

## ***Plectranthus scutellarioides* (L.) Reduces the Rectal Temperature of Diphtheria-Pertussis-Tetanus Vaccine-Induced Mice**

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### **Abstract**

The pharmacological activities of *Plectranthus sp* include anti-oxidant, antimicrobial, inhibition on cyclooxygenase (COX) and xanthine oxidase, and cytotoxic activity. The aim of this work was to study the antipyretic activity of *P. scutellarioides* (L.) extract on animal model. Twenty seven Swiss Webster male mice were divided into nine groups and were pyrexia-induced by using 0.1 ml of diphtheria-pertussis-tetanus (DPT) vaccine. Their body temperature was measured every 60 minutes. After 4 hours of DPT vaccine injection, the mice were treated with (I) Arabic gum suspension (PGA) 2%; (II) paracetamol 16.25 mg/kg BW; (III) paracetamol 32.5 mg/kg BW; (IV) paracetamol 65 mg/kg BW; (V) paracetamol 130 mg/kg BW; (VI) *P. scutellarioides* extract 70 mg/kg BW; (VII) *P. scutellarioides* extract 140 mg/kg BW; (VIII) *P. scutellarioides* extract 280 mg/kg BW; (IX) *P. scutellarioides* extract 560 mg/kg BW. Data were analyzed using SPSS 21. Result showed that the body temperature of the mice started to rise at 2.5 hours and reached its peak at 4 hours after vaccine induction. The *P. scutellarioides* extract dose 280 mg/kg of BW indicated an 80.47% decrease ( $\alpha=0.05$ ) compared to paracetamol dose 130 mg/kg of BW which decreases 85.43% of the animals' fever. Interestingly, higher dose of the extract indicated a decrease in the antipyretic effect. We concluded that *P. scutellarioides* might possess antipyretic activity which started as early as 5 hours post vaccine-induced and was maintained for 3 hours. This plant needs further scientific exploration

**Keywords:** Diphtheria-pertussis-tetanus vaccine, anti-pyretic, rectal temperature, Webster mice

### **Introduction**

*Plectranthus scutellarioides* (L.) R.Br. (*Lamiaceae* or *Labiatae* family) (Figure 1), locally known as jawer kotok in West Java, Indonesia, is a native plant in South East

Asia. This plant could also be cultivated in tropical and temperate regions.<sup>1</sup> In Indonesia, this plant has been empirically used to cure various diseases, by consuming the water-

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boiled of the leaves until the symptoms is reduced. The leaves of *P. scutellarioides* are single (undivided), heart-shaped and have brownish-purple color which is predicted due to its secondary metabolites content, e.g. terpenoids, tannins, and flavonoids, terpenoids, saponins and anthraquinones, polyphenols, flavonoids, saponins, and quinones.<sup>2,3,4</sup>

Pharmacology activities of *Plectranthus sp* had been reported, although limited, in which included anti-oxidant<sup>5,6</sup>, antimicrobial<sup>6,7,8</sup>, inhibition on COX and xanthine oxidase<sup>4</sup>, larvicidal activity against 2<sup>nd</sup>–3<sup>rd</sup> instar larvae of *Aedes aegypti*<sup>9</sup>, and cytotoxic activity<sup>6</sup>. However, none of those studies reported on antipyretic activity of *P. scutellarioides*.

It has been known that non-steroidal anti-inflammatory drugs (NSAIDs) generally possess antipyretic activity, which was suggested due to the ability of NSAIDs to inhibit prostaglandin synthesis.<sup>10</sup> DPT is preferred in many developing countries due to its more efficacy. The DTP vaccine may cause mild fever, drowsiness, loss of appetite, and vomiting.<sup>11</sup> Encephalopathy was also reported as a case in the administration of DPT vaccine on a 4-month-old male infant.<sup>12</sup>

Our work studied the antipyretic activity of *P. scutellarioides* (L.) R.Br. leaves extract on DPT vaccine-induced Swiss Webster mice.

