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ORIGINAL ARTICLE

Effectiveness of transdermal patch formulation of *Hibiscus rosa-sinensis* L. leaf extract as an antipyretic in male rats induced with DPT vaccine

Nessya Try Natasya Br Ketaren[1], Astriani Natalia Br Ginting[2*], Rena Meutia[3], Asyrun Alkhari Lubis[2]

ABSTRACT

Fever is characterized by an elevation of body temperature above the normal range of 37.5°C and represents a physiological response to various health conditions such as infections or other diseases. Antipyretic drugs, including paracetamol and ibuprofen, are commonly used to manage fever. Ethanol extract of Hibiscus rosa-sinensis L. (kembang sepatu) leaves contains bioactive compounds such as tannins, flavonoids, saponins, carbohydrates, steroids, phenols, glycosides, quinones, terpenoids, cyclopeptides, and alkaloids, which may confer natural antipyretic properties. In this study, 3% and 5% (w/w) Hibiscus rosa-sinensis leaf ethanol extracts were formulated into transdermal patches and evaluated for their antipyretic efficacy in male rats induced with the Diphtheria-Pertussis-Tetanus (DPT) vaccine. The dried Hibiscus rosa-sinensis leaves were macerated using 96% ethanol. The concentrated extract was formulated into a hydroxypropyl methylcellulose (HPMC)-based transdermal patch. Phytochemical screening of the extract, stability testing of the transdermal patch, and statistical analysis of the antipyretic effect on DPT-induced male rats were conducted. Phytochemical screening of the 96% ethanol extract confirmed the presence of alkaloids, flavonoids, tannins, steroids, and saponins. The transdermal patch formulation demonstrated good stability over 21 days of storage. The 5% extract patch exhibited the highest antipyretic activity, significantly reducing fever in the experimental rats and showing effectiveness comparable to the positive control (paracetamol).

Keywords: Hibiscus rosa-sinensis leaf, transdermal patch, antipyretic

Introduction

Fever, or pyrexia, is a physiological process characterized by an elevation of body temperature beyond the homeostatic range, defined as a temperature of ≥38°C. Fever represents a systemic response involving the activation of the immune system to combat the invasion of foreign antigens.[1,2] Excessive elevation of body temperature may lead to complications such as seizures; therefore, the reduction of fever is typically managed by administering antipyretic agents such as paracetamol and ibuprofen.[3,4] Antipyretics exert their effect centrally by inhibiting the hypothalamic thermoregulatory center, thereby reducing heat production.[5] The use of natural plant-derived compounds with antipyretic properties offers a promising strategy to minimize the risk of overdose and the adverse effects associated with synthetic chemicals. Numerous plants have been reported to possess antipyretic efficacy, including turmeric, papaya, soursop, and hibiscus.[5–8]

Affiliation
[1]Undergraduate Program in Clinical Pharmacy, Universitas Prima Indonesia
[2]Department of Clinical Pharmacy, Universitas Prima Indonesia
[3]Undergraduate Program in Clinical Pharmacy, Universitas Syiah Kuala

***Corespondence:**
astrianinataliabrginting@unprimdn.ac.id

The hibiscus plant (*Hibiscus rosa-sinensis* L.) is known to have various health benefits attributed to its flowers, leaves, roots, and stems. Its bioactive properties include anti-inflammatory, antipyretic, anti-obesity, natural coloring, hepatoprotective effects, among others.[9] This study focuses on utilizing hibiscus leaf extract as an antipyretic agent, targeting phytochemicals such as alkaloids, flavonoids, tannins, steroids, and saponins present in the leaves. The extract is incorporated into a transdermal patch formulation to potentially achieve more rapid fever reduction through percutaneous absorption. Transdermal patches are dosage forms comprising a flexible adhesive matrix containing the active compound, designed for prolonged administration and controlled drug release via the skin.[10–12]

The aim of this research is to evaluate the efficacy of hibiscus leaf extract as an antipyretic and to identify the optimal formulation within a transdermal patch system. The transdermal delivery approach is innovated to facilitate rapid temperature reduction and to enhance patient compliance through ease of use. The extraction process employed maceration with 96% ethanol as the solvent, and hydroxypropyl methylcellulose (HPMC) was used as the patch matrix base.

Method

Study Design

This research constitutes an experimental laboratory study conducted from December 2024 to February 2025 at the Integrated Laboratory of the Faculty of Medicine, Dentistry, and Health Sciences, Universitas Prima Indonesia.

