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Evaluation of Antipyretic Activity of Ethanol, Ethyl Acetate, and *n*-Butanol Extracts of *Pometia pinnata* Fruit Peel

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ABSTRACT

Fever is a regulated biological response to infection and inflammation, characterized by an elevation in body temperature mediated by endogenous pyrogens. Although synthetic antipyretics such as paracetamol are effective, prolonged use may cause adverse effects, prompting the search for safer, plant-derived alternatives. This study evaluated the antipyretic activity of *Pometia pinnata* fruit peel extracts prepared using three solvents of different polarity (ethanol, *n*-butanol, and ethyl acetate) under ultrasound-assisted extraction (UAE; 40 kHz, 20 min, 25 °C). Antipyretic activity was assessed in male Wistar rats using a brewer's yeast-induced pyrexia model (eleven groups; n=6 per group) at doses of 125, 250, and 500 mg/kg BW, with rectal temperature recorded every 30 min for 4 h. Data were analyzed using one-way ANOVA (Tukey) at each time point and two-way ANOVA (Sidak) to evaluate dose, time, and interaction effects ($p<0.05$). Phytochemical screening showed that the ethanol extract (EEPP) contained saponins, alkaloids, flavonoids, phenolics, tannins, terpenoids, and triterpenoids; the *n*-butanol extract (NBPP) contained alkaloids, terpenoids, and steroids; and the ethyl acetate extract (ETPP) contained flavonoids, phenolics, and steroids. All extracts exhibited significant dose- and time-dependent antipyretic activity. The highest percentage of fever reduction was achieved by ETPP (89.52 ± 15.28%), followed by NBPP (89.12 ± 7.90%) and EEPP (71.93 ± 6.07%). These findings indicate that *Pometia pinnata* fruit peel has strong antipyretic potential, and the antipyretic activity profile is influenced by extraction solvent polarity.

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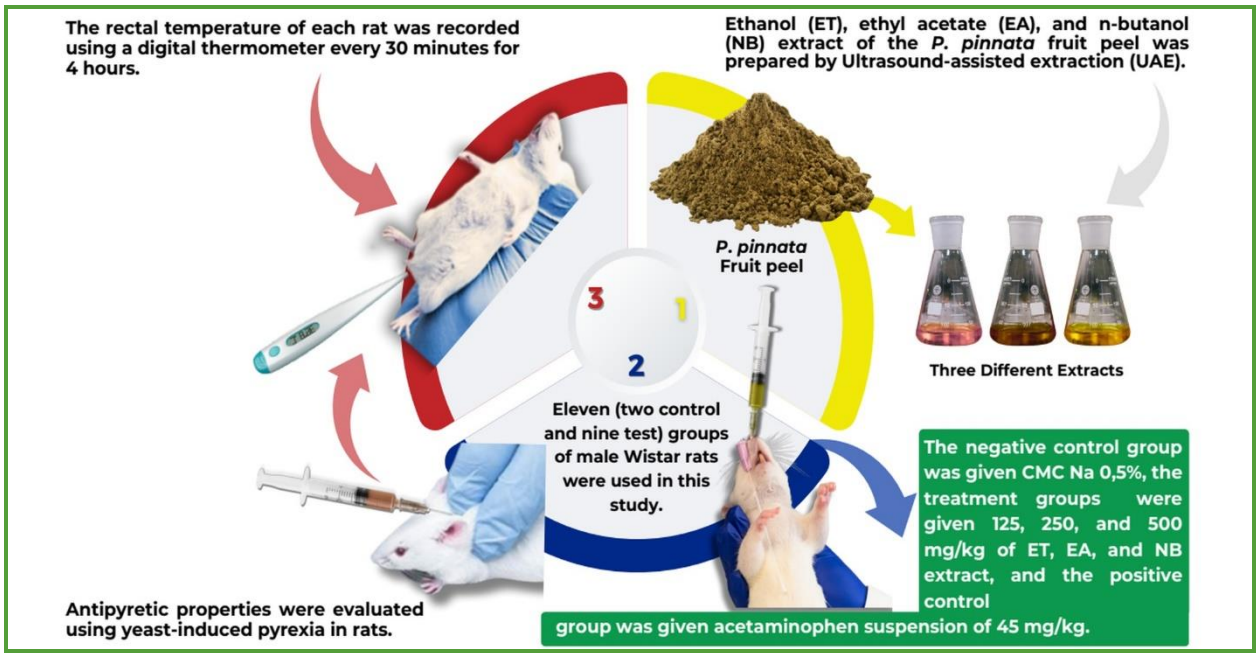
Graphical Abstract



