ethyl acetate extract (3.9%). Based on the results shown in Table I, it is known that both extracts contain flavonoid, alkaloid, saponin, tannin, and steroid, while terpenoid is only found in ethanol preparation. The total flavonoid content from each extract is presented in Figure 1 (B). The higher flavonoid levels are obtained from the ethyl acetate extract (249.7mg QE/g extract) than that of ethanol extract (51.7mg QE/g extract).

Table I: Phytochemical screening of *Bandotan* herb extracts

Compound group	Result of phytochemical screening	
	Ethanol extract	Ethyl acetate extract
Flavonoid	+	+
Alkaloid	+	+
Saponin	+	+
Tanin	+	+
Steroid	+	+
Terpenoid	+	-

Note: (+) sign indicates the presence of such a component, and (-) indicates no such component

Figure 2 demonstrates the changes in the rectal temperature of mice hour by hour. Fever in mice occurred at four hours after yeast induction with an

average temperature increase of 1.5°C. It is known that the temperature decreases gradually until four hours after the treatments of both extracts, except for the negative control group.

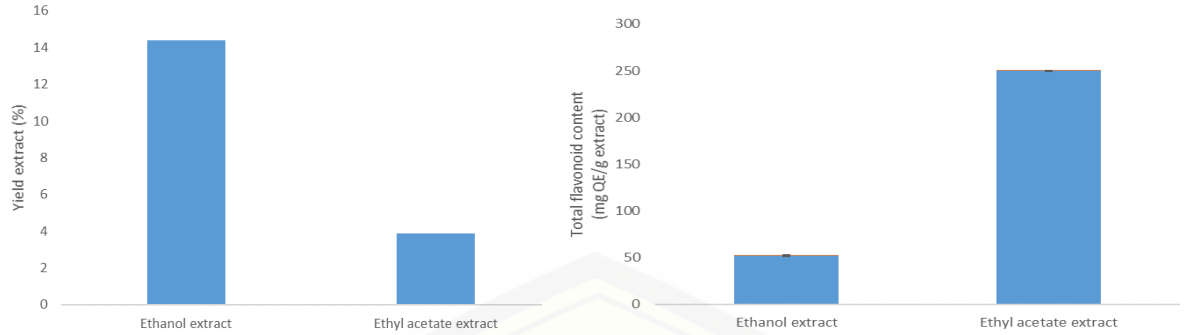

Figure 1: Yield extract (A) and total flavonoid content (B) of *Bandotan* herb in different solvent

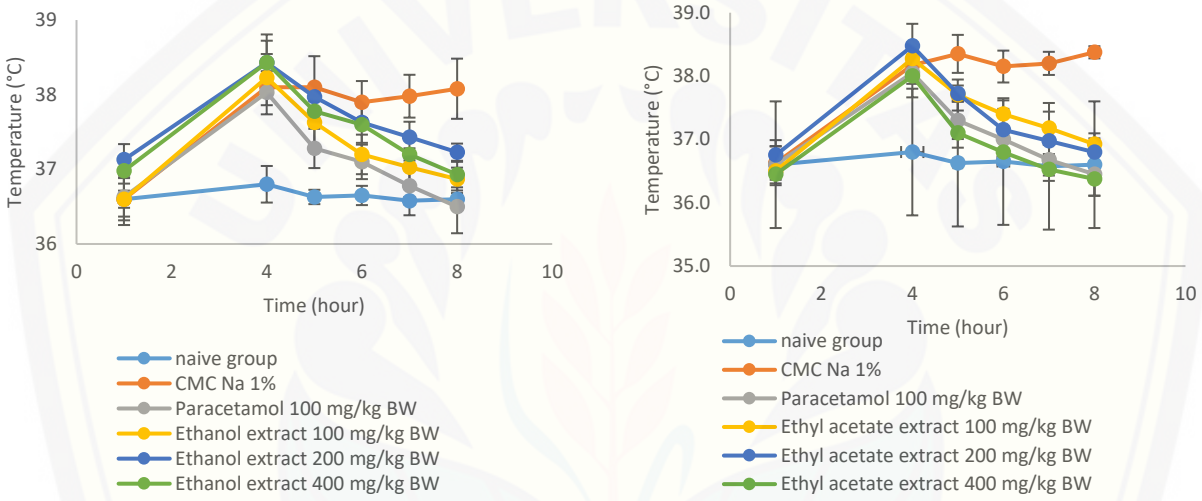

Figure 2: Temperature changes after the treatment with ethanol (left) and ethyl acetate (right) extract of *Bandotan* herb

From the picture, ethanol and ethyl acetate extract shows the same trend of temperature change. It was also found that a dose of 400mg/kg BW in each section was the optimum dose in reducing fever. Therefore, the comparison of the antipyretic activities of the two preparations was carried out at a dose of 400mg/kg BW. Then, the calculation of the hourly percentage of pyrexia inhibition in each extract was compared, as shown in Table II.