Equipment and Materials

The following equipment was utilized in this study: a digital balance (ACIS) for precise weighing, mortar and stamper for sample preparation, PYREX graduated cylinders for accurate volume measurement, Petri dishes for sample cultivation, and an oven (JOUAN TYPE IG 150) to maintain controlled heating conditions. Additionally, a GILLETTE shaving device was used for specific sample preparations, while a laboratory scale (ACIS) was employed for weighing laboratory mice. Temperature was monitored using a TERMOONE thermometer. A dropper pipette facilitated precise liquid handling. A STUART rotary evaporator was employed for solvent removal, and sample homogenization was performed using a MIYAKO blender. Glass jars served for sample storage. An analytical balance (OHAUS) was used for fine measurements, and the OHAUS BENCHTOP STARTER 3100 pH meter was applied for pH determination. Sample injection utilized ONEMED syringes. Samples were stored in a desiccator to prevent moisture contamination, and test tubes were used for various experimental procedures.

The study employed Hibiscus rosa-sinensis (roselle) leaves as the primary plant material. Other chemical reagents included hydroxypropyl methylcellulose (HPMC), glycerol, oleic acid, methylparaben, and Tween 80 as formulation agents. Solvents used were 96% and 70% ethanol along with distilled water. Analytical standards such as paracetamol and sodium carboxymethyl cellulose (CMC-Na) were also used. Biological materials included the DPT vaccine. Additional materials comprised aluminum foil, laboratory animal feed and drinking water specific for mice, filter paper for filtration processes, concentrated hydrochloric acid (HCl), and magnesium powder.

Procedures

Preparation of simplisia powder

Hibiscus rosa-sinensis leaves, intended as raw materials, were collected from Tanah Karo, North Sumatra. Fresh leaves, specifically young leaves, were selected and subjected to botanical identification at the Herbarium Medanense (MEDA) Laboratory, University of North Sumatra. The powder preparation process comprised several steps. Initially, 4 kg of fresh Hibiscus rosa-sinensis leaves were harvested, washed with clean water, drained, and then dried. Drying was performed by sun-drying the leaves under black cloth covering to protect from direct sunlight. Once dried, the leaves were ground into powder using a blender and subsequently sieved through a 40-mesh screen.

Extraction

A total of 500 g of Hibiscus rosa-sinensis leaf powder was extracted by maceration with re-maceration over 12 days using 96% ethanol as the solvent. Following extraction, the solvent was evaporated to obtain a concentrated extract of the hibiscus leaves.

Phytochemical Screening

Phytochemical screening began with the flavonoid test, where four grams of powdered simplisia were ground and mixed with ether, then filtered. The filtrate was treated with 10% NaOH, separated into layers, acidified with 2N HCl until colorless, and extracted with ether. The ether layer was mixed with ethanol and divided into two tubes; one received magnesium and concentrated HCl. A yellow to red color indicated flavonoids, while the other tube served as control. For the saponin test, four grams of powder were shaken with 5 mL distilled water. Persistent foam lasting one minute indicated saponins. In testing for steroids and triterpenoids, four grams of powder were mixed with chloroform and filtered onto a spot plate. After drying, acetic acid and concentrated H2SO4 were added. Green to blue color indicated steroids, while red to purple indicated triterpenoids. The alkaloid test involved treating powdered sample with dilute H2SO4 followed by Dragendorff's reagent. An orange to brick-red precipitate showed alkaloids. Finally, tannins were detected by mixing powder with ferric chloride solution; a dark green or blue color confirmed their presence.

Preparation of transdermal patch formulations

The ethanol extract of Hibiscus rosa-sinensis leaves was formulated into transdermal patches at two extract concentrations (references for formulation methodology to be inserted).

