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# Analgesic and Anti-pyretic activities of *Phyllanthus acidus* and *Averrhoa bilimbi* fruits methanolic extracts in mice

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**ABSTRACT: Background:** There is a greater global interest in non-synthetic analgesic and anti-pyretic derived from herbal sources due to their better tolerance and minimum adverse drug reactions. **Aim:** This study was conducted to determine analgesic and anti-pyretic activities of *Phyllanthus acidus* and *Averrhoa bilimbi* methanolic extracts. **Methodology:** Analgesic activity was evaluated using hot plate and tail immersion methods meanwhile anti-pyretic activity was evaluated by yeast-induced pyrexia technique. **Results:** Analgesic study showed that *P. acidus* and *A. bilimbi* methanolic extracts significantly effective in combating pain. However, anti-pyretic study revealed that only *Phyllanthus acidus* methanolic extract effective in combating fever but not *A. bilimbi.* **Conclusion:** As a conclusion, *P. acidus* methanolic extract exhibited analgesic and anti-pyretic activities. Meanwhile, *A. bilimbi* methanolic extract possesses a significant analgesic activity but not anti-pyretic.

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## INTRODUCTIONS:

Regular use of synthetic analgesic and anti-pyretic, namely aspirin and acetaminophen, increases the risk of hearing loss in men [1]. Acetaminophen also associate with gastro-intestinal ulceration and renal disorders [2]. Hence, there is an urgent need to discover an alternative new and safer analgesic and anti-pyretic agent. In recent year, there is a growing interest in herbal supplements which provide health benefits and are alternative to modern medicine. Medicinal plants have been used as a source of remedies from the beginning of civilization.

The current investigation, represent another effort to screen Malaysia natural tropical heritage for alternative therapeutics against pain and fever.

*Phyllanthus acidus* commonly known as 'cermai' in Malaysia belongs to the family of Phyllanthaceae. Phyllanthus species have been used in traditional medicine for more than 3000 years and useful in the treatment of kidney diseases, urinary bladder disturbances, diabetes, pain management and several sexually transmitted diseases [3]. Previous authors reported that Phyllanthus species is rich with secondary metabolites namely saponins, tannins, alkaloids and steroids [4]. Meanwhile, *Averrhoa bilimbi* or 'belimbing buluh' in Malaysia belongs to the family of Oxalidiaceae. The *Averrhoa bilimbi* tree is long lived and reaches 5 to 10 in height meanwhile the leaves is alternate, imparipinnate and cluster at branch extremities [5]. Traditionally in the rural area of Malaysia, *Averrhoa bilimbi* is used for treating cough, cold, itches, sexually transmitted diseases, management of blood glucose and blood pressure.

To date, there is a lack of scientific data concerning the effects of *Phyllanthus acidus* and *Averrhoa bilimbi* in pain management and on fever. The present study was designed to test the hypothesis that *Phyllanthus acidus* and *Averrhoa bilimbi* methanolic extracts could reduce pain sensitivity and reduce body core temperature of mice with fever.

## MATERIALS AND METHODS:

### Chemicals:

Methanol (analytical grade) was obtained from Kollins Chemicals, USA. Aspirin was obtained from LNK International Inc, USA. Acetaminophen was obtained from HoventaPharma, India.

### Collection of Plant materials and Extraction:

In the current study, *Phyllanthus acidus* and *Averrhoa bilimbi* fruits were collected from their natural habitats in Selangor, Malaysia.

The fruits were air dried and then in hot air oven to remove the moisture. The dried fruits were subjected to size reduction using an electrical blender (Pensonic PB-3203L, Malaysia), then soaked in 95 % methanol (Kollins Chemicals, USA) for 3 days and filtered using Whatman filter paper Grade 1 (Sigma-Aldrich, Germany). The filtrates then were evaporated using the rotary evaporator (Buchi Rotavapor R-210, Switzerland) to remove methanol [6].

