



# Antipyretic Efficacy of a Combined Ethanol Extract of Papaya (*Carica papaya*) and Basil (*Ocimum basilicum*) Leaves in White Mice (*Mus musculus*)

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**Abstract:** Fever is a condition characterized by an increase in body temperature above 37°C. It typically occurs as a response of the immune system to combat infections caused by bacteria and viruses. Previous studies have shown that papaya leaves (*Carica papaya*) and basil leaves (*Ocimum basilicum*) contain flavonoid compounds. Scientifically, the individual extracts of these two plants have demonstrated antipyretic activity. Flavonoids play an important role in inhibiting the cyclooxygenase-2 (COX-2) enzyme in the biosynthesis of prostaglandins (PGE), which act as mediators of fever. This study aimed to evaluate the antipyretic effectiveness of a combination of ethanol extract of papaya and basil leaves in mice induced with 5% peptone. This research employed a true experimental design. A total of 25 male mice were used and divided into five groups: a positive control group (Paracetamol 1.3 mg/20 g body weight), a negative control group (Na-CMC), and three treatment groups receiving combinations of ethanol extract of papaya and basil leaves at doses of 400 mg/kg BW : 200 mg/kg BW (4:2), 600 mg/kg BW : 300 mg/kg BW (6:3), and 800 mg/kg BW : 400 mg/kg BW (8:4), respectively. All treatments were administered orally. Body temperature was measured at 30, 60, and 90 minutes after induction. The results showed no significant difference between the combination treatments and the positive control group, indicating that the combination of ethanol extract of papaya and basil leaves is effective as an antipyretic agent.

**Keywords:** Antipyretic; Basil Leaves (*Ocimum basilicum*); Ethanol Extracts of Papaya (*Carica papaya*)

## Introduction

According to the World Health Organization (WHO) in 2020, the number of fever cases worldwide reached approximately 11–20 million, with an estimated 128,000–161,000 deaths occurring annually (Jeon, 2023; Raman et al., 2025). In Indonesia, it is estimated that between 800 and 100,000 people suffer from fever each year. Fever cases are predominantly found in children, accounting for 91% of cases among individuals aged 3–

19 years (WHO, 2020)

The South Sulawesi Provincial Health Office reported in 2019 that fever ranked third among the most common diseases in hospitalized patients, with 1,895 recorded cases and 17 deaths. In Makassar City particularly, 135 cases of fever were reported, with one resulting in death (South Sulawesi Provincial Health Office, 2019). Meanwhile, the Palopo City Health Office recorded 1,223 cases of fever in 2018, making it one of the top ten most prevalent diseases in the city.

### How to Cite:

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Papaya (*Carica papaya* L.) is a medicinal plant known for its therapeutic properties. One of the medicinal parts of the papaya plant is its leaves (O'Hare & Williams, 2013; Pinnamaneni, 2017; Singh et al., 2020). Traditionally, papaya leaves are believed to help treat fever, vaginal discharge, acne, increase appetite, enhance breast milk production, and relieve toothache (Hariono et al., 2021). Papaya leaves (*Carica papaya* L.) contain alkaloids, papain, saponins, flavonoids, and tannins (Luciana et al., 2025; Puspitasari et al., 2024). According to a study by Ningsih & Rejeki (2018), ethanol extract of papaya leaves contains flavonoid compounds that exhibit antipyretic activity.

According to a study by Nigrum et al., the administration of ethanol extract of papaya leaves at doses of 200 mg/kg BW, 400 mg/kg BW, and 800 mg/kg BW resulted in a decrease in the rectal temperature of febrile mice. The administration of varying doses of ethanol extract of papaya leaves produced different effects, with Duncan's test showing that the 800 mg/kg BW dose had the most effective antipyretic activity, followed by the 400 mg/kg BW and 200 mg/kg BW doses. Phytochemical screening of papaya leaves revealed the presence of compounds such as flavonoids, alkaloids, tannins, and saponins (Ningrum, 2024).

Basil (*Ocimum basilicum*) is a type of herbal medicine known to be effective in treating various diseases. In animal models, basil leaf extract has been shown to reduce both chronic and acute inflammation, as well as exhibit antipyretic and analgesic properties. A study by Ceriana et al. (2022) also demonstrated that basil leaf extract possesses antipyretic effects, as evidenced by a reduction in body temperature in mice. Basil contains compounds with the potential to inhibit prostaglandin biosynthesis. Essential oils, tannins, flavonoids, and triterpenoid steroids are present in basil leaves (Romano et al., 2022). Flavonoids have the ability to inhibit lipooxygenase, which is an initial step in the synthesis of eicosanoid hormones such as prostaglandins and thromboxanes (Ceriana et al., 2022). According to research by Kumalasari & Andiarna (2020), ethanol extract of basil leaves contains flavonoids and tannins that can help reduce fever in mice.

