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Secondary metabolite and antipyretic effects of Maja (*Crescentia cujete* L.) in fever-induced mice

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Abstract

Objectives: Fever is a condition when the body experiences an increase in average body temperature above normal level. Maja fruit (*Crescentia cujete* L.) contains chemical compounds including alkaloid, flavonoid, saponin, and terpenoid, suspected as potential antipyretics.

Methods: The study aimed to determine the antipyretic activity of ethanol extract of Maja fruit. A total of 25 male white mice of the DDY strain (20–30 g). These treatments divided into three groups with a dose extract of 125, 250, 500 mg/kg BW, standard groups of ibuprofen 400 mg/kg BW, and control groups of CMC-Na 1%. Mice were injected intraperitoneally with 0.1 cc of DPT vaccine-induced. Observations were made by measuring the rectal temperatures of mice using a digital thermometer before DPT vaccine injected or average temperatures, at 0 min (after DPT vaccine injected), 60, 120, 180, and 240 min after administering the test material. The differences between the positive control group, test group, and the negative control group were compared using statistical analysis using one-way variance analysis (ANOVA). The results were considered statistically when the value is ($p<0.05$).

Results: The above phytochemical screening results showed that alkaloids, flavonoids, and saponins were present in the Maja fruit powder and extract (*C. cujete* L.). Based on the results of the statistical analysis obtained, i.e., Group II was not significantly different from Group III and Group IV ($p\leq0.05$) and was significantly different from Group I and Group V. Group I was significantly different from Group II, Group III and Group IV and was not significantly different from Group V ($p\geq0.05$).

Conclusions: The study showed that Maja fruit mice's antipyretic behavior at doses of 125, 250, and 500 mg/kg BW was confirmed as a result in reducing the body temperature of male mice. The 500 mg/kg BW dosage of Maja fruit extract (*C. cujete* L) effectively reduced fever.

Keywords: antipyretics; *Crescentia cujete* (L.); *Diphtheria-pertussis-tetanus* vaccine; fever.

Introduction

The globe is facing the Covid-19 pandemic, which significantly affects people's mobility to carry out activities. As one of the afflicted nations, Indonesia imposes large-scale social sanctions to break the chain of transmission. The transmission is caused by microorganisms, including viruses that invade the body, impair organ functions, and cause a risk of death if not treated properly. Medical effects that are developed due to microorganism infection can send signs, including fever, to the body. A stimulation of cells that are responsible for the immune system will increase the body temperature [1], namely the production of cytokinesis (IL-1, IL-6, and IFN-a). By growing the PGE-2 synthesis pathway, leading to a rise in body temperature, this activation stimulates the hypothalamus to improve cytokines. If the state of the body decreases, microorganisms will defeat the immune system within the body [2]. When body temperature increases above normal levels, people take paracetamol and ibuprofen as antipyretic drugs which function by inhibiting prostaglandin production. Drugs, however, have many side effects and might potentially damage tissues, such as tissues in the liver and kidneys, when taken for a long term [3, 4].

Several researchers in Indonesia are searching for medicines during the pandemic to maintain patients' endurance and avoid consequences of microorganisms within patients that have been infected or exposed to the virus. Many Indonesian researchers are devising a treatment therapy to strengthen the immune system to avoid the exposure to microorganisms. Starting from evolving medicines that have been circulating to looking for new drugs from different varieties of plants that are predicted to produce compounds used as drug action targets that can pharmacologically improve the immune system [5]. Many plants have pharmacological activities from various studies that had been conducted, including Maja (*Crescentia cujete* L), which

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reportedly provides pharmacological activity in overcoming bronchitis, influenza, asthma, pain, diarrhea [6], anti inflammation [7], stimulating insulin production [8], lowering blood pressure [8], and increasing antioxidants [9] and antimicrobials [10]. There are fewer side effects from the use of alternative medicine [11].

Maja fruit has a lot of identified pharmacological activities. Fortunately, the efficacy of Maja fruit compounds as an antipyretic has not been documented. Therefore, if Maja's fruit ability to relieve fever is demonstrated, various previous studies on its pharmacological action linked to the immune system, such as antioxidants, analgesics, anti inflammatory, and antimicrobials, will be sponsored. This study aims to include knowledge about its pharmacological ability to dramatically improve the body's immune system by creating new medicines.