## PHARMACOLOGICAL SCREENING:

### Experimental protocol and animals:

Meanwhile, animals were purchased from commercial supplier (Chenur Supplier Sdn Bhd, Malaysia) and maintained under supervision of Research Ethics Committee of the Faculty of Applied Sciences, Universiti Teknologi MARA (UiTM Care: 179/2017). The mice were approximately 6 to 8 weeks olds and ranged from 25 to 30 g in bodyweight (bwt). They were housed in standard polypropylene cages and kept in a well-ventilated area. All animals were fed on standard rodent pellet (Golden Coin Feedmills Sdn Bhd, Malaysia) and water *ad libitum*.

### Analgesic activity:

Analgesic activity study was conducted as previously described [7-8]. Albino mice of either sex were selected and divided into four groups with six animals each. The first group was served as a control and received saline with dose of 2 ml/kg bwt of mice. Second group was treated with Aspirin and served as a positive control at a dose of 10 mg/kg bwt of mice. Meanwhile group 3 and 4 were treated with *Phyllanthus acidus* and *Averrhoa bilimbi* methanolic extracts at a dose of 500 mg/kg bwt of mice, respectively. All groups were treated by oral route and subjected to the following test.

#### *Hot plate method*:

The test was carried out using hot plate apparatus maintained at 55 ˚C [7]. The mouse was placed on the hot plate after the standard drug or extract was given orally at the time interval of 0, 30, 60, 90, 120 and 150 min respectively. The time until the mouse demonstrated pain responses (jumping, licking or withdrawal of the paws) or pain latency was recorded. The reaction time of the mouse to the thermal stimulus, taken to be the interval in between the instant the mouse reached the hot plate to the time the mouse licked its paws, withdrawal of the paws or jump off the hot plate.

#### *Tail immersion method*:

The test was carried out by measuring tail withdrawal time from hot water [8]. About 2 cm of the tail was dipped into hot water maintained at the temperature of 55˚C. The time taken for the mouse to flick the tail known as the pain reaction time or pain latency was recorded for all mice.

### Anti-pyretic activity:

The antipyretic activity study was carried out as

previously described [9]. The anti-pyretic activity of *Phyllanthus acidus* and *Averrhoa bilimbi* fruits methanolic extracts was screened by using brewer's yeast induced pyrexia. Fever was induced in mice by the administration of brewer's yeast suspension at a dose of 0.1 g/kg bwt of mice. Eighteen hours following fever induction, rectal temperature of mice was measured and mice that showed a rise in temperature for at least 0.7 °C were included for the anti-pyretic study. Selected mice with fever were divided into four groups with six animals each. Group I was treated with distilled water at a dose of 2 ml/kg bwt of mice and served as a negative control, Group II was treated with Acetaminophen at a dose of 10 mg/kg bwt of mice and served as a positive control, Group III and IV were as treated with *Phyllanthus acidus* and *Averrhoa bilimbi* at a dose of 500 mg/kg bwt of mice. Rectal temperatures were then measured and recorded hourly for the maximum of 5 h.

**Statistical analysis:**

All data were later presented as mean±standard error of mean (SEM) and were analysed by ANOVA. In all cases, $p<0.05$ was considered statistically significant.

## RESULTS AND DISCUSSIONS:

**Analgesic activity:**

As represent in Table 1 and 2, which demonstrated that the Aspirin at a dose of 10 mg/kg bwt of mice and *Phyllanthus acidus* and *Averrhoa bilimbi* at a dose of 500 mg/kg bwt of mice was significantly increased ($p<0.05$) the pain response time or pain latency to a heat stimulus 30 min after the treatment and persisted until 150 min in comparison to the control group. The pain response time or pain latency was higher ($p<0.05$) in a group treated with 10 mg/kg bwt Aspirin compared to the group treated with 500 mg/kg bwt *Phyllanthus acidus* or *Averrhoa bilimbi* methanolic extracts. Both plants extract also demonstrated a similar capability in increasing pain latency in animal model. The analgesic activity in *Phyllanthus acidus* was comparable ($p>0.05$) to that of *Averrhoa bilimbi*.