Flavonoids have been reported to exert antipyretic activity by inhibiting the cyclooxygenase (COX) enzyme, thereby suppressing prostaglandin synthesis from arachidonic acid, which is a key mediator in fever response (Mbaubedari et al., 2021). Tannins also exhibit antipyretic potential through their antioxidant properties and ability to scavenge free radicals, thus contributing to the reduction of inflammatory responses (Sedu et al., 2020).

According to a study conducted by Wati et al. (2024), the results of the study showed the ethanol

extract of basil (*Ocimum spp.*) leaves exhibited antipyretic effects at all three doses (32.5 mg/kg BW, 65 mg/kg BW, and 130 mg/kg BW in mice), with the 130 mg/kg BW dose demonstrating efficacy comparable to that of paracetamol (Wati & Fadhillah, 2023). Based on the aforementioned findings, the researcher is interested in conducting a study on the antipyretic effectiveness of a combination of ethanol extracts of papaya leaves (*Carica papaya*) and basil leaves (*Ocimum basilicum*) in white mice (*Mus musculus*) (Wati & Fadhillah, 2023).

## Method

### Materials

The materials used in this study were papaya leaves (*Carica papaya*) and basil leaves (*Ocimum basilicum*), which were obtained from Palopo City, South Sulawesi.

### Preparation of extract

A total of 200 g of papaya leaves and 200 g of basil leaves were weighed separately and placed into different macerators. Each was then soaked in 750 mL of 96% ethanol as the extraction solvent. The mixtures were left to stand for 3 × 24 hours, protected from direct sunlight, and stirred occasionally. After maceration, the mixtures were filtered to separate the filtrate from the residue. The residues were then re-macerated using 500 mL of 96% ethanol. The resulting extracts were concentrated using a rotary evaporator at 50°C, followed by evaporation in a water bath until a thick extract was obtained. The extracts were then weighed to determine their mass (Yuniarto & Selifiana, 2018).

### Pytochemical analysis

#### Alkaloid Test

Approximately 2–3 drops of the extract were placed into a test tube and mixed with 5 drops of concentrated ammonia (NH<sub>3</sub>). Then, 2 N sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) was added and the mixture was shaken until two layers formed. The resulting solution was divided into two portions. To the first tube, 3 drops of Mayer's reagent were added, and to the second tube, 3 drops of Dragendorff's reagent were added. The presence of alkaloids was indicated by the formation of a precipitate.

#### Flavonoid Test

Three drops of the extract were placed into a test tube and mixed with 2–3 drops of 96% methanol. The mixture was then heated at 50°C. After cooling, a small piece of magnesium metal and 4–5 drops of concentrated hydrochloric acid (HCl) were added. The presence of flavonoids was indicated by a color change in the filtrate to red or orange.

*Saponin Test*

Approximately 5–6 drops of the test solution (from the flavonoid assay) were placed into a test tube and shaken vigorously in a vertical motion for 10 seconds. The presence of saponins was indicated by the formation of a stable foam layer measuring 1–10 cm in height, which remained stable for approximately 15 minutes and did not disappear upon the addition of a drop of 2 N hydrochloric acid (HCl).

*Tannin Test*

Three drops of the extract were placed into a test tube, followed by the addition of 3–4 drops of distilled water and 2–3 drops of ferric chloride (FeCl<sub>3</sub>) solution. The presence of tannins was indicated by the appearance of a dark blue or black coloration.

*Preparation of the paracetamol suspension*

The preparation of the paracetamol suspension begins with the preparation of a colloidal Na-CMC solution. Specifically, 0.5 g of Na-CMC is accurately weighed and gradually added to 50 mL of hot distilled water while stirring until a colloidal solution is formed. The final volume is then adjusted to 100 mL. 10 paracetamol tablets are weighed, and their average weight is calculated. The tablets are then pulverized in a mortar, and the amount corresponding to the required paracetamol dose is accurately measured. 1% Na-CMC colloidal solution is then gradually added to the powdered tablets while triturating until a homogeneous mixture is obtained. The mixture is transferred to a volumetric flask, and the volume is adjusted to 100 mL with the 1% Na-CMC colloidal solution (Viani & Hijratul, 2016).