## Methods

### *Plant materials*

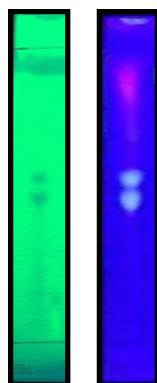
The fresh plant was purchased from The Research Institute for Spices and Medicinal Plants (Balittro) Manoko Lembang, West Java, Indonesia (<http://balittro.litbang.pertanian.go.id/?p=993&lang=en>). The specimen was authenticated by a certified botanist at Laboratory of Identification and Determination, School of Life Sciences and Technology, Bandung Institute of Technology, Indonesia, and confirmed as *P. scutellarioides* (L.) R.Br. (family *Lamiaceae*) (the confirmation letter No. 1011/I1.CO2.2/PL). The leaves were washed with tap water to remove dirt and soil and were shade dried for 4 days. The dried materials were powdered and passed through a 10-mesh sieve. The sieved materials were stored in an air tight container for further use.

### *Vaccine and chemicals*

DPT vaccine was purchased from PT. Biofarma (<http://www.biofarma.co.id/produk/vaksin-dtp-bacterial-vaccines-2/>), ethanol 70 % technical grade



**Figure 1.** *Plectranthus scutellarioides* (L.) R.Br. (*Lamiaceae* or *Labiatae* family)



**Figure 2.** Thin layer chromatograms of ethanolic leaves extract of *P. scutellarioides* (a) observed under UV 254 nm; and (b) sprayed with  $\text{AlCl}_3$  a specific reagent for flavonoids. Three spots were detected ( $\text{Rf}_1 = 0.48$ , pale yellow;  $\text{Rf}_2 = 0.56$ , pale yellow;  $\text{Rf}_3 = 0.90$ , magenta).

Spot 1 and 2 were predicted belongs to flavonoids.

for extraction was purchased from <http://www.bratachem.com/>, paracetamol drug was purchased from Kimia Farma (<http://www.kimiafarma.co.id/en/product/generic.html>). All other chemicals used for the study were obtained commercially.

#### Animals

Swiss Webster male mice (30-40 g in weight, 2-3 months age) were purchased from Laboratory of Pharmacology, Faculty of Medicine, Universitas Padjadjaran, Indonesia for further use of the experiment. The animals were kept in clean and dry plastic cages,

with 12h: 12h light dark cycle at  $26 \pm 2^\circ\text{C}$  temperature. The animals were fed with standard pellet diet and water was given ad libitum for a week to acclimatized the animals for research condition. Animals handling and treatment in this work had been approved by the Research Ethical Committee of the Faculty of Medicine Universitas Padjadjaran, Indonesia (No. 393/UN6.C.1.3.2/KEPK/PN/2016). This study was performed in June to December 2016 (the animal preparation, acclimatization of the animals, and antipyretic activity assay).

**Table 1. The Effect of Ethanolic Leaves Extract of *P. scutellarioides* on Mice Rectal Temperature**

Treatment	Baseline of rectal temperature ( $^\circ\text{C}$ )	Rectal temperature ( $^\circ\text{C}$ ) 4 hours after DPT vaccine-induced pyrexia	Rectal temperature ( $^\circ\text{C}$ ) after DPT vaccine-induced pyrexia		
			5 h	6 h	7 h
PGA 2 %	$37.5 \pm 0.2$	$38.5 \pm 0.2$	$38.6 \pm 0.5$	$38.4 \pm 0.5$	$38.3 \pm 0.1$
Paracetamol 16.25 mg/kg BW	$36.5 \pm 0.2$	$38.4 \pm 0.1$	$37.7 \pm 0.2$	$37.6 \pm 0.3$	$37.2 \pm 0.2$
Paracetamol 32.5 mg/kg BW	$36.6 \pm 0.2$	$38.5 \pm 0.3$	$37.2 \pm 0.6$	$37.5 \pm 0.5$	$37.0 \pm 0.7$
Paracetamol 65 mg/kg BW	$36.6 \pm 0.2$	$38.4 \pm 0.5$	$37.6 \pm 0.5$	$37.6 \pm 0.3$	$36.8 \pm 0.4$
Paracetamol 130 mg/kg BW	$36.4 \pm 0.3$	$38.9 \pm 0.2$	$37.1 \pm 0.7$	$36.5 \pm 0.5$	$36.4 \pm 0.1$
Extract 70 mg/kg BW	$36.6 \pm 0.1$	$39.3 \pm 0.4$	$37.5 \pm 0.2$	$36.9 \pm 0.1$	$37.1 \pm 0.1$
Extract 140 mg/kg BW	$36.7 \pm 0.2$	$39.3 \pm 0.4$	$37.8 \pm 0.2$	$37.1 \pm 0.2$	$37.0 \pm 0.4$
Extract 280 mg/kg BW	$36.7 \pm 0.2$	$38.6 \pm 0.6$	$36.7 \pm 0.2$	$37.0 \pm 0.4$	$37.0 \pm 0.4$