Introduction

Fever is a complex biological response triggered by the release of endogenous pyrogens, such as interleukin-1 and prostaglandin E_2, produced in response to infection or inflammatory processes. While fever plays a critical role in the body's immunological defense, prolonged elevation of body temperature can lead to complications, thereby requiring therapeutic intervention [1,2]. Synthetic antipyretics such as paracetamol and ibuprofen are widely used to reduce fever but are associated with potential hepatotoxic and nephrotoxic effects, particularly with long-term use [3,4]. This has driven the search for safer and more effective natural antipyretics, especially those derived from medicinal plants rich in bioactive compounds. Several studies have demonstrated that medicinal plants possess promising antipyretic activity with minimal or no side effects [5,6]. However, most of these studies have employed only a single solvent type and conventional extraction methods. Moreover, few investigations have examined how solvent polarity affects the antipyretic activity of plant extracts. Since solvent polarity influences the profile of extracted bioactive compounds, it can significantly impact the pharmacological activity of the extract [7].

Conventional extraction techniques such as maceration or reflux often require extended durations and yield relatively low extract quantities [8]. Furthermore, maceration with heating or reflux may lead to degradation of thermolabile compounds due to prolonged exposure to high temperatures [9]. In recent years, ultrasound-assisted extraction (UAE) has emerged as a more efficient alternative, enhancing solvent diffusion into plant matrices and promoting the release of active compounds through cavitation effects [8,10]. Nonetheless, the application of UAE for extracting bioactive compounds from the fruit peel of Indonesian native plants, particularly *Pometia pinnata* (matoa), remains largely unexplored. Several studies on *Pometia pinnata* have reported various bioactivities and phytochemical findings,

with research predominantly focusing on leaves, seeds, or whole fruit extracts [11–13]. Investigations specifically targeting the fruit peel remain limited and have primarily addressed non-antipyretic endpoints, such as metabolic or larvicidal activities [13,14]. No previous study has evaluated the antipyretic activity of *Pometia pinnata* fruit peel, despite fever being a common manifestation of inflammatory and infectious conditions [1,15]. Moreover, existing studies have not systematically examined how extraction solvent polarity influences the phytochemical composition of *Pometia pinnata* fruit peel extracts [7,16,17].

In addition, most antipyretic studies on medicinal plants rely on conventional extraction techniques and a single solvent [2]. Ultrasound-Assisted Extraction has emerged as a green and efficient extraction technique capable of improving extraction efficiency while reducing time and solvent consumption [8,9]. From a green chemistry perspective, UAE is advantageous due to its lower energy requirements and reduced consumption of organic solvents compared with conventional extraction methods. However, its application to *Pometia pinnata* fruit peel in the context of antipyretic evaluation has not been reported. Therefore, the present study addresses these gaps by comparatively investigating *Pometia pinnata* fruit peel extracts obtained using three solvents of different polarity (ethanol, *n*-butanol, and ethyl acetate) under UAE conditions, integrating phytochemical screening with rectal temperature changes and percent fever reduction (PFR) analyses in a yeast-induced pyrexia model. This integrated approach provides a rational basis for establishing the potency ranking of the extracts and supports future bioactive compound isolation and safety evaluations.

Experimental

Materials

Simplicia of *Pometia pinnata* fruit peel simplicia was obtained from UPT Balai Materia Medika Batu, Indonesia, under official identification letter No. 074/631/102.20-A/2022. Other materials used included brewer's yeast, 0.9% NaCl infusion (Sanbe Farma), CMC-Na, distilled water (Brataco), ethanol (96%), *n*-butanol, ethyl acetate, n-hexane (Smartlab), HCl, Wagner's reagent, Mayer's reagent, 5% $FeCl_3$ solution, 10% $FeCl_3$ solution, and H_2SO_4 (E. Merck).