Table 1. Composition of transdermal patch formulations containing Hibiscus Rosa-Sinensis leaf extract

Ingredient	K (+)	F0/ K(-)	F1	F2	Function
Hibiscus leaf extract	-	-	3%	5%	Active compound
Paracetamol	0,0030%	-	-	-	Antipyretic
HPMC	-	10%	10%	10%	Base
Glycerol	-	10%	10%	10%	Plasticizer
Methylparaben	-	0,1%	0,1%	0,1%	Preservative
Oleic acid	-	5%	5%	5%	Penetration enhancer
Tween 80	-	1,2%	1,2%	1,2%	Penetration enhancer
Etanol 70% ad	-	100%	100%	100%	Solvent

Notes: K (+) = Positive control (paracetamol), K (-) = Negative control/F0 (patch base without extract), F1 = Formulation 1 (3% extract concentration), F2 = Formulation 2 (5% extract concentration)

The patch preparation involved dispersing HPMC in hot water at a ratio of 1:20, followed by mixing with glycerol until homogeneous. The ethanolic extract of Hibiscus leaves, previously dissolved partly in ethanol, was added and mixed thoroughly. Methylparaben was dissolved in a small amount of ethanol then mixed with oleic acid and Tween 80. This mixture was then homogenized. Subsequently, the remaining 70% ethanol was added gradually with continuous stirring until a uniform solution was obtained. The resulting mixture was cast into molds measuring 2 x 2 cm, left to evaporate at room temperature for 3 hours, then oven-dried at 50°C for 5 hours. Once dried, the patches were removed from the molds.

Stability testing of the transdermal patch

The stability testing of the transdermal patch involved several evaluations to ensure quality and skin compatibility. First, the patches were visually checked for shape, color, and odor. Next, weight uniformity was assessed by weighing four randomly selected patches from each formulation and calculating the average weight. For pH measurement, 10 milliliters of CO2-free distilled water was added to a patch and left for an hour; the pH was then recorded using indicator paper, with an acceptable range of 5 to 6.5 to prevent skin irritation. Thickness was measured at three points per patch using a ruler or micrometer, with a maximum allowed thickness of 1 mm. Finally, folding endurance was tested by repeatedly folding the patch at the same spot until it broke, with a minimum acceptable value of 300 folds, indicating sufficient flexibility and durability.

Evaluation of antipyretic effectiveness of the transdermal patch

The evaluation of the antipyretic effectiveness of the transdermal patch was conducted using 24 male white rats, which were randomly divided into four groups, each containing six rats. The sample size was determined based on Federer's formula, ensuring that the product of the number of groups minus one and

the sample size minus one was at least 15. This calculation confirmed that having six rats per group met the minimum requirements for an adequate sample size.

Before starting the experiment, each rat was weighed and marked for identification. They were then allowed to acclimate in their environment for seven days to minimize stress and physiological variations. On the eighth day, the hair on the dorsal area of each rat was shaved to expose the skin, and the rats were left for an additional day to avoid any inflammation that might be caused by shaving.

The rats were then assigned to one of four treatment groups. The first group received a DPT vaccine injection of 0.2 mL and a transdermal patch without any herbal extract, serving as the negative control. The second group was given the same DPT vaccine injection plus oral paracetamol at a dose of 500 mg/kg body weight as the positive control. Group three received the vaccine along with a transdermal patch containing 3% Hibiscus leaf extract, while group four received the vaccine plus a patch with 5% Hibiscus leaf extract.

The study commenced by measuring the baseline rectal temperatures of each rat one hour before the intraperitoneal injection of the DPT vaccine, given in a volume of 0.2 mL to induce fever. One hour after this injection, rectal temperatures were measured again to confirm the induction of fever. The designated transdermal patches were then applied to the shaved dorsal area of each rat and secured with Hypafix adhesive to ensure proper contact.

Temperature monitoring was intensive and frequent. Measurements were taken every 15 minutes during the initial two hours following patch application and subsequently every 30 minutes until the end of the three-hour observation period. This schedule allowed for detailed tracking of the temperature changes and evaluation of the antipyretic effects of the different treatments over time.

Results

Results of the Hibiscus rosa sinensis L. leaf simplisia

The amount of concentrated extract obtained during maceration, using 500 g of simplisia extracted with 5000 mL of 96% ethanol, is presented as a percentage yield in Table 2.

Table 2. Extract Yield of Hibiscus rosa sinensis L. Leaf

Sample	Simplisia Weight	Extract Weight	% Yield
Daun kembang Sepatu (*Hibiscus rosa sinensis L.*)	500gram	65gram	13 %

The yield results indicate that the extract meets the required standards, as a good yield is defined as ≥10%.

Phytochemical screening results

Phytochemical screening of the Hibiscus rosa sinensis L. leaf extract revealed the presence of secondary metabolites, as summarized in Table 3.