Materials and methods

Materials

Ethanol 70% (C_2H_5OH), Sodium Hydroxide (NaOH) 1 N (Merck), *Diphtheria, Pertussis, Tetanus* (DPT) Vaccine (Pentabio), Alumunum Chloride ($AlCl_3$) 1% (Merck), Hydrochloric Acid (HCl) 2 N (Merck), Mayer reagent, Dragendorf reagent, Ferrous (III) Chloride ($FeCl_3$) 1% (Merck), Sodium Carboxymethylcellulose (CMC-Na) 1% (Merck), Acetate Anhydrase ($C_4H_6O_3$) (Merck), Sulfuric Acid (H_2SO_4) (Merck).

Preparation, extraction of plant materials

The Maja fruit was collected from the South Jakarta National Institute of Science and Technology, Jakarta. The determination of the desired species of *C. cujete* L. was confirmed by the Indonesian Institute of Science, Bogor, Biology Research Center, West Java. The confirmed desired species are *Crescentia cute* L. and the Bignoniaceae tribe (Certificate Number of 2108/IPH.1.01/1f.07/XI/2019). Five kilograms of the fruit was washed under running water. Then, the clean fruit was peeled and separated from the peel, sliced thinly, and dried at 60 °C for 48 h Six hundred and fifty grams of fine Maja fruit powder was filtered with a mesh with a size of 40, resulting in 2 kg of dried fruit powder, which was then pollinated. The dried Maja fruit powder was macerated in ethanol 70% at a room temperature for 3 days and shielded from the sunlight. The extract was filtered and concentrated under vacuum using a rotary evaporator until the ethanol in the extract evaporated.

Preliminary phytochemical test

The preliminary phytochemical screening went through the identification of alkaloid, flavonoid, saponin, and tannin using the extract. The screening aimed to establish pharmacognosy standards.

Alkaloids, 1 g of Maja fruit powder and extract were moistened in a beaker glass with 5 mL of 25% ammonia (NH_3). Twenty millilitres of chloroform were applied until the substance immersed, then stirred, heated, and filtered over a water bath. The filtrate was then halfway evaporated. The residue was poured into a test tube and added with 1 mL of 2 N hydrochloric acids (HCl), then shaken and left to form two layers; the formed transparent layer was taken and equally placed in three test tubes. The Mayer reagent was attached to the first tube, the second tube with the Bouchard reagent, and the third tube with the Dragendorff reagent. The deposition of white deposits in the Mayer reagent, brown deposits in the Bouchard at reagent, and red sediments in the Dragenddorff reaction suggest alkaloids. Alkaloid screening can be declared as positive if at least two experiments with the reagents form deposits [12].

Tannin, 1 g of Maja fruit powder and extract were separated and purified in 100 mL of hot water. Five millilitres of filtrate was inserted into a test tube and a few drops of 1% ferric chloride ($FeCl_3$) solution were applied. If a green–purple or black color is produced, the test substance involves tannins [12].

Flavonoids, 1 g of Maja fruit powder and extract were filtered in 100 mL of hot water. In the test tube, 5 mL of filtrate was applied, then 1 mL of 5% sodium nitrite solution ($NaNO_3$) and 1 mL of 10% ammonium chloride solution ($AlCl_3$) were added and shaken, and 2 mL of 1 N hydroxide solution was applied. If the test substance contains flavonoids, the color will turn red or orange [12].

Saponin, 1 g of Maja fruit powder and extract were extracted and purified in 100 mL of hot water. In the test tube, 10 mL of filtrate was mounted and shaken vertically for 10 s. The production of steady foam with the height of 1–10 cm suggests saponin. If the test substance includes saponin with the addition of one drop of 2 N HCl, a stable ±1 cm foam will be formed [12].

Male DDY mice weighing 20–30 g, between 2 and 3 months, were collected from the IPB University Faculty of Animal Science, Indonesia. The UPNVJ Health Research Ethics Committee (Letter Number B/2378/1/2020/KEPK) has accepted all experimental protocols for pharmacological trials to support researchers in the preservation of research ethics and attempts to protect human rights and the welfare of animals as research objects. The mice were previously acclimatized for one week, which helped the animals adapt with the laboratory environment. Before checking the antipyretic activity of the ethanol extract of the Maja fruit, an experimental test was done in advance.