Extraction and phytochemical analysis

The powdered *Pometia pinnata* fruit peel simplicia was extracted using Ultrasound-Assisted Extraction (UAE) at a frequency of 40 kHz for 20 min at 25 °C. Extraction was performed using three solvents of varying polarity: ethanol (96%), *n*-butanol, and ethyl acetate, at a solvent-to-solid ratio of 1:20 (b/v). The resulting extracts were concentrated using a rotary evaporator at 50 °C to obtain viscous extracts. Phytochemical screening was conducted to identify major classes of phytoconstituents following standard protocols [18,19].

Animal experiment and antipyretic evaluation

Antipyretic activity was evaluated with slight modifications to previously established protocols [20,21]. Male Wistar rats aged 2–3 months (150–200 g) were housed under standard laboratory conditions (temperature 22–25 °C, relative humidity 50–60%, 12 h light/dark cycle) with free access to standard pellet diet and water. The animals were acclimatized for 7 days before experimentation. Ethical clearance was granted by the Health

Research Ethics Committee, Faculty of Medicine, Universitas Islam Malang (Approval No. 083/LE.003/III/01/2024). The animals were randomly assigned into eleven groups (n = 6 per group). Baseline rectal temperatures were recorded before fever induction. Fever was induced via a subcutaneous injection of 20% (b/v) brewer's yeast suspended in 0.9% NaCl solution at a dose of 10 mL/kg BW. 18 h post-injection, rectal temperatures were measured again to confirm fever induction. Treatment groups received one of the following: negative control: 0.5% CMC-Na; positive control: paracetamol (45 mg/kg BW); ethanol extract of *Pometia pinnata* peel (EEPP): 125, 250, and 500 mg/kg BW; *n*-butanol extract (NBPP): 125, 250, and 500 mg/kg BW; ethyl acetate extract: 125, 250, and 500 mg/kg BW. The extract doses were selected based on preliminary studies and previous reports evaluating the pharmacological activity of plant extracts in rodent models, covering low, medium, and high dose ranges to assess dose-dependent effects**.** After treatment, rectal temperatures were recorded at 1 h and subsequently every 30 min for a total duration of 4 h. The percent of fever reduction was calculated using Equation 1:

$$PFR = \frac{\text{Post fever temperature} - \text{Temperature at each time point}}{\text{Post fever temperature} - \text{Normal temperature}} \times 100\ \% \quad (1)$$

Statistical analysis

One-way ANOVA followed by Tukey's multiple comparison test was used to compare treatment groups at individual time points. Two-way ANOVA followed by Sidak's post hoc test was applied to evaluate the effects of dose, time, and their interaction. Values of $p < 0.05$ were considered statistically significant. Prior to ANOVA, data were assessed for normality and homogeneity of variance, and all datasets met the assumptions required for parametric analysis. All statistical analyses were performed using GraphPad Prism version 10.

Results and Discussion

Phytochemical screening

Qualitative phytochemical screening of *Pometia pinnata* fruit peel extracts revealed distinct secondary metabolite profiles depending on the extraction solvent used (Table 1). The ethanolic extract (EEPP) contained the most diverse range of bioactive constituents, including saponins, alkaloids, flavonoids,

Table 1. Phytochemical screening results of *Pometia pinnata* fruit peel extracts

No.	Phytochemical constituent	EEPP	NBPP	ETPP
1	Saponins	+	–	–
2	Alkaloids	+	+	–
3	Flavonoids	+	–	+
4	Phenolics	+	–	+
5	Tannins	+	–	–
6	Terpenoids	+	+	–
7	Steroids	–	+	+
8	Triterpenoids	+	–	–

Note: (+) = presence of compound; (–) = absence of compound. EEPP = ethanolic extract of Pometia pinnata fruit peel; NBPP = n-butanol extract of Pometia pinnata fruit peel; and ETPP = ethyl acetate extract of Pometia pinnata fruit peel.