Table 3. Phytochemical Screening of Hibiscus rosa sinensis L. Leaves

Compound	Reagent	Positive Result	Identification Result	Remarks
Alkaloids	Dragendorff's reagent	Red precipitate	Red precipitate	+
Flavonoids	Mg powder and HCl	Brick-red color	Red color	+
Tannins	$FeCl_3$	Green or dark blue	Dark green	+
Steroid	Chloroform 98%, Anhydrous acetic acid 98%	Green	Green	+
Saponins	Distilled water and 1 M HCl	Foam formation	Foam formation	+

Based on the data in Table 3, the phytochemical screening of the Hibiscus rosa sinensis L. leaf extract was positive for alkaloids, flavonoids, tannins, steroids, and saponins.

Stability testing of the transdermal patch preparation

Two formulations of the transdermal patch with different extract concentrations were prepared and evaluated for physical stability. The organoleptic test revealed distinct characteristics for each formula. Formula F0 exhibited an elastic and chewy texture, accompanied by a characteristic odor and a transparent color. Formula F1 presented a soft texture that was slightly elastic, with no noticeable odor and a pale green color. Formula F2 had a slightly elastic texture, was odorless, and displayed a green color.

When stored at cold temperatures between 1º and 4ºC, all three formulas—F0, F1, and F2—underwent noticeable changes in their texture, odor, and color. In contrast, when kept at room temperature, ranging from

25º to 28ºC, none of the formulas showed any changes in these organoleptic properties. However, exposure to elevated temperatures of around 40ºC caused changes in all formulas. Based on these observations, storing the formulas at room temperature was found to be the optimal condition for preserving their original organoleptic qualities.

Weight uniformity was assessed in quadruplicate for each formula, with the results summarized as mean values accompanied by their standard deviations (SD), as shown in Table 4. For Formula F0, all four replicates consistently measured 0.07, resulting in a mean of 0.07 with no variation (± 0). Formula F1 demonstrated more variability, with replicate weights of 0.08, 0.03, 0.03, and 0.08, leading to a mean value of 0.05 and an SD of 0.028. Formula F2 showed replicate weights of 0.06, 0.03, 0.04, and 0.05, yielding a mean of 0.04 with an SD of 0.013.

Table 4. Mean values and standard deviations of formulas across four replications

Formula	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Mean ± SD	Status
F0	0,07	0,07	0,07	0,07	0,07 ± 0	MS
F1	0,08	0,03	0,03	0,08	0,05± 0,028	MS
F2	0,06	0,03	0,04	0,05	0,04± 0,013	MS

Note: MS is meets standard

Despite some variation, all formulas meet the standard for acceptable weight uniformity, as their standard deviations are equal to or below the threshold of 0.05 set by Hermanto et al. (2019), indicating that each formula satisfies the criteria for good weight uniformity.

The pH values were measured to determine whether the formulations complied with the accepted pH range of 4.5 to 6.5, as referenced by Hermanto et al.[13] Initially, formulations F0, F1, and F2 all displayed a pH of 6. When stored at cold temperatures between 1º and 4ºC, the pH of F0 decreased to 5, indicating a shift toward increased acidity under refrigeration. However, when kept at room temperature, ranging from 25º to 28ºC, no significant changes in pH were observed across any of the formulations, suggesting stability under these conditions. At an elevated temperature of 40ºC, the pH of F0 rose to 7, becoming more alkaline, while the pH values of F1 and F2 dropped to 5, reflecting increased acidity. These fluctuations in pH between the control formulation (F0) and those containing the extract (F1 and F2) were attributed to the inherently acidic nature of the extract. Based on these findings, storing the formulations at room temperature is deemed optimal and acceptable for maintaining the desired pH balance in the patch formulations.

Patch thickness was measured and required to meet an SD ≤ 0.05 for compliance (Cahyani et al., 2024). All formulations satisfied the thickness uniformity requirement (see Table 5). The folding endurance test was conducted on the formulations labeled F0, F1, and F2, revealing that each one demonstrated exceptional flexibility and durability. All three formulations successfully withstood at least 300 folds, confirming their robustness and suitability for use as patches. This high folding endurance underscores the strength and resilience of the materials, ensuring they can endure repeated bending without damage.