**Table 2. The Temperature Index and Percentage of Antipyretic of *P. scutellarioides* Leaves Extracts Compared to Those of Paracetamol.**

Treatment	Temperature index	Antipyretic (%)
PGA 2 %	10.7	--
Paracetamol 16.25 mg/kg BW	6.1	43.28
Paracetamol 32.5 mg/kg BW	4.5	58.42
Paracetamol 65 mg/kg BW	3.9	63.18
Paracetamol 130 mg/kg BW	1.6	85.43
Extract 70 mg/kg BW	4.3	59.82
Extract 140 mg/kg BW	3.7	65.43
Extract 280 mg/kg BW	2.1	80.47
Extract 560 mg/kg BW	3.7	65.43

#### *Preparation of the extract*

The dried sieved materials (1.2 kg) were submitted to sequential maceration with ethanol (70%) for 3 x 24 hours at room temperature. After each step, the extracts were collected and the solvents were evaporated under reduced pressure using rotary evaporator at 40-600 C, 65 rpm. The yield of viscous ethanol extract was 234.61 g (19.55%).

#### *Phytochemical screening*

Phytochemical screening was performed according to standard method of Tiwari and collaborators using specific reagents to detect secondary metabolites (alkaloids, flavonoids, polyphenols/tannins, terpenoids,

quinones, and saponins) in *P. scutellarioides* (L.) R.Br. leaves extracts and the dried sieved materials.<sup>13</sup>

#### *Antipyretic activity assay*

Antipyretic activity on Swiss Webster mice was studied using DPT vaccine-induced pyrexia. The mice were divided into nine groups @3 mice and fed uniformly till 24 hours. Food was withdrawn before giving drugs. After measuring rectal temperature of the mice by introducing 1.5 cm of digital thermistor thermometer in the rectum, pyrexia was induced by injecting DPT vaccine subcutaneously at a dose of 0.1 ml. After 4 hours of DPT vaccine injection, all mice showed a rise in temperature and were

**Table 3. Data Normality ( $\alpha=0.05$ )**

	Kolmogorov-Smirnova			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Standardized Residual	0.072	144	0.064	0.989	144	0.305

a. Lilliefors Significance Correction

$H_0$ : Data are normally distributed if Sig. value >  $\alpha$

**Table 4. Homogeneity of Variances ( $\alpha=0.05$ )**

Temperature			
Levene Statistic	df1	df2	Sig.
0.95	7	136	0.47

$H_0$ : Data are homogenic if Sig. value >  $\alpha$

treated with (I) Arabic gum suspension (PGA) 2%; (II) paracetamol 0.325 mg/20 g BW; (III) paracetamol 0.65 mg/20 g BW; (IV) paracetamol 1.3 mg/20 g BW; (V) paracetamol 2.6 mg/20 g BW; (VI) *P. scutellarioides* extract 1.4 mg/20 g BW; (VII) *P. scutellarioides* extract 2.8 mg/20 g BW; (VIII) *P. scutellarioides* extract 5.6 mg/20 g BW; (IX) *P. scutellarioides* extract 11.2 mg/20 g BW.

#### Statistical Analysis

Data were analysed using SPSS 21 ( $\alpha=0.05$ ) by employing one-way ANOVA: one-sample Kolmogorov-Smirnov test, Levene test, and Student-Newman-Keuls ( $\alpha=0.05$ ) for data distribution, data homogeneity, and test of between-subjects for antipyretic effect, respectively.

### Results and Discussion

#### Phytochemical screening

Phytochemical screening of both *P. scutellarioides* leaves extracts and the dried sieved materials revealed positive results for flavonoids (Figure 2), saponin, phenolic compounds, and quinons.

#### Antipyretic activity

The effect of ethanolic leaves extract of *P. scutellarioides* on mice rectal temperature is

presented in Table 1, whereas the temperature index and antipyretic ratio of *P. scutellarioides* leaves extracts compared to those of paracetamol is in Table 2.