phenolics, tannins, terpenoids, and triterpenoids. In contrast, the *n*-butanol extract (NBPP) contained alkaloids, terpenoids, and steroids, whereas the ethyl acetate extract (ETPP) was characterized by the presence of flavonoids, phenolic, and steroids. These differences in phytochemical composition are consistent with solvent polarity effects, following the principle of "like dissolves like", whereby polar solvents preferentially extract hydrophilic compounds, while less polar solvents favor lipophilic constituents [7,16]. Ethanol, as a polar protic solvent, efficiently solubilizes phenolic, flavonoids, tannins, and other polar or semi-polar phytochemicals, while ethyl acetate, with lower polarity, preferentially extracts moderately lipophilic compounds such as aglycone flavonoids and steroids [17,22]. Semi-polar solvents such as *n*-butanol exhibit intermediate extraction behavior, enriching compounds of moderate polarity depending on solvent–solute interactions [7,23]. In addition to qualitative differences, extraction yields varied markedly among the solvents. Ethanol produced the highest extraction yield (30.54%), followed by *n*-butanol (5.78%) and ethyl acetate (1.34%). This trend reflects the broader solvation capacity of ethanol toward a wide spectrum of plant secondary metabolites, whereas *n*-butanol and ethyl acetate provide more selective extraction of specific metabolite classes rather than maximizing total extract mass [24-26]. Overall, solvent polarity strongly influenced both the phytochemical profiles and extraction yields of *Pometia pinnata* fruit peel extracts, supporting the use of multiple solvents to obtain complementary chemical information.

Antipyretic activity of Pometia pinnata fruit peel extract

Changes in rectal temperature

Rectal temperature measurements demonstrated that all *Pometia pinnata* fruit peel extracts significantly reduced yeast-induced pyrexia in a dose- and time-dependent manner compared to the negative control. For each observation time point, differences among treatment groups were analyzed using one-way ANOVA followed by Tukey's multiple comparison test ($p<0.05$; Table 2), while the overall effects of dose, time, and their interaction were evaluated using two-way ANOVA followed by Sidak's post hoc test. A gradual decrease in rectal temperature was observed starting approximately 60–90 min after oral administration, with the antipyretic effect persisting up to 240 min. These findings indicate that the extracts exert antipyretic activity during the early phase of the febrile response and maintain their temperature-lowering effects throughout the observation period [2,5].

Values are expressed as mean ± SD (n=6). Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparison test. Means with different superscript letters (a–e) within the same column indicate significant differences among groups ($p<0.05$). The control group received 0.5% CMC-Na suspension (negative control); ACH=acetaminophen (45 mg/kg BW; positive control); EEPP=ethanolic extract of *Pometia pinnata* fruit peel; NBPP=*n*-butanol extract of *Pometia pinnata* fruit peel; ETPP=ethyl acetate extract of *Pometia pinnata* fruit peel.

A comparative evaluation revealed differences in both the onset and magnitude of temperature reduction among the extracts. At corresponding time points, statistically significant differences were observed among treatment groups ($p<0.05$), as indicated in Table 2. ETPP produced a more rapid initial decrease in rectal temperature, particularly at higher doses, whereas NBPP exhibited a consistent temperature-lowering effect across the observation period. EEPP also significantly reduced rectal temperature, although with a

Table 2. Changes in rectal temperature over time to assess the antipyretic effects of *Pometia pinnata* fruit peel extracts in yeast-induced fever model