Table II: Percentage of pyrexia inhibition of *Bandotan* herb extract in different solvent

Group	Average of Percent Inhibition Pyrexia (%) ± SD			
	1 hour[ǂ]	2 hours[ɪ]	3 hours[ǂ]	4 hours[ɪ]
CMC Na 1%	-9.9 ± 16.3[a]	2.6 ± 10.6[a]	-0.2 ± 12.2[a]	-12.6 ± 3.9[a]
Paracetamol 100mg/kg BW	53.4 ± 17.9[b]	72.7 ± 4.3[b]	94.8 ± 6.1[b]	110.2 ± 8.7[b]
Ethanol extract (400mg/kg BW)	43.3 ± 16.6[b]	53.9 ± 23.2[b]	82.7 ± 15.8[b]	105.7 ± 21.9[b]
Ethyl acetate extract (400mg/kg BW)	57.9 ± 6.0[b]	79.2 ± 10.2[b]	95.6 ± 9.4[b]	103.7 ± 12.5[b]

Note: [ǂ]ANOVA followed by LSD test; [ɪ]Kruskal-Wallis followed by Mann-Whitney test, Different superscript letters ([a,b]) indicate that there are significant differences between groups ($p < 0.05$).

The result of the statistical analysis using ANOVA or Kruskal-Wallis showed significant differences ($p > 0.05$) between groups. The post hoc test also showed that all treatment groups were statistically significant compared to a control group. Administration with *Bandotan* herb preparation was not significantly different ($p > 0.05$) from paracetamol treatment. There was also no significant difference between *Bandotan* ethanol extract and ethyl acetate extract in the percentage of pyrexia inhibition. It was concluded that both extracts had antipyretic activities, and they were as effective as paracetamol in reducing fever.

Discussion

The variation in extracts yields could be attributed to the difference in solvent polarities, determined by the dielectric constant value. Ethanol is more polar than ethyl acetate, with dielectric constant values of 24.5 and 6.02, respectively (Maryott & Smith, 1951). Since ethanol is known to be a universal solvent, almost all components of *Bandotan* Herb were also attracted in the extraction process. It is also in line with the research conducted by Effendi and colleagues (2017). However, other studies have proved slightly different results, where the ethyl acetate extract of *Bandotan* leaves contains flavonoids, p-hydroquinone, terpenoids, and steroids (Sugara *et al*., 2016). It is due to differences in plant origin, parts used, and the extraction process.

In this study, flavonoids are the main phytochemical component targeted. Flavonoid glycosides are readily soluble in water, methanol, and ethanol, while flavonoids aglycones are only soluble in methanol and ethanol. Nevertheless, less polar flavonoids such as isoflavones, flavanones, methylated flavones, and flavonols can be extracted with solvents such as chloroform and ethyl acetate (Andersen & Markham, 2006). The flavonoid content of the ethyl acetate extract was higher than that of the ethanol extract. It indicates that non-polar compounds dominate the flavonoid content in *Bandotan* herbs. However, both extracts met the requirements of the Indonesian Herbal Pharmacopoeia, where the flavonoid content of *Bandotan* extract is not less than 1.40% (Ministry of Health Republic of Indonesia, 2017).

Although the extract yield and total flavonoid of the two extracts were different, their antipyretic activity was equivalent. It was assumed that there is a synergistic action of various phytochemical components in the extract. The pharmacological activity of the *Bandotan* herb extract was thought due to the presence of flavonoid compounds, such as nobiletin, kaempferol, quercetin, acacetin, and chalcone. Flavonoids could inhibit PGE2 as the last mediator of fever (Blomqvist & Engblom, 2018). Nobiletin is a typical compound from *Bandotan* (Ministry of Health Republic of Indonesia, 2017), which can suppress the production of PGE2 induced by proinflammatory cytokines such as IL-1α, IL-1β, IL-6, and TNF-α in mouse macrophages and also selectively downregulates COX-2 (Lin *et al*., 2003). Kaempferol and quercetin inhibit the activation of NFKβ, which encodes the formation of TNF-α (Bernstein *et al*., 2018), represses phospholipase A2 and decreases COX-2 expression (D'mello *et al*., 2011). Quercetin can also inhibit the release of iNOS, which triggers the formation of the inflammatory mediator IL-1 (Steiner & Branco, 2001). Acacetin and chalcone decrease the expression of iNOS and COX-2 in macrophages (Xiao *et al*., 2011).

In addition to flavonoids, alkaloids showed significant antipyretic activity from several early laboratory studies (Ahmad *et al*., 2017). Alkaloids are known to inhibit prostaglandin synthesis. According to Aronof and Neilson (2001), effective antipyretics can affect pyrogen associated with peripheral inflammation or central pyrogenic signalling due to PGE2 production.

However, both extracts had equivalent antipyretic activity, as well as when compared with paracetamol. Ethanol and ethyl acetate have the same ability to attract various fever-reducing components based on their non-toxic, volatile and readily available properties. Moreover, further research is still needed to determine the best promising candidate for the development of a herbal antipyretic product.

Conclusion

This study concluded that ethanol and ethyl acetate extracts have the same ability in reducing hyperpyrexia in mice. Both extracts can be developed as an alternative antipyretic agent through a series of advanced preclinical and clinical studies.

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