*Preparation of 5% Peptone Induction Solution*

1 gram of peptone was accurately weighed and transferred into a beaker, then dissolved in 20 mL of distilled water with continuous stirring. Peptone is a protein compound used to induce fever in experimental animals. As a pyrogenic substance, peptone can increase the body temperature of test animals (Viani & Hijratul, 2016).

*Antipyretic Activity Test*

In this study, a total of 25 mice were divided into five treatment groups: one negative control group (Na-CMC), one positive control group (paracetamol), and three treatment groups receiving a combination of ethanol extracts of papaya leaves and basil leaves at doses of (P1= 400 mg/kgBW : 200 mg/kgBW), (P2=600 mg/kgBW : 300 mg/kgBW), and (P3= 800 mg/kgBW : 400 mg/kgBW), respectively. All groups were first induced with a 5% peptone solution, and body temperature measurements were taken three hours after the induction. Temperature was measured using an rectal thermometer on the rectum area of the mice (normal themprature :37,5<sup>0</sup>C) (Widyasari et al., 2018). The temperature data obtained were then used to calculate the percentage of antipyretic activity using the Formula 1 (Widyasari et al., 2018).

$$\% \text{ Antipyretic activity} = \frac{t_0 - t_n}{t_0 - t_{(initial)}} \times 100\% \quad (1)$$

Note:

*t<sub>initial</sub>*: Initial body temperature before induction with 5% peptone

*t<sub>0</sub>*: Fever temperature after induction with 5% peptone

*t<sub>n</sub>*: Body temperature at 30, 60, and 90 minutes after treatment administration (Ahmad et al., 2023)

*Data Analysis SPSS (Statistical Package for the Social Sciences)*

The research data were analyzed using one-way ANOVA followed by Tukey's post hoc test to determine significant differences between groups. The level of significance (α) was set at 0.05 (5%), indicating that results were considered statistically significant if p < 0.05.

**Result and Discussion**

*Phytochemical screening*

Phytochemical screening showed that the ethanol extracts of papaya leaves and basil leaves contain several secondary metabolites, such as flavonoids, alkaloids, saponins, and tannins. These data are presented in Tables.

**Table 1.** Results of Phytochemical Screening of Ethanol Extract of Papaya Leaves (*Carica papaya*)

Compound	Reagent	Literature Result	Observation Result	Note
Alkaloids	Mayer	White/yellow precipitate	White/yellow precipitate	+
	Dragendorff	Brick-red precipitate	Brick-red Precipitate	+
Flavonoids	Mg + HCl	Red/orange coloration	Red or orange coloration	+
Saponins	Hot distilled water	Presence of foam	Presence of foam	+
Tannins	FeCl <sub>3</sub>	Dark blue/black coloration	Dark blue/black coloration	+

Phytochemical screening revealed that papaya leaves contain several secondary metabolites, including alkaloids, flavonoids, saponins, and tannins. The purpose of conducting phytochemical screening is to identify the classes of secondary metabolites present in plants (Vifta & Advistasari, 2018). These findings are consistent with previous studies, which reported that papaya leaves contain alkaloids, tannins, saponins,

flavonoids, and several volatile compounds (Alara et al., 2020). In addition, phytochemical screening of basil leaves showed the presence of active compounds such as alkaloids, flavonoids, saponins, and tannins. This is in line with the study by larasati, which found that basil leaves contain essential oils, flavonoids, alkaloids, saponins, tannins, triterpenoids, steroids, and phenols.

**Table 2.** Results of Phytochemical Screening of Ethanol Extract of Basil Leaves (*Ocimum basilicum*)

Compound	Reagent	Literature Result	Observation Result	Note
Alkaloids	Mayer	White/yellow	White/yellow	+
	Dragendorff	Precipitate Brick-red	precipitate Brick-red	+
Flavonoids	Mg + HCl	Precipitate Red/orange	precipitate Red or orange	+
Saponins	Hot distilled water	Coloration	coloration	+
Tannins	FeCl <sub>3</sub>	Presence of foam	Presence of foam	+
		Dark blue/black	Dark blue/black	+
		Coloration	coloration	

*Antipyretic activity test*

In the antipyretic activity test, the results showed a decrease in body temperature over a period of 90 minutes. The administration of a combination of papaya leaf extract and basil leaf extract in groups P2 and P3 resulted in a temperature reduction that was not

significantly different from the positive control group. These data are presented in Table 3 and 4. Furthermore, the results of the ANOVA and Tukey tests indicated a significant difference between the negative control group and the P2, P3, and positive control groups. These findings are shown in Table 3.