Table 5. Thickness Test

Formulation	Dimensi sediaan			SD
	Length (cm)	Width (cm)	Thickness (µm)	
F0	8	3	0,05	
F1	8	3	0,04	≤0,05
F2	8	3	0,06	

Table 6. Antipyretic Activity of Hibiscus rosa sinensis L. leaf extract transdermal patch

Time	Mean±SD			
	Negative	Positive	F1	F2
1 hour before induction	32.75 ± 0.41	33.57 ± 0.29	32.57 ± 0.43	32.60 ± 0.14
1 hour after induction	37.95 ± 0.24	37.97 ± 0.41	38.00 ± 0.09	38.00 ± 0.09
1 hour 15 minutes	36.67 ± 0.64	33.95 ± 0.14	37.42 ± 0.39	34.70 ± 0.43
1 hour 30 minutes	37.57 ± 0.33	33.27 ± 0.50	36.85 ± 0.48	33.95 ± 0.14
1 hour 45 minutes	38.35 ± 0.31	33.27 ± 0.50	36.15 ± 0.42	33.95 ± 0.14
2 hours	39.68 ± 0.86	32.53 ± 0.36	33.60 ± 0.46	33.48 ± 0.37
2 hours 30 minutes	40.17 ± 0.52	32.38 ± 0.26	33.33 ± 0.46	33.28 ± 0.24
3 hours	41.55 ± 1.10	32.12 ± 0.12	33.15 ± 0.42	33.18 ± 0.10

Note: Group 1: Negative control (vehicle only), Group 2: Positive control (Paracetamol 500 mg/kg body weight), Group 3: Transdermal patch with 3% leaf extract (F1), and Group 4: Transdermal patch with 5% leaf extract (F2)

Antipyretic Activity Test

The antipyretic efficacy of the Hibiscus rosa sinensis L. leaf extract transdermal patches was evaluated in animal models. Both extract-containing formulations reduced fever effectively, with formulation F2 demonstrating superior heat reduction compared to F1. The results are summarized in Table 6.

Temperature measurements were recorded 1 hour before and after fever induction and subsequently every 15 minutes during the first 2 hours, then every 30 minutes up to 3 hours. Temperature changes were calculated as percentages using the following formula:

$$\% \text{ Fever reduction} = \frac{(\text{Initial temperature} - \text{Next temperature})}{(\text{Initial temperature})} \text{x } 100$$

The average temperature rise in groups 1 through 4 exceeded 37.5°C one hour post-induction. Subsequently, temperature declined in groups 2 through 4, whereas the negative control group exhibited continuous temperature increase due to lack of therapeutic intervention.

Table 7. Percentage increase and decrease in fever

Time	Mean±SD			
	Negative	Positive	F1	F2
1 hour before induction	0.00%	0.00%	0.00%	0.00%
1 hour after induction	↑ 15.88%	↑ 13.11%	↑ 16.67%	↑ 16.56%
1 hour 15 minutes	↑ 11.97%	↓ 1.13%	↓ 14.89%	↓ 6.44%
1 hour 30 minutes	↑ 14.72%	↓ 0.89%	↓ 13.14%	↓ 4.14%
1 hour 45 minutes	↑ 17.10%	↓ 0.89%	↓ 10.99%	↓ 4.14%
2 hours	↑ 21.16%	↓ 3.10%	↓ 3.16%	↓ 2.70%
2 hours 30 minutes	↑ 22.66%	↓ 3.54%	↓ 2.33%	↓ 2.09%
3 hours	↑ 26.87%	↓ 4.32%	↓ 1.78%	↓ 1.78%

The changes in fever percentage and the corresponding graph illustrate the thermal response profile across all experimental groups.