The hot plate and tail immersion are preferential methods to screen centrally acting analgesic drugs [10]. In the present study, *Phyllanthus acidus* and *Averrhoa bilimbi* successfully attenuated the thermal-induced pain sensation suggesting the methanolic extract of both plants have an ability to inhibit pain at the level of central (supra-spinally) nervous system. The present results also strongly suggested that, at least in part, the analgesic activity demonstrated by *Phyllanthus acidus* and *Averrhoa bilimbi* fruits methanolic extracts could be due to the inhibition of synthesis, release or response of prostaglandins at the level of central (supra-spinally) and/or peripheral nervous system. Prostaglandins are potent hyperalgesic mediators which modulate multiple sites along the nociceptive pain pathway and enhance both transduction (peripheral sensitizing effect) and transmission (central sensitizing effect) [11]. However, this hypothesis should be confirmed by the experiments on the mechanism of action of both plants extract.

**Table 1. Analgesic activities of 10 mg/kg bwt Aspirin, *P. acidus* and *A. bilimbi* at 500 mg/kg bwt on thermally induced pain (hot plate method) in mice.**

| Groups | Mean pain latency (s) | | | |
|---|---|---|---|---|
| | I | II | III | IV |
| 0 min | $3.3\pm0.1^{a}$ | $3.4\pm0.2^{a}$ | $3.3\pm0.1^{a}$ | $3.3\pm0.2^{a}$ |
| 30 min | $3.2\pm0.1^{a}$ | $6.2\pm0.1^{b}$ | $5.3\pm0.3^{c}$ | $5.4\pm0.4^{c}$ |
| 60 min | $3.1\pm0.4^{a}$ | $6.7\pm0.3^{b}$ | $5.9\pm0.1^{c}$ | $5.7\pm0.3^{c}$ |
| 90 min | $3.2\pm0.5^{a}$ | $6.2\pm0.3^{b}$ | $5.4\pm0.2^{c}$ | $5.2\pm0.2^{c}$ |
| 120 min | $3.1\pm0.1^{a}$ | $6.1\pm0.2^{b}$ | $5.1\pm0.3^{c}$ | $5.1\pm0.3^{c}$ |
| 150 min | $3.2\pm0.3^{a}$ | $6.2\pm0.1^{b}$ | $5.2\pm0.2^{c}$ | $5.1\pm0.4^{c}$ |

**Groups I, II, III and IV are control, aspirin, *P. acidus* and *A. bilimbi*. Values with different superscript letters within the same column shows significant difference at $p<0.05$ (n=6). Values are presented as mean±standard error of mean (n=6).**

**Table 2. Analgesic activities of 10 mg/kg bwt Aspirin, *P. acidus* and *A. bilimbi* at 500 mg/kg bwt on thermally induced pain (tail immersion method) in mice.**

| Groups | Mean pain latency (s) | | | |
|---|---|---|---|---|
| | I | II | III | IV |
| 0 min | $3.1\pm0.2^{a}$ | $3.2\pm0.1^{a}$ | $3.1\pm0.1^{a}$ | $3.1\pm0.3^{a}$ |
| 30 min | $3.1\pm0.2^{a}$ | $6.5\pm0.2^{b}$ | $5.8\pm0.4^{c}$ | $5.7\pm0.2^{c}$ |
| 60 min | $3.3\pm0.3^{a}$ | $6.7\pm0.5^{b}$ | $5.8\pm0.4^{c}$ | $5.8\pm0.4^{c}$ |
| 90 min | $3.4\pm0.3^{a}$ | $6.3\pm0.6^{b}$ | $5.3\pm0.1^{c}$ | $5.2\pm0.3^{c}$ |
| 120 min | $3.5\pm0.1^{a}$ | $6.2\pm0.3^{b}$ | $5.3\pm0.3^{c}$ | $5.2\pm0.5^{c}$ |
| 150 min | $3.4\pm0.2^{a}$ | $6.3\pm0.4^{b}$ | $5.4\pm0.7^{c}$ | $5.3\pm0.1^{c}$ |