**Table 3.** Results of Average Rectal Temperature Measurements in Mice

Treatment	Ta (Initial Temp)	t1	t30	t60	t90	Temperature Decrease
Negative Control	37.04	38.02	37.58	37.52	37.60	0.42 °C
Positive Control	37.32	38.04	37.16	37.14	37.04	1.00 °C
P1	37.04	38.02	37.30	37.20	37.10	0.92 °C
P2	37.10	38.00	37.28	37.04	37.00	1.00 °C
P3	37.02	38.02	37.10	37.02	37.00	1.02 °C

Note:

Ta: Initial temperature

t1: Temperature after peptone induction

t30: Temperature at 30 minutes after treatment

t60: Temperature at 60 minutes after treatment

t90: Temperature at 90 minutes after treatment

The antipyretic effectiveness test showed a decrease in the average body temperature of the test animals over a period of 90 minutes. In the negative control group, which was given 0.1% Na CMC (Negative Control) suspension, the temperature decreased by 0.42 °C. Meanwhile, the Paracetamol (Positive Control), and treatment group P3 showed a temperature reduction of 1.02 °C, group P2 showed a reduction of 1.00 °C, and group P1 showed a reduction of 0.92 °C. The temperature decrease in the positive control group, which received paracetamol, occurred because the peak plasma concentration of paracetamol is reached within 30 minutes, with a plasma half-life of 1 to 3 hours (Wells et al., 2009).

Furthermore, in groups P1, P2, and P3, which were administered a combination of ethanol extracts of papaya leaves and basil leaves, a decrease in body temperature was observed. This reduction is presumed to be due to the presence of active flavonoid compounds in papaya leaves, which have antipyretic properties. This finding is consistent with the study by Dwi Ningsi and Endang in 2018, which reported that ethanol extract of papaya leaves contains flavonoids with antipyretic effects (Ningsih & Rejeki, 2018). In addition, the presence of flavonoids and tannins in basil leaves is also known to contribute to antipyretic activity (Ceriana et al., 2022). This is supported by the Kumalasari & Andiarna (2020), which stated that ethanol extract of basil leaves contains

flavonoids and tannins that help reduce fever in mice. These results suggest that the combination of ethanol extracts of papaya and basil leaves exhibits antipyretic effects comparable to those of the positive control (paracetamol).

Among the three tested concentrations, the P3 (800 mg/kg BW : 400 mg/kg BW in mice) exhibited the greatest reduction in body temperature, with a decrease of 1.02 °C. This result is attributed to the synergistic effect of the bioactive compounds present in papaya and

basil leaves. Papaya leaves contain active constituents such as flavonoids (quercetin), alkaloids (carpaine), tannins, and saponins, which possess antipyretic properties primarily through the inhibition of prostaglandin E2 (PGE2) synthesis (Hariono et al., 2021; Ningsih & Rejeki, 2018). Meanwhile, basil leaves are rich in flavonoids, essential oils (eugenol and linalool), tannins, and triterpenoids, which antipyretic effects by inhibiting the cyclooxygenase (COX) enzyme (Ceriana et al., 2022; Romano et al., 2022).

**Table 4.** Result of Anova

Antipyretic	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	96.160	4	24.040	4.906	0.006
Within Groups	98.000	20	4.900		
Total	194.160	24			

The results of the one-way ANOVA showed a statistically significant difference, with a p-value of 0.006 < 0.05, indicating that there were meaningful differences among the treatment groups. Furthermore, the higher the concentration administered, the greater the antipyretic effect. Flavonoids act by inhibiting the activity of cyclooxygenase (COX) enzymes, thereby reducing the production of prostaglandins from arachidonic acid, which in turn leads to a reduction or prevention of fever (Mbaubedari et al., 2021). Similarly, tannins have the ability to inhibit cyclooxygenase enzymes involved in the biosynthesis of prostaglandins, thus potentially preventing the onset of fever (Mbaubedari et al., 2021).

**Conclusion**

The combination of ethanol extracts of papaya (*Carica papaya* L.) leaves at 600 mg/kg body weight and basil (*Ocimum* spp.) leaves at 300 mg/kg body weight exhibited a greater antipyretic effect in peptone-induced mice. It is recommended that future studies compare the antipyretic efficacy of this combination not only to paracetamol but also to other standard antipyretics, such as ibuprofen and aspirin, to further assess its potential for clinical application.

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**Author Contributions**

All authors played an active role in the development of this manuscript. Each contributed meaningfully to various aspects, including data collection, analysis, and the drafting of specific

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**Conflicts of Interest**

The authors declare no conflicts of interest related to the publication of this manuscript. This statement upholds the transparency and integrity of the research process, ensuring that the reported findings are free from external bias or undue influence.

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