Treatment groups	Initial (°C)	Fever (°C)	1 h (°C)	1.5 h (°C)	2 h (°C)	2.5 h (°C)	3 h (°C)	3.5 h (°C)	4 h (°C)
Control group	36.42 ± 0.13^{a}	38.67 ± 0.31^{a}	38.77 ± 0.10^{a}	38.78 ± 0.10^{a}	38.75 ± 0.14^{a}	38.70 ± 0.09^{a}	38.58 ± 0.15^{a}	38.67 ± 0.10^{a}	38.53 ± 0.12^{a}
Yeast + ACH	36.23 ± 0.23^{a}	38.65 ± 0.12^{a}	37.43 ± 0.16^{b}	37.23 ± 0.34^{b}	37.08 ± 0.26^{b}	36.93 ± 0.22^{b}	36.85 ± 0.29^{b}	36.55 ± 0.23^{b}	36.50 ± 0.20^{b}
Yeast + EEPP 125	36.23 ± 0.18^{a}	38.45 ± 0.53^{a}	38.25 ± 0.63^{a}	38.15 ± 0.61^{a}	38.03 ± 0.54^{a}	37.95 ± 0.50^{a}	37.83 ± 0.52^{a}	37.72 ± 0.48^{a}	37.47 ± 0.58^{a}
Yeast + EEPP 250	35.97 ± 0.38^{a}	37.80 ± 0.28^{b}	37.67 ± 0.21^{b}	37.57 ± 0.23^{b}	37.42 ± 0.22^{b}	37.32 ± 0.29^{b}	37.23 ± 0.25^{b}	37.05 ± 0.36^{b}	36.95 ± 0.33^{b}
Yeast + EEPP 500	36.23 ± 0.64^{a}	38.07 ± 0.83^{b}	37.78 ± 0.87^{b}	37.63 ± 0.82^{b}	37.58 ± 0.87^{b}	37.38 ± 0.90^{b}	37.17 ± 0.81^{b}	37.00 ± 0.65^{b}	36.83 ± 0.62^{b}
Yeast + NBPP 125	36.43 ± 0.54^{a}	38.20 ± 0.37^{a}	38.08 ± 0.35^{a}	38.03 ± 0.40^{a}	37.88 ± 0.50^{b}	37.77 ± 0.45^{b}	37.53 ± 0.37^{b}	37.35 ± 0.38^{b}	37.18 ± 0.39^{b}
Yeast + NBPP 250	36.43 ± 0.68^{a}	38.72 ± 0.30^{a}	38.23 ± 0.35^{a}	38.05 ± 0.47^{a}	37.72 ± 0.37^{b}	37.65 ± 0.40^{b}	37.28 ± 0.51^{b}	37.10 ± 0.54^{b}	37.02 ± 0.40^{b}
Yeast + NBPP 500	36.48 ± 0.31^{a}	38.83 ± 0.16^{a}	38.27 ± 0.43^{a}	37.80 ± 0.44^{b}	37.67 ± 0.39^{b}	37.50 ± 0.37^{b}	37.32 ± 0.41^{b}	37.12 ± 0.44^{b}	36.83 ± 0.35^{b}
Yeast + ETPP 125	35.95 ± 0.10^{a}	37.93 ± 0.55^{b}	37.77 ± 0.55^{b}	37.70 ± 0.52^{b}	37.62 ± 0.56^{b}	37.48 ± 0.47^{b}	37.33 ± 0.52^{b}	37.13 ± 0.52^{b}	36.67 ± 0.51^{b}
Yeast + ETPP 250	36.75 ± 0.41^{a}	38.63 ± 0.23^{a}	38.32 ± 0.40^{a}	38.13 ± 0.35^{a}	38.08 ± 0.29^{a}	37.88 ± 0.39^{a}	37.55 ± 0.40^{a}	37.43 ± 0.40^{a}	37.27 ± 0.43^{a}
Yeast + ETPP 500	35.95 ± 0.27^{b}	38.05 ± 0.18^{b}	37.28 ± 0.27^{b}	37.12 ± 0.23^{b}	36.87 ± 0.23^{b}	36.75 ± 0.29^{b}	36.43 ± 0.21^{c}	36.32 ± 0.20^{c}	36.18 ± 0.16^{c}

relatively slower onset compared to ETPP and NBPP. These variations suggest differences in pharmacodynamic profiles of the extracts, potentially related to their distinct phytochemical compositions [7,16]. Based on these findings, rectal temperature analysis confirms the antipyretic potential of all extracts, while comparative potency among treatments is further evaluated using percent fever reduction.

Percent reduction of fever

Percent fever reduction analysis demonstrated that all *Pometia pinnata* fruit peel extracts produced dose- and time-dependent antipyretic effects compared to the negative control (Figure 1). The negative control exhibited negligible changes in PFR throughout the observation period, while acetaminophen (positive control) showed a rapid onset and high maximal effect at 4 h, consistent with its well-established clinical antipyretic efficacy [15].

