The potential effects of *Melicope ptelefolia* root extract as an anti-nociceptive and anti-inflammatory on animal models


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**KEYWORDS**

Analgesic; Tengek burung; Edema; Pain

**Abstract** The demand of herbal medicine has been increasing for the purpose of reducing the side effects of modern medicine. *Melicope ptelefolia* (*M. ptelefolia*) is a local Malaysian plant claimed to have many benefits for health. This study was performed to evaluate the potential of *M. ptelefolia* root extract as anti-nociceptive and anti-inflammatory agents in rats. The anti-nociceptive activity of *M. ptelefolia* root extracts (50 and 100 mg/kg) was evaluated using acetic acid-induced writhing and tail immersion test, while the anti-inflammatory activity was studied using carrageenan-induced paw edema test. *M. ptelefolia* root extract significantly inhibited the pain stimulant in acetic acid-induced writhing test, however it did not exert any significant change in the tail immersion test. Nevertheless, the mean reaction time in tail immersion test of *M. ptelefolia* root extract increased as the doses of extract increased. Furthermore, *M. ptelefolia* root extract at both doses showed significant anti-inflammatory activity by reducing paw edema volume (*p* < 0.05). In conclusion, methanol root extract of *M. ptelefolia* possesses anti-inflammatory effects and anti-nociceptive effects in rats.

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1. **Introduction**

The use of contemporary medicines as anti-inflammatory and anti-nociceptive agents is becoming controversial due to their multiple side effects such as gastro-intestinal bleeding and ulcers, renal disorders and adverse cardiovascular events.
Therefore, herbal medicines are currently in demand and their popularity as health supplements has been increasing throughout the world. \textsuperscript{5} Melicope ptelefolia (\textit{M. ptelefolia}) is from the Rutaceae family which is commonly found in several Asian countries as well as in Malaysia. \textsuperscript{7} Locally it is known as ‘teng-gek burung’, ‘pauh-pauh’, ‘medang beberas’, ‘cubang tiga’ and ‘tapak itik’. As for Javanese people in Indonesia, they called it ‘sampang’ while the Siamese called it as ‘Uam, Sam Ngam’. \textsuperscript{6}

\textit{M. ptelefolia} is claimed to have many advantages in terms of health care. The leaves of \textit{M. ptelefolia} have gained a great deal of popularity over the years as a traditional fresh vegetable among Malaysians. \textsuperscript{7} \textit{M. ptelefolia} leaf extract was reported to have anti-inflammatory, antipyretic, analgesic and antioxidant properties \textsuperscript{8} as well as antimicrobial activities. \textsuperscript{9} Besides, other parts of this plant have also been used traditionally to treat pathological conditions such as fever, wounds, stomach ache and rheumatism. \textsuperscript{10} To our knowledge, no research has been done on the potential of anti-nociceptive and anti-inflammatory effect of \textit{M. ptelefolia} root extract. Therefore, this study was done to evaluate the anti-nociceptive and anti-inflammatory activity of \textit{M. ptelefolia} methanolic root extract.

2. Materials and methods

2.1. Chemicals

Indomethacin was bought from ChemLab, USA and other chemicals were bought from Sigma Chemical Co, USA.

2.2. Plant materials and extraction

The plant was obtained from Institute of Bioscience, UPM, Serdang, Selangor, Malaysia with reference number UPM/IBS/UB/H14-13. The extraction method with slight modification is based on Sulaiman et al. \textsuperscript{11} Briefly, freshly collected roots of \textit{M. ptelefolia} were air-dried for 48 h and ground into a fine powder. The powder was extracted with methanol with the ratio of 1:5 (sample/solvent) (w/v). The mixture was allowed to stand for 24 h, filtered and evaporated using rotary evaporator (Rotavapor\textsuperscript{\textregistered} R-210, BUCHI, Switzerland) under controlled temperature of 55 °C. The resultant extract was then dried using freeze dryer (Alpha 1–2 LD plus freeze dryer, SciQuip, UK) for 24 h. Dry extract was stored in a refrigerator at 4 °C until further use.