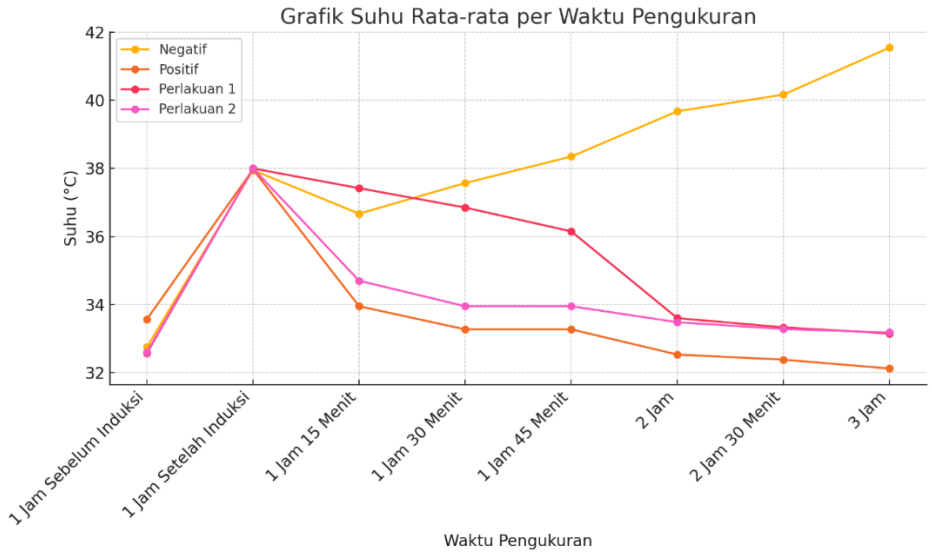

Figure 1. Graph depicting the reduction of fever

The analysis results indicate that the induction procedure significantly increased the measured parameter values (evidenced by $p < 0.001$) (see Table 8). The administration of transdermal patches containing Hibiscus rosa-sinensis leaf extract at concentrations of 3% and 5% exhibited a dose-dependent effect, as Treatment Groups 1 to 3 showed progressively distinct reductions. The negative control group remained stable, serving as the baseline reference for normality.

Table 8. One-Way ANOVA results

Time	Mean±SD			
	Negative	Positive	F1	F2
1 hour before induction	32.75 ± 0.41	33.57 ± 0.29	32.57 ± 0.43	32.60 ± 0.14
1 hour after induction	37.95 ± 0.24	37.97 ± 0.41	38.00 ± 0.09	38.00 ± 0.09
1 hour 15 minutes	36.67 ± 0.64	33.95 ± 0.14	37.42 ± 0.39	34.70 ± 0.43
1 hour 30 minutes	37.57 ± 0.33	33.27 ± 0.50	36.85 ± 0.48	33.95 ± 0.14
1 hour 45 minutes	38.35 ± 0.31	33.27 ± 0.50	36.15 ± 0.42	33.95 ± 0.14
2 hours	39.68 ± 0.86	32.53 ± 0.36	33.60 ± 0.46	33.48 ± 0.37
2 hours 30 minutes	40.17 ± 0.52	32.38 ± 0.26	33.33 ± 0.46	33.28 ± 0.24
3 hours	41.55 ± 1.10	32.12 ± 0.12	33.15 ± 0.42	33.18 ± 0.10
p-value	<.001	<.001	<.001	<.001

Table 9. Post Hoc test results

Time	Mean±SD			
	Negative	Positive	F1	F2
1 hour before induction	32.75 ± 0.41*b	33.57 ± 0.29*a,c,d	32.57 ± 0.43*b	32.60 ± 0.14*b
1 hour after induction	37.95 ± 0.24	37.97 ± 0.41	38.00 ± 0.09	38.00 ± 0.09
1 hour 15 minutes	36.67 ± 0.64*b,c,d	33.95 ± 0.14*a,c,d	37.42 ± 0.39*a,b,d	34.70 ± 0.43*a,b,c
1 hour 30 minutes	37.57 ± 0.33*b,c,d	33.27 ± 0.50*a,c,d	36.85 ± 0.48*a,b,d	33.95 ± 0.14*a,b,c
1 hour 45 minutes	38.35 ± 0.31*b,c,d	33.27 ± 0.50*a,c,d	36.15 ± 0.42*a,b,d	33.95 ± 0.14*a,b,c
2 hours	39.68 ± 0.86*b,c,d	32.53 ± 0.36*a,c,d	33.60 ± 0.46*a,b	33.48 ± 0.37*a,b
2 hours 30 minutes	40.17 ± 0.52*b,c,d	32.38 ± 0.26*a,c,d	33.33 ± 0.46*a,b	33.28 ± 0.24*a,b
3 hours	41.55 ± 1.10*b,c,d	32.12 ± 0.12*a,c,d	33.15 ± 0.42*a,b	33.18 ± 0.10 a,b
p-value	<.005	<.005	<.005	<.005

The p-values for body temperature measured at one hour before induction, one hour after induction, and three hours after induction were determined based on the non-parametric post hoc LSD analysis. The results revealed a significant difference specifically at one hour after induction. The non-parametric post hoc analysis indicated significant differences as follows: (a) compared to the Negative Control group ($p < 0.05$); (b) compared to the Positive Control group treated with paracetamol ($p < 0.05$); (c) compared to the Treatment Group 1 receiving a 3% Hibiscus leaf extract transdermal patch ($p < 0.05$); and (d) compared to the Treatment Group 2 receiving a 5% Hibiscus leaf extract transdermal patch ($p < 0.05$).