This experimental test helped decide what needs to be done in the final test to achieve more reliable and precise data. The preliminary test included the administration of a dosage of DPT and a dosage of Maja fruit extract. In this test, there were four groups consisted of two mice for each group; a wireless thermometer was used to measure the original rectal temperature before the DPT vaccine was triggered. The DPT vaccine, a fever inducer, was administered in the intraperitoneal (IP) and intramuscular (IM) routes with separate volumes of 0.1 and 0.2 cc to each mouse in each group; the temperature was again determined after 30 min of the induction of the DPT vaccine. A fever was identified by the initial increase of body temperature. The second preliminary test was carried out to evaluate the initial dosage of the test substance to be used on the mice for the antipyretic behavior test. The initial dosage of 250 and 500 mg/kg BW of Maja fruit extract (*C. cujete* L.) were used along with the oral administration route in this preliminary research. BW, 300, and 600 mg/kg BW had an antipyretic effect with the highest antipyretic effect obtained at a dosage of 600 mg/kg BW [13] and Maja fruit (*Aegle marmelos* L. Corr.) anti inflammatory test with doses of 100, 200, and 400 mg/kg BW [14]. The initial dose of 250 and 500 mg/kg BW, based on the preliminary

examination, indicated that antipyretic activity occurred in the form of a decrease in the mice's body temperature following a fever with the DPT vaccine induction. The additional dose of 125 mg/kg BW is the lowest dose among the original doses intended to perform antipyretic action.

The mice consisted of randomly chosen healthy five male mice. Fever using DPT vaccine 0.1 cc was induced intraperitoneally for 1 h to achieve a fever.

Evaluation of antipyretic potential

Based on the preliminary test, the amount and route of administering the DPT vaccine used in the antipyretic activity test were 0.1 cc (IP) as a fever induction dose, and 250 and 500 mg/kg BW as the initial dose of Maja fruit extract (*C. cujete* L.). The five research groups in this test are as follows:

No.	Groups	Treatment
1	I	0.5% CMC-Na (Control Groups), orally
2	II	Ibuprofen 400 mg/kg BW (Standard Drug), Orally
3	III	Extract-125 mg/kg BW suspended in 0.5% CMC-Na, Orally
4	IV	Extract-250 mg/kg BW suspended in 0.5% CMC-Na, Orally
5	V	Extract-500 mg/kg BW suspended in 0.5% CMC-Na, Orally

The rectal temperature was measured by a digital thermometer for 240 min for every 60 min after the mice was treated. Both mice with various temperature changes, varying from 1.3 to 1.8 °C, were infected by the DPT vaccine fever.

Data analysis

The data obtained from this research is quantitative. A decline in fever temperature is a quantitative data. The results were then examined statistically. The differences between the positive control group, test group, and the negative control group were compared using statistical analysis using ANOVA if the data were normally distributed and homogeneous, followed by a Tukey's test. The results were considered statistically when the value is ($p<0.05$).

Results

This study's test material was the volleyball-shaped fruit of Maja (*C. cujete* L.) with a diameter of 25 cm; with smooth and shiny green skin that was hard and woody, and soft white and fragrant flesh. The dried Maja fruit was collected and sorted to be separated from impurities that were left in the dried fruit and unwanted plant components. The above phytochemical screening results showed that alkaloids, flavonoids, and saponins were present in the Maja fruit powder and extract (*C. cujete* L.). This shows that the Maja fruit extract (*C. cujete* L.) contains secondary metabolites thought to have pharmacological effects as antipyretics. As shown in Table 1.

The IP route had a higher absorption rate than IM as the route of administration directly penetrated the abdomen and had a large absorption surface. The medication could quickly enter the systemic bloodstream, providing a faster response. The DPT vaccine, which functioned as a pyrogen, induced the release of endogenous pyrogens (IL-1 and TNF-α) released by polymorphonuclear cells by increasing the temperature in mice. These pyrogens released arachidonic acid in the anterior hypothalamus's chemoreceptive region, which activated prostaglandin production and affected the increase of body temperature/fever [15]. Compared with positive and negative control therapies, the mice treated with oral administration of Maja fruit extract (*C. cujete* L.) showed numerous temperature changes as shown in Table 2.

Based on the effects of the average rectal temperature calculation data on mice, the average rectal temperature decrease of mice was determined to assess the potential of treatments I, III, IV, and V to decrease the mice's rectal temperature. The average decrease in temperature indicates the test substance's antipyretic role in reducing the body temperature of mice, obtained by measuring the temperature 60 min after the induction of the DPT vaccine minus the temperature at a certain point after treatment. Table 2 shows the effects of the average decrease in the mice's rectal temperature. The findings of the average decrease in mice's rectal temperature, based on Table 2, demonstrate a difference in the temperature drop in 240 min per 60 min of each group. The drop in temperature

Table 1: Result for phytochemical constituents of Maja fruit.

Constituents	Theory [12]	Test	Result	
			Extract	Powder
Alkaloid	Mayer: Formed white deposits	White deposits	(+)	(+)
	Bouchardat: formed brown deposits	Brown deposits	(+)	(+)
	Dragendorff: formed red brick deposits	Redbrick deposits	(+)	(+)
Flavonoid	Reddish orange	Reddish orange color	(+)	(+)
Saponin	Formed froth ±1 cm stable	Stable ±1 cm froth	(+)	(+)
Tannin	Formed greenish black color	Greenish black color	(+)	(+)

Table 2: Effect of plant extracts on Maja fruit induced pyrexia in mice male.