2.3. Animals

Adult female Sprague Dawley rats ($n = 48$), weighing approximately between 180 and 230 g were used in this study. All rats were maintained at room temperature 22 ± 2 °C (12 h light/12 h dark) cycle during the experiment\textsuperscript{12}, housed in polypropylene cages with access to standard pellet and water \textit{ad libitum}. The rats were acclimatized for at least seven days prior to environmental adaption period. All the experiments were conducted in accordance with the ethical guidelines on animal experimentation with resolution number: UPM/FPPSK/PADS/BRU/00221 by Institution Animal Care and Use Committee (IACUC) FMHS, Universiti Putra Malaysia (UPM), Malaysia. All rats were randomly divided into four groups which consist of six animals per group for each antinociceptive and anti-inflammatory effect. Normal saline group: The negative control rats received 5 ml/kg of 0.9% normal saline solution; 50 mg/kg mp group: rats received 50 mg/kg of methanol root extract of \textit{M. ptelefolia}; 100 mg/kg mp group: rats received 100 mg/kg of methanol root extract of \textit{M. ptelefolia}; aspirin group: rats received 100 mg/kg of aspirin as a positive control for antinociceptive effects and indomethacin group: rats received 10 mg/kg of indomethacin as a positive control for anti-inflammatory effects. All substances were given orally before 30 min prior to test.

2.4. Acetic acid-induced writhing test

The peripheral analgesic activity was tested by glacial acetic acid-induced writhing test in rats. \textsuperscript{13} Writhes were induced by intraperitoneal injection of 0.6% (v/v) 10 ml/kg acetic acid. \textsuperscript{5} The number of abdominal constrictions, which consist of constriction of abdominal part together with full stretching of both hind limbs were counted and recorded over a period of 20 min, starting 5 min after acetic acid injection. \textsuperscript{13} The percentage inhibition of writhing was calculated and evaluated statistically.

2.5. Tail immersion test

The central analgesic activity was tested by tail immersion test in rats. \textsuperscript{13} Basal reaction time was taken by immersing the tip (last 2 cm) of the tail in one liter of water at 55 ± 2 °C. \textsuperscript{14} Reaction times were chosen as the time when the animals completely withdraw their tails from the hot water. The tails should only allow to be immersed for not more than 10 s to prevent tissue damage to the tail of the animals. \textsuperscript{5} The initial reading was taken immediately before administration of extracts and 90 min after the administration. The mean difference of reaction time was calculated and evaluated statistically.

2.6. Carrageenan-induced paw edema method

Inflammation was induced by injecting 0.1 ml of 1% (w/v) carrageenan into the sub-plantar area of the right hind paw of rats. The increase in paw edema volume was measured by plethysmometer (Ugo Basile, Canada) every hour for five hours. The edema volume and the percentage inhibition of paw edema was calculated and evaluated statistically.

2.7. Statistical analysis

The obtained data were statistically analyzed using statistical package for social science (SPSS) version 20. After confirming the normality and homogeneity of variance of data, the mean differences were established by a one-way analysis of variance (ANOVA) followed by Fisher’s LSD post hoc multiple comparisons. The results were statistically significant if $p < 0.05$. All data were expressed as mean ± standard error (SEM).
3. Results

3.1. Effect of *M. ptelefolia* extracts on the acetic acid-induced writhing test

Table 1 shows the effect of *M. ptelefolia* root extract on acetic acid-induced writhing test in rats. *M. ptelefolia* extracts significantly reduced the number of writhes compared to normal saline at doses of 50 and 100 mg/kg at *p* < 0.05. The number of writhes was reduced as the doses of extracts increased. However, aspirin showed the highest reduction in the number of writhes and it was statistically significant when compared to the normal saline group and *M. ptelefolia* extracts group (*p* < 0.005). The percentage of writhing inhibition is also shown in Table 1. The aspirin group accounts for the highest percentage of writhing inhibition followed by 100 mg/kg *M. ptelefolia* and 50 mg/kg *M. ptelefolia* with approximately 89.5%, 49.7% and 46.7%, respectively.