Panels (a–g) exhibit PFR at 1, 1.5, 2, 2.5, 3-, 3.5, and 4 h post-treatment, respectively. Bars represent mean ± SD (n = 6). Statistical analysis was performed using one-way ANOVA followed by Tukey's test. Bars with different letters (a–e) differ significantly ($p< 0.05$); those sharing a letter are not significantly different ($p>0.05$). The control group is the negative control; acetaminophen (ACH) is the positive control. Among the extracts, EEPP showed the slowest onset and the lowest maximal PFR, with only the highest dose exceeding the 50%

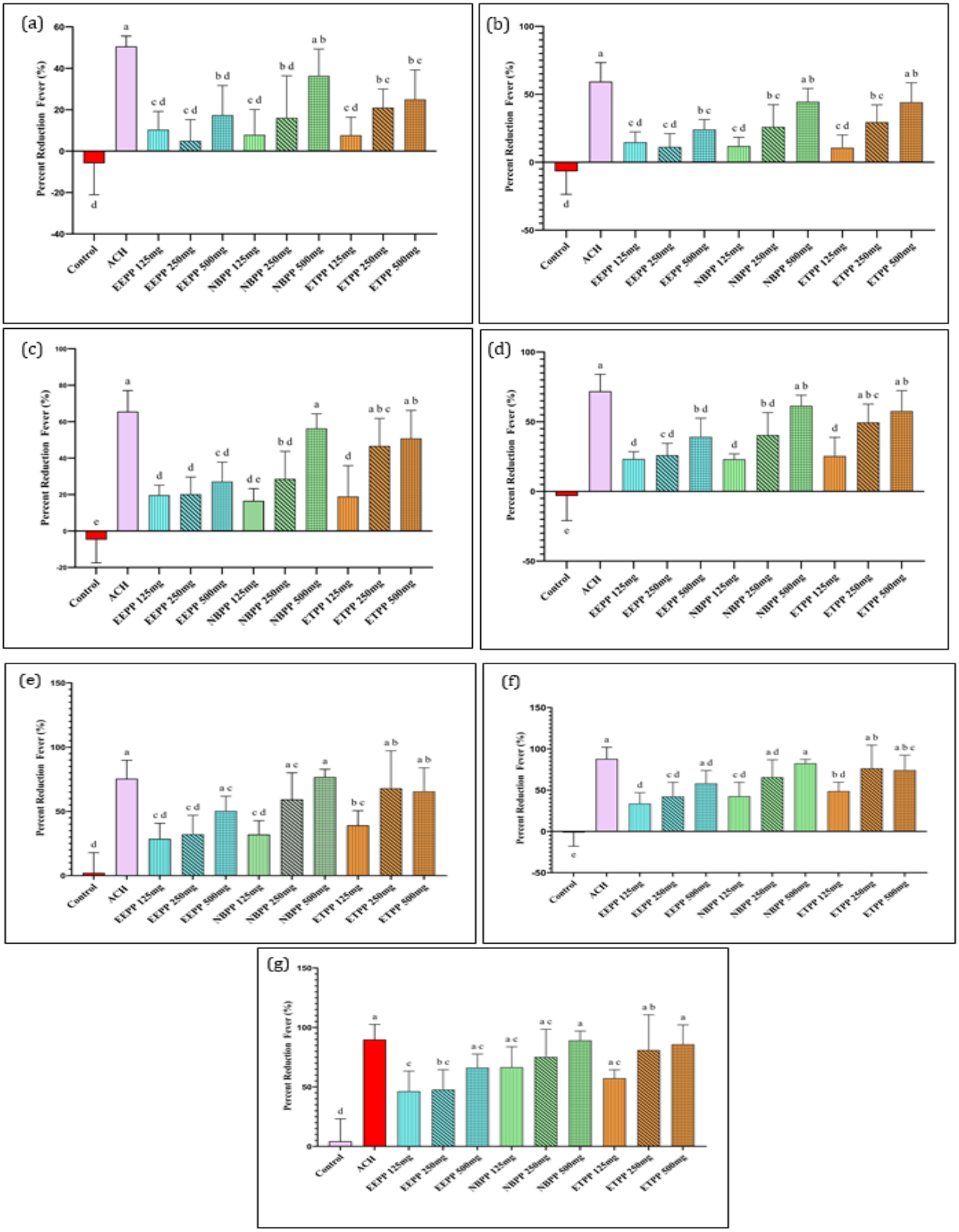


Figure 1. Percent fever reduction over time following treatment

threshold during the later observation periods. In contrast, NBPP produced a faster onset and higher PFR values across doses, indicating greater antipyretic efficacy than EEPP. ETPP exhibited the most rapid onset and the highest overall PFR profile, achieving effects comparable to acetaminophen at the highest dose. These differences suggest that solvent polarity influences the enrichment of bioactive constituents associated with antipyretic efficacy, as reported in previous phytochemical and pharmacological studies [7,16].