Groups	Treatment-dose, mg/kg p.o	Temperature at 1 h after induction	Rectal temperature (°C) at different hours after the treatment with the extract					p-Value Tukey's test
			+1 h	+2 h	+3 h	+4 h	Average	
I	Control-Ibuprofen 400	38.54 ± 1.40	37.42 ± 1.12	37.04 ± 0.38	36.54 ± 0.5	36.84 ± 0.30	36.96 ± 0.42*	0.424*
II	Standard-Na CMC 1%	38.76 ± 2.02	38.64 ± 0.12	38.54 ± 0.10	38.32 ± 0.22	38.60 ± 0.28	38.52 ± 0.04	0.038
III	Extract-125	38.26 ± 1.42	37.50 ± 0.76	37.92 ± 0.42	38.08 ± 0.16	38.00 ± 0.08	37.87 ± 0.06	0.062
IV	Extract-250	38.50 ± 1.36	38.02 ± 0.48	37.08 ± 0.94	38.08 ± 1	38.02 ± 0.06	37.80 ± 0.12	0.118
V	Extract-500	39.06 ± 1.86	38.30 ± 0.76	37.78 ± 0.52	37.54 ± 0.24	36.90 ± 0.64	37.63 ± 0.54*	0.538*

*Significantly different when compared to Group II (Standard); III (Extract 125 mg/kg); IV (Extract 250 mg/kg). Statistically significant $p<0.05$ analyzed by *one-way* ANOVA followed by the Tukey test.

after the treatment was not the same for each mouse, even in the same treatment category. The antipyretic activity of the Maja (*C. cujete* L.) fruit extract has increased in quantity depending on the dosage. There was a greater antipyretic effect in Group V compared to Group I, II, III, and IV. However, antipyretic behavior was not substantially different from the statistical findings obtained from the control groups, I groups, and V groups.

Based on the results of the statistical analysis obtained, i.e., Group II was not significantly different from Group III and Group IV ($p \leq 0.05$) and was significantly different from Group I and Group V. Group I was significantly different from Group II, Group III, and Group IV and was not significantly different from Group V ($p \geq 0.05$). It could also be inferred that the results on the decrease of fever temperature in the mice revealed that there was a substantial difference between Group II, Group III, and Group IV on the reduce of fever temperature; on the other hand, Group I and Group V did not have a significant difference in the reduce of fever.

The next step was to observe the medication's average onset of action and the average duration of drug action obtained from the beginning of antipyretic therapy and the amount of time needed by the treatment to give therapeutic activity, as shown in Table 3.

It can be seen in Table 3 that Group III, IV, and V have the same average onset of action at minute 60. Duration of drug action was different; Group V had an overall duration of drug action that varied. Compared to Group III with a duration of drug action of 120 min and treatment Group IV with a duration of drug action of 180 min shorter than treatment Group I and V. This suggests that Group V had the best dose compared to Group III and IV as the median initiation and duration of action of the medicinal product were close to those of Group V.

Table 3: Onset and duration of action extract of Maja fruit induced pyrexia.

Groups	Treatment-dose, mg/kg p.o	Onset, min	Durasi, min
I	Control-Ibuprofen 400	60′	240′
II	Extract-125	60′	120′
III	Extract-250	60′	180′
IV	Extract-500	60′	240′

Discussion

Several active compounds were believed to be responsible for the antipyretic activity caused by the Maja fruit ethanol extract (*C. cujete* L.), namely flavonoids, tannins, and saponins. Based on the antipyretic test findings, Maja fruit extract (*C. cujete* L.) produced antipyretic activity in DPT vaccine-induced mice. It also reported that Alkaloid [10, 16–19] saponin [16, 17, 20], tanin [16–18, 21], flavonoid [16–18, 21], steroid [18, 21], antrakuinon [8, 16], fenol [10, 16], cardenolides [16, 20], fitosterol [17], glikosida resin [18], apigenin, and nephtoquinon [21].

The results of this study were also supported by previous research, which proved that plants containing flavonoids (a compound of the phenolic group) could inhibit cyclooxygenase. The flavonoid mechanism functioned by inhibiting eicosanoids, which could induce cyclooxygenase pathway blockade to disrupt changes in endoperoxide arachidonic acid, contributing to the production of prostaglandin E2 in peripheral tissues that could not directly with the brain, resulting in a reduction in the set point in the hypothalamus [22]. Tannins may have antipyretic properties by inhibiting arachidonic acid in prostaglandin biosynthesis [23].