<table>
<thead>
<tr>
<th>Groups</th>
<th>No. of writhing/20 min</th>
<th>Percentage of inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal saline</td>
<td>39.8 ± 12.5</td>
<td>0.0</td>
</tr>
<tr>
<td>50 mg/kg mp</td>
<td>21.2 ± 4.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>46.7</td>
</tr>
<tr>
<td>100 mg/kg mp</td>
<td>20.0 ± 4.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>49.7</td>
</tr>
<tr>
<td>Aspirin</td>
<td>4 ± 1.3&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>89.5</td>
</tr>
</tbody>
</table>

<sup>a</sup> Is significantly different compared with normal saline at *p* < 0.05.

<sup>b</sup> Is significantly different compared with 50 mg/kg mp, 100 mg/kg mp at *p* < 0.05.

![Figure 1](image-url) **Figure 1** Effect of *M. ptelefolia* extract on tail immersion time in rats. *a* is significantly different compared with normal saline and 50 mg/kg mp at *p* < 0.05.

3.2. Effect of *M. ptelefolia* extracts on the tail immersion time

The effect of methanolic extract of the root of *M. ptelefolia* on mean reaction time by tail immersion test in rats is presented in Table 1. The aspirin group showed the highest mean reaction time with significant changes when compared to normal saline and *M. ptelefolia* extracts at doses of 50 and 100 mg/kg at *p* < 0.05. *M. ptelefolia* extracts at doses of 50 and 100 mg/kg did not exert any significant changes in the reaction time against the thermal stimulus-induced pain of the tail immersion test. However, according to these results, the reaction time was increased as the doses of extracts increased.

3.3. Effect of *M. ptelefolia* extracts on the carrageenan-induced paw edema

The *M. ptelefolia* extracts elicited anti-inflammatory activity as shown in Fig. 2. *M. ptelefolia* extracts at doses of 50 mg/kg and 100 mg/kg showed significant reduction in edema volume compared with the normal saline group at *p* < 0.05 and *p* < 0.001, respectively. The indomethacin also showed anti-inflammatory activity by significantly reducing edema volume compared with normal saline group (*p* < 0.001). Interestingly, *M. ptelefolia* extract at dose of 100 mg/kg showed the highest effects as anti-inflammatory agent when compared with indomethacin.

![Figure 2](image-url) **Figure 2** Effect of *M. ptelefolia* extract on paw edema volume in rats. *a* is significantly different compared with normal saline group (*p* < 0.05); *b* is significantly different compared with normal saline group (*p* < 0.001).

Table 2 shows the percentage of edema volume inhibition in rats. The percentage of edema volume inhibition increased as the duration increased for *M. ptelefolia* extracts and indomethacin. At the fifth hour, *M. ptelefolia* extract at dose of 100 mg/kg accounts for the highest percentage of inhibition followed by indomethacin and 50 mg/kg *M. ptelefolia* with 85.8%, 10.28% and 63.7%, respectively.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Paw Edema Volume (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Saline</td>
<td>0.3</td>
</tr>
<tr>
<td>50 mg/kg mp</td>
<td>0.25</td>
</tr>
<tr>
<td>100 mg/kg mp</td>
<td>0.2</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>0.15</td>
</tr>
</tbody>
</table>

4. Discussion

Tail immersion test and acetic acid-induced writhing test have been found to be suitable for the evaluation of central and peripheral analgesic activities of the rats, respectively. The tail immersion test measures the complex response of non-inflammatory and acute nociceptive input. It is an
Table 2 Effects of *M. ptelefolia* extract on percentage inhibition of edema volume (%) on carrageenan-induced paw edema in rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Percentage of inhibition (%)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>1 h</td>
</tr>
<tr>
<td>Normal saline</td>
<td>0.0</td>
</tr>
<tr>
<td>50 mg/kg mp</td>
<td>41.2</td>
</tr>
<tr>
<td>100 mg/kg mp</td>
<td>59.2</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>43.3</td>
</tr>
</tbody>
</table>

established fact that any agent that causes a prolongation of the tail immersion latency using this method must be acting centrally.\textsuperscript{17} Centrally acting analgesics not only elevate the threshold for pain, but also alter the physiological response to pain and suppress the patient’s anxiety and apprehension.\textsuperscript{13} In the present study, administration of *M. ptelefolia* extracts showed potential in reducing pain stimulant in rats, thus possesses antinociceptive effects in a dose dependent manner. The potential activity might be due to the presence of secondary bioactive compounds such as flavonoids and saponins. Flavonoids and saponins have been shown to exert analgesic effect on acetic acid-induced writhing test.\textsuperscript{18,19} Therefore, the effects of the *M. ptelefolia* root extract on this test could be due to its ability to inhibit the release of inflammatory mediators.