The DPT vaccine elevated the mice rectal temperature after 4h of administration. Treatment with *P. scutellarioides* extract at dose of 70, 140, 280, 560 mg/kg BW lower the rectal temperature of the animals. We observed that *P. scutellarioides* extract at a dose of 280 mg/kg BW caused significant lowering of mice rectal temperature at 5th hour post vaccine-inducing ( $36.7 \pm 0.2$ ), whereas dose 560 mg/kg BW successfully decreased mice rectal temperature at 7th hour ( $36.8 \pm 0.3$ ). The antipyretic activity started as early as 5th hour post vaccine-inducing and was maintained for 3 hours (Table 1). Furthermore, the *P. scutellarioides* extract dose 280 mg/kg of BW indicated an 80.47% decrease compared to paracetamol dose 130 mg/kg of BW which decreases 85.43% of the animals' fever. Interestingly, the highest dose of the extract (560 mg/kg of BW) indicated a decrease in antipyretic effect (Table 2).

The standard drug (paracetamol dose of 130 mg/kg BW) reached its maximum antipyretic activity in 7th hour ( $36.4 \pm 0.1$ ). This NSAID has been well-known in possessing anti-

Table 5. Test of Between-Subjects Effects ( $\alpha=0.05$ )

Dependent Variable: Temperature					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	35.133a	47	0.748	4.965	0
Intercept	200450.214	1	200450.214	1331403.633	0
Treatment	20.093	7	2.87	19.066	0
Time	6.977	5	1.395	9.269	0
Treatment*time	8.063	35	0.23	1.53	0.054
Error	14.453	96	0.151		
Total	200499.8	144			
Corrected Total	49.586	143			

R Squared = .709 (Adjusted R Squared = .566)

pyretic and analgesic activities with minimal anti-inflammatory activity. It may selectively inhibit specific COX isoform in the CNS to inhibit prostaglandin synthesis to achieve its antipyretic effect.<sup>14</sup>

Statistical data analysis was provided in Table 3-5. Kolmogorov-Smirnov statistical analysis (Table 3) shows that the distribution of the data is normal (sig. value=0.064 which is  $> \alpha$ ; H<sub>0</sub> is accepted). Homogeneity of variances test (Table 4) shows that our data are homogenic (sig. value=0.470 which is  $> \alpha$ ; H<sub>0</sub> is accepted). Test of between-subject effects (Table 5) indicates that there is a significant effect of antipyretic activity between subjects (sig. value=0.000 which is  $< \alpha$ ; H<sub>0</sub> is rejected).

This antipyretic activity of *P. scutellarioides* leaves extracts might be caused by flavonoids, which has been proven contained in this plant (Figure 2). Flavonoids in various plants have been proven to have antipyretic activity as well as inhibit pain perception.<sup>15</sup> Flavonoids, glycosides and tannins found to be present in the aqueous extract of Jwarhar mahakashay confirmed its antipyretic-analgesic effect with very low ulcerogenicity and toxicity.<sup>16</sup> The extract of *Gratiola officinalis* L. which contains abundant level of flavonoids exhibits its antipyretic effect.<sup>17</sup> The ethanol extract of root of *Asparagus racemosus* possessed significant antipyretic effect which may be attributed to the presence of flavonoids and saponins.<sup>18</sup> The methanol leaf extract of *Bombax malabaricum* DC, which showed the presence of steroids, carbohydrates, tannins, triterpenoids, deoxysugar, flavonoids and coumarin glycosides, exhibited antipyretic activity in Baker yeast-induced Wistar rats.<sup>19</sup> Other study concluded that the ethanolic and aqueous leaves extract of *Gnetum africanum* plant, which contained alkaloids, flavonoids, glycosides, saponin, tannins, steroids, terpe-

noid, and carbohydrates, possessed an antipyretic property.<sup>20</sup> Moreover, flavonoids contained in *Dalbergia* species had proven in inhibiting prostaglandin synthetase.<sup>21</sup>

Our study revealed that DPT vaccine markedly elevated the mice rectal temperature after 4h of administration. Moreover, *P. scutellarioides* leaves extracts (dose 280 mg/kg of BW) showed 80.47% antipyretic activity compared negative control (PGA 2%). This result can be beneficial for plant-based drug discovery. In particular, this study will help other researchers to uncover the critical areas of antipyretic drug discovery on DPT-vaccine induced animals that many researchers were not able to explore. Thus, a new theory on antipyretic phytopharmaceutical may be arrived at.

## Conclusion

*P. scutellarioides* might possess antipyretic activity which started as early as 5 hours post vaccine-induced, moreover, this activity was maintained for 3 hours. This plant needs further scientific exploration.

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None.

## Conflict of Interest:

None declared.

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