Antipyretic activity existed due to secondary alkaloid metabolites, which suppressed the COX of the enzyme [24], thereby disrupting the synthesis of prostaglandin. Alkaloids of the matrine form were thought to suppress dopamine release or block dopaminergic receptors to

interact with prostaglandin synthesis [25], revealed that the boldine alkaloid mechanism indicated that alkaloid was an important prostaglandin biosynthesis inhibitor. The study revealed that punarnavine, an alkaloid isolated from B *Diffusa*, decreased the rise in mouse levels of lipopolysaccharide-induced pro inflammatory cytokines such as TNF-, IL-1, and IL-6. Prostaglandin E2, the primary mediator of fever, was enhanced by releasing inflammatory mediators such as IL-1 and IL-6 in the body [26]. The mechanism of lowering the rectal temperature in fever-induced laboratory mice, the quality of flavonoids, hormones, tannins, and saponins was thought to function synergistically [27].

The use of vaccine-induced volumes of 0.1 and 0.2 cc DPT increased mice's body temperature within the 0.7–1.6 °C range [13, 14]. With the mice's temperature, the DPT pyrogen-induced vaccine could facilitate endogenous pyrogen production (IL-1 and TNF alpha) produced by polymorphonuclear cells. These pyrogens functioned to release arachidonic acid in the anterior hypothalamus's chemoreceptive region, inducing prostaglandin synthesis, which influenced the increase in body temperature [15]. Based on previous antipyretic, the amount and route of administration of 0.1 and 0.2 cc DPT vaccines were used. The use of DPT vaccine volumes of 0.1 and 0.2 cc allowed the increase in mice's body temperature within the range of 0.7–1.6 °C [28]. The DPT vaccine volume was 0.1 and 0.2 cc i.p, depending on these test results, with a temperature rise of 1.2–1.7 °C [27].

The drop in rectal temperature in each mouse after the treatment was not the same, even in one treatment category. Variables such as hormones, climate, and gastric condition were responsible for this decrease. Psychological causes, such as tension endured due to repetitive measurements in the rectum of mice, might have also induced it [29]. The dose-antipyretic activity of Maja fruit extract (*C. cujete* L.) improved as the dose increased. Group V showed more substantial antipyretic effects than Group I, II, III, and IV. However, there was no substantial difference in statistical findings between treatment Group I and V that provided antipyretic activity.

The isolation of alkaloid from *B. Diffusa* decreased lipopolysaccharide-induced elevated levels of TNF-1, IL-1, and IL-6 pro inflammatory cytokines in mice [26]. The release of inflammatory mediators such as IL-1 and IL-6 within the body facilitated the prostaglandin E2 synthesis, the primary fever mediator [26]. Alkaloids such as bolidine could minimize rising temperatures by inhibiting the synthesis of prostaglandin E2 [30]. Cyclooxygenase and 5-lipoxygenase inhibition pathways and eicosanoid biosynthesis inhibition along with their ability to block neutrophil degradation [25]. The inhibition of arachidonic acid peroxidation was also seen by flavonoids and their corresponding compounds, resulting in a decrease in prostaglandin levels, thereby minimizing fever and pain [31]. Saponin was involved in the mechanism of the opioid receptor [32] using antagonistic activity [33] by binding the sensory nerve terminals. Flavonoids, steroids, tannins, and saponin were the most important bioactive compounds of plants and were suspected to function synergistically in the process of rectal temperature reduction in fever-induced laboratory mice [34].

These compounds had a wide variety of pharmacological activities, such as anthelmintic [35], antidepressant and anti anxiety [36], anti inflammatory [7, 37], antibacterial [10, 38], antifertility [39], anti arthritis [40], and anti-diarrheal [6, 41]. The 500 mg/kg BW dosage of Maja fruit extract (*C. cujete* L) reduced fever. These results can be added to the study on Maja fruit's pharmacological activity and continued to be formulated as a novel drug, particularly as an antipyretic via the pharmacological mechanisms of the secondary metabolites present in Maja. However, for further research, the active components of the Maja fruit shall remain through the compound isolation stage to improve research results.

Conclusions

The study showed that Maja fruit mice's antipyretic behavior at doses of 125, 250, and 500 mg/kg BW was confirmed as a result in reducing the body temperature of male mice. The 500 mg/kg BW dosage of Maja fruit extract (*C. cujete* L) effectively reduced fever. However, for further research, the active components of the Maja fruit shall remain through the compound isolation stage to improve research results.

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