Discussion

This study demonstrates that the administration of the ethyl acetate fraction from the leaves of Hibiscus rosa-sinensis L. exerts a significant antipyretic effect in rats induced with the DPT vaccine. Body temperature measurements were conducted one hour prior to induction and one hour post-induction. Subsequently, temperatures of male white rats were monitored at 15-minute intervals during the first two hours, followed by 30-minute intervals up to three hours post-induction. Following the injection of 0.2 ml DPT vaccine, a significant elevation in body temperature was observed, particularly in the negative control and treatment groups, confirming the successful establishment of the pyrexia model.

The group treated with Hibiscus rosa-sinensis leaf extract showed a significant reduction in temperature over time, with the most pronounced effect observed from 1 hour and 15 minutes up to 3 hours post-induction. This temperature reduction exhibited a dose-dependent pattern, wherein the group receiving two transdermal patches containing 5% extract exhibited the most substantial antipyretic effect, closely approximating the efficacy of the positive control (paracetamol).

Statistical analysis employing one-way ANOVA followed by post hoc LSD tests revealed significant differences between groups ($p < 0.001$). These findings support the hypothesis that Hibiscus rosa-sinensis leaf extract contains bioactive compounds such as flavonoids, saponins, and alkaloids, which contribute to its antipyretic activity, as corroborated by phytochemical screening results. The present study aimed to evaluate the antipyretic potential of transdermal patches formulated with ethanol extract of Hibiscus rosa-sinensis leaves, using a DPT vaccine-induced fever model in male rats. The DPT vaccine was selected for its ability to trigger the release of proinflammatory cytokines, including IL-1β, IL-6, and TNF-α, which stimulate the hypothalamic thermoregulatory center via prostaglandin pathways.[14]

Transdermal patches containing 3% and 5% Hibiscus rosa-sinensis leaf extract demonstrated a significant reduction in body temperature starting at 1 hour and 15 minutes post-application. The higher concentration (5%) yielded a more pronounced antipyretic response, indicating a clear dose-response

relationship. This efficacy aligns with previous reports by Abate and Belay[15], which documented that Hibiscus rosa-sinensis leaf and flower extracts possess both antipyretic and antioxidant activities. The synergistic action of flavonoids, saponins, tannins, steroids, and alkaloids appears to inhibit cyclooxygenase-2 (COX-2) enzyme activity, suppress prostaglandin E_2 (PGE_2) production, and reduce systemic inflammation.[16] Additionally, transdermal delivery bypasses first-pass metabolism, thereby enhancing the bioavailability of active compounds and providing controlled, sustained release.[17] Compared to paracetamol, the 5% extract patch exhibited comparable antipyretic efficacy, albeit with a slower onset; the stable release profile of the transdermal system thus represents a promising alternative therapeutic approach for fever management.

The mechanism underlying the antipyretic effect of the ethanol extract-based transdermal patch of Hibiscus rosa-sinensis involves the delivery of bioactive compounds such as flavonoids, saponins, and tannins through the skin layers into the bloodstream. These compounds inhibit the activity of COX-2, leading to reduced synthesis of PGE_2, a primary mediator of fever acting on the hypothalamus. The consequent decrease in PGE_2 levels facilitates the resetting of the body's thermostat to normal, thereby alleviating febrile symptoms.

Conclusion

The study demonstrated that the ethanolic extract of *Hibiscus rosa-sinensis* L. leaves, formulated into transdermal patches, exhibits significant antipyretic activity in male rats induced with fever by the Diphtheria-Pertussis-Tetanus (DPT) vaccine. Both 3% and 5% extract concentrations in the transdermal patches effectively reduced body temperature in a dose-dependent manner, with the 5% extract patch (F2) showing the highest antipyretic effect comparable to the positive control, paracetamol. The phytochemical constituents such as alkaloids, flavonoids, tannins, steroids, and saponins likely contribute to this effect by inhibiting prostaglandin synthesis through COX-2 enzyme suppression. Additionally, the transdermal delivery system provided stable, controlled release and good skin compatibility, making it a promising alternative for fever management. Overall, the findings support the potential use of *Hibiscus rosa-sinensis* leaf extract transdermal patches as a natural and effective antipyretic treatment.

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