Statistical analysis using one-way ANOVA followed by Tukey's multiple comparison test revealed significant differences among treatment groups at corresponding time points ($p<0.05$), as indicated in Figure 1. Collectively, the PFR data establish the antipyretic potency ranking as ETPP> NBPP>EEPP, consistent with the rectal temperature findings.

Correlation between secondary metabolite compounds and antipyretic mechanism

Based on the phytochemical screening results (Table 1), the three extracts exhibited distinct secondary metabolite profiles that corresponded with their observed antipyretic performance. ETPP, characterized mainly by flavonoids and phenolic compounds, produced the fastest onset and the highest percent fever reduction. This observation suggests that these compounds may contribute to the antipyretic effect, potentially through modulating key inflammatory mediators involved in fever regulation. Prostaglandin E_2 (PGE_2) is a principal pyrogenic mediator responsible for elevating the hypothalamic temperature set point during fever, and suppression of PGE_2 synthesis represents a well-established mechanism of antipyretic action [1,15]. Previous studies have reported that flavonoids can inhibit cyclooxygenase-2 (COX-2) activity and reduce PGE_2 production, thereby contributing to the reduction of fever [27-29]. The rapid antipyretic response observed for ETPP is therefore consistent with the potential involvement of flavonoid-mediated modulation of this pathway.

NBPP, despite containing fewer classes of secondary metabolites, demonstrated strong and consistent antipyretic effects. This pronounced response may be associated with its alkaloid and steroid content. Alkaloid-containing extracts have been reported to exert antipyretic effects through modulating inflammatory mediators, including pro-inflammatory cytokines such as interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α), which are known to stimulate PGE_2 synthesis in the central nervous system [30-32]. This interpretation aligns with the rapid and sustained temperature-lowering effect observed for NBPP despite its relatively limited metabolite diversity.

EEPP also demonstrated significant antipyretic activity, although with a slower onset compared to ETPP and NBPP. This delayed response may be related to the presence of saponins and tannins, which have been reported to exert anti-inflammatory and antipyretic effects through modulating inflammatory signaling pathways, including cytokine release and downstream prostaglandin production [33-36]. The combined action of these compounds may explain the stable but delayed antipyretic response observed for EEPP.

Overall, the correlation between secondary metabolite composition and antipyretic activity highlights the role of solvent-dependent extraction in shaping pharmacodynamic profiles. However, as this study evaluated antipyretic activity using crude extracts without isolating individual bioactive compounds and employed a single experimental fever model with a limited sample size, the proposed mechanisms remain associative rather than confirmatory. Further studies involving bioassay-guided compound isolation, biomarker

validation, and expanded experimental models are therefore warranted.

Conclusion

Pometia pinnata fruit peel extracts exhibited significant, dose- and time-dependent antipyretic activity in the yeast-induced pyrexia model. Overall, the antipyretic potency followed the order ETPP> NBPP> EEPP, with ETPP at 500 mg/kg BW producing a fever reduction comparable to paracetamol. These findings are consistent with solvent-dependent differences in secondary metabolite profiles. Future studies should focus on bioassay-guided isolation and identification of active constituents, as well as safety evaluations through acute and subacute toxicity studies, to support further development.

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Authors' Contributions

Conceptualization, Andri Tilaqza; methodology, Andri Tilaqza and Merlita Herbani; validation, Andri Tilaqza; formal analysis, Andri Tilaqza and Merlita Herbani; investigation, Andri Tilaqza and Merlita Herbani; resources, Faculty of Medicine, University of Islam Malang; data curation, Merlita Herbani; writing—original draft preparation, Andri Tilaqza; writing—review and editing, Merlita Herbani; visualization, Andri Tilaqza; supervision, Andri Tilaqza and Merlita Herbani; project administration, Andri Tilaqza; funding acquisition, Medical Faculty, University of Islam Malang.

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