Pain sensation from injection of acetic acid mainly causes the release of free arachidonic acid from tissue phospholipid.\textsuperscript{20} It is via endogenous mediators, such as prostaglandins, as well as increase in lipoxygenase (LOX) production in the peritoneal that eventually stimulate local peritoneal nociceptors.\textsuperscript{21} The present study demonstrated that *M. ptelefolia* root extract reduced the amount of abdominal writhing. This finding was in line with previous study done by Johnson et al.\textsuperscript{8} who found that *M. ptelefolia* leaves extract showed antinociceptive activities.

Aspirin has been proven to have higher antinociceptive activity than the methanolic extracts of the roots of *M. ptelefolia*. Aspirin is an NSAIDs, non-selective COX inhibitor which inhibit prostaglandins synthesis.\textsuperscript{22} Prostaglandins that are produced by COX-2 are associated with pain and all the signs of inflammation. Therefore, inhibition of prostaglandins reduces the inflammatory signs and pain as well. Aspirin acts both centrally and peripherally to block the transmission of pain impulse in order to relieve pain.\textsuperscript{23} However, methanol extracts of the roots of *M. ptelefolia* also showed the potential in reducing the pain sensation in rats when compared to the normal saline group. The mechanism of *M. ptelefolia* root extract might be linked to the inhibition of LOX and/or COX in peripheral tissues, thereby reducing prostaglandins synthesis and interfering with the mechanism of transduction in primary afferent nociceptors. This was in line with Sulaiman et al.\textsuperscript{11} who also concluded that the possible mechanism of action of *M. ptelefolia* was due to inhibition of LOX and COX.

Administration of *M. ptelefolia* root extract reduced the carrageenan-induced paw edema volume significantly. The percentage of inhibition in paw edema volume was most effective at the dose of 100 mg/kg, thus showing this extract might possess anti-inflammatory effects. According to Johnson et al.,\textsuperscript{8} *M. ptelefolia* leaves extract possess anti-inflammatory effect in a dose dependent manner, where two different doses were used in the research, 50 mg/kg and 100 mg/kg respectively. The two major active compounds such as evoidine and leptonol found in the *M. ptelefolia* extract increased as the dose increased. Therefore, these active compounds might also be present in the root extract of *M. ptelefolia*, thus giving the anti-inflammatory effect in carrageenan-induced paw edema. Furthermore, doses used in this research are within the safety range based on the acute toxicity test which was done by Sulaiman et al.\textsuperscript{11}

Preliminary qualitative phytochemical screening in previous studies revealed the presence of flavonoids, alkaloids, glycosides, saponins and acyphloroglucinol, 2, 4, 6-trihydroxy-3-ger anylacetophenone (HGA) in *M. ptelefolia*\textsuperscript{24–26}. Recent report indicates that antioxidants are able to reduce pain and inflammation induced by chemical and thermal stimuli.\textsuperscript{27} Flavonoids are a class of phenolic compounds widely distributed in plants and reported to have a role in analgesic and anti-inflammation activity mainly by targeting prostaglandins.\textsuperscript{28,29} As for alkaloids, Uche and Apioku\textsuperscript{30} had mentioned that alkaloids are well known for their ability to inhibit pain perception. Therefore, it is believed that these compounds might be responsible for the observed analgesic and anti-inflammatory activities in methanol root extract of *M. ptelefolia*.

5. Conclusion

The methanol root extract of *M. ptelefolia* used in this study showed a potential antinociceptive and anti-inflammatory effects in rats. Identifying the major phytochemicals content of *M. ptelefolia* roots as well as the higher dose range should be considered for further biological studies.

Conflict of interest

The Authors declares that there is no conflict of interest.

Acknowledgements

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