**Groups I, II, III and IV are control, aspirin, *P. acidus* and *A. bilimbi*. Values with different superscript letters within the same column shows significant difference at $p<0.05$ (n=6). Values are presented as mean±standard error of mean (n=6).**

**Anti-pyretic activity:**

Brewer's yeast induces pyrexia by increasing the synthesis of prostaglandin. The inhibition of prostaglandin synthesis could be the possible mechanism of antipyretic action as that of Acetaminophen [12]. In addition, the inhibition of prostaglandin can be achieved

by blocking the cyclo-oxygenase enzyme activity. Our data revealed that *Phyllanthus acidus* methanolic extract (500 mg/kg bwt) significantly ($p<0.05$) attenuated rectal temperature of mice with yeast induced pyrexia (Table 3). Thus, it can be postulated that *Phyllanthus acidus* methanolic extract contained pharmacologically active compounds that inhibit the synthesis or secretion of prostaglandin. On the other hand, *Averrhoa bilimbi* methanolic extracts was unable to reverse yeast-induced pyrexia in mice (Table 3).

**Table 3. Analgesic activities of 10 mg/kg bwt Aspirin, *P. acidus* and *A. bilimbi* at 500 mg/kg bwt on yeast-induced pyrexia in mice.**

| Groups | Rectal temperature (˚C) of mice | | | | |
|---|---|---|---|---|---|
| | I | II | III | IV | V |
| 0 h | $36.3\pm0.1^{a}$ | $38.6\pm0.1^{b}$ | $38.2\pm0.3^{b}$ | $38.5\pm0.6^{b}$ | $38.4\pm0.2^{b}$ |
| 1 h | $36.2\pm0.3^{a}$ | $38.8\pm0.2^{b}$ | $37.7\pm0.3^{c}$ | $38.1\pm0.2^{b}$ | $38.5\pm0.3^{b}$ |
| 2 h | $36.3\pm0.4^{a}$ | $38.9\pm0.3^{b}$ | $36.5\pm0.1^{c}$ | $37.7\pm0.1^{d}$ | $39.1\pm0.5^{e}$ |
| 3 h | $35.8\pm0.3^{a}$ | $38.6\pm0.1^{b}$ | $36.1\pm0.4^{a}$ | $37.2\pm0.3^{c}$ | $38.8\pm0.2^{b}$ |
| 4 h | $35.7\pm0.4^{a}$ | $38.5\pm0.2^{b}$ | $35.3\pm0.7^{a}$ | $36.3\pm0.5^{c}$ | $38.4\pm0.1^{b}$ |
| 5 h | $35.9\pm0.2^{a}$ | $38.3\pm0.3^{b}$ | $35.1\pm0.4^{a}$ | $35.8\pm0.1^{a}$ | $38.2\pm0.3^{b}$ |

**Groups I, II, III, IV and V are Healthy + Saline, Fever + Saline, Fever + Acetaminophen, Fever + *P. acidus* and *A. bilimbi*. Values with different superscript letters within the same column shows significant difference at $p<0.05$ (n=6). Values are presented as mean±standard error of mean (n=6).**

## CONCLUSION:

In conclusion, the methanolic extract from fruits of *Phyllanthus acidus* exhibited analgesic and anti-pyretic activities. Meanwhile methanolic extract from fruits of *Averrhoa bilimbi* possesses analgesic activity but not anti-pyretic. Further studies are in progress to elucidate the mechanism of the observed effects.

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