INTRODUCTION

Labisia pumila (LP) is a popular herb from the family Myrsinaceae. It is a small woody and leafy plant that grows and can be found widely in the lowland and hill forests of peninsular Malaysia at an altitude of 300-700 m and other countries like Indochina, Sumatra, Java and Borneo. In Malaysia, this plant is locally known as "Kacip Fatimah" and is quite popularly known among the folk practitioners or womenfolk to relief pain during menstruation. This plant has worn out. Silica gel column chromatography was employed in this study to isolate the entire five bioactive components namely fraction A to fraction E from the DCM extracts of the plant LP.

MATERIALS AND METHODS

Materials

The leaves of LP (Kacip Fatimah) were purchased from University Putra Malaysia, Serdang, Selangor Darul Ehsan. The plant was specifically identified by Mr. Shamsul Khamis, a research officer (plant taxonomy) from the Laboratory of Natural Products (NATPRO), Institute of Bioscience in University Putra Malaysia.

Preparation of extracts

The leaves were air-dried for almost 3 weeks and were then grounded into fine powder using a miller. An extraction with dichloromethane was carried out by successive maceration at room temperatures of 35°C until dryness to maximize the proportion of desired bioactive fractions contained in the dichloromethane. The process of extraction, filtration and concentration was repeated several times until there were maximum yield of crude DCM extracts and the plant has worn out. Silica gel column chromatography was used to isolate the five bioactive components namely fraction A to fraction E from the DCM extracts of the plant LP.

Animals

Healthy young adult Sprague-Dawley rats of both sexes weighing 200 g were purchased from IMR were used in this study. The rats were screened and housed in standard polypropylene cages (three rats per cages), maintained under standard laboratory conditions (i.e. 12:12 hour light and dark cycle; at an ambient temperature of 25 ± 5°C; 50-70 % of relative humidity); the animals were fed with standard rat pellet diet and water was made available at all times.

Drugs

The LP extracts (100mg/kg each) was suspended in vehicle [2% of tragacanth powder, 2 drops of glycerol and Tween®40 in saline solution for rats respectively]. Morphine sulphate (1mg/kg) was dissolved in saline solution and aspirin (100 mg/kg) was dissolved in 0.1 ml of absolute ethanol. These analgesic drugs were used as antinociceptive reference drug. Drugs were freshly prepared on the day of the experiment.

Experimental Protocol

Hot plate test

In this method, a 24 cm diameter glass cylinder was placed on a hot plate with temperature set at 55 ± 0.5 °C. Latency of the rats was determined before and after the treatment. The latency was recorded at the time before and 15, 30, 45, and 60 minutes after intraperitoneal administration of the extracts or drugs. Each rat was placed on the hot plate in order to obtained the animals response to heat-induce antinociceptive pain stimulus. Response was defined as licking, or biting of the paw, or jumping where all four paws leave the plate. Time taken for each response was noted and recorded in seconds. A latency period of 30s was fixed as the cut off time to prevent tissue damage to the rats. Eight group of rats (n=5) received dichloromethane extracts and fractions of LP (100mg/kg), 0.9% saline (1ml/kg) and morphine (10mg/kg) each.
**Table 1: Effect of Hot plate test on rats and its Maximum Possible Effect (MPE) % versus treatment**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Time (min)</th>
<th>MPE (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control (Saline)</td>
<td>-</td>
<td>0</td>
<td>4.60±3.16</td>
</tr>
<tr>
<td>Positive control (Morphine)</td>
<td>100 mg/kg</td>
<td>15</td>
<td>4.32±2.41</td>
</tr>
<tr>
<td>Fraction A</td>
<td>100 mg/kg</td>
<td>30</td>
<td>21.5±1.56</td>
</tr>
<tr>
<td>Fraction B</td>
<td>100 mg/kg</td>
<td>45</td>
<td>5.57±2.29</td>
</tr>
<tr>
<td>Fraction C</td>
<td>100 mg/kg</td>
<td>60</td>
<td>5.72±4.58</td>
</tr>
<tr>
<td>DCM Extract</td>
<td>100 mg/kg</td>
<td>30</td>
<td>4.98±3.47</td>
</tr>
<tr>
<td></td>
<td></td>
<td>45</td>
<td>5.97±1.78</td>
</tr>
</tbody>
</table>

Values given with respect to the mean ± SD, n=5 rats. Asterisks indicated significant difference from control. *p<0.05, **p<0.01, ***p<0.001 (ANOVA followed by paired t-test). MPE were calculated as percentage (%).

**Table 2: Effect of Formalin test on rats and its Percentage of Inhibition**

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg)</th>
<th>Number of licking</th>
<th>Early Phase (0-5min)</th>
<th>% inhibition</th>
<th>Late Phase (15-30)</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control</td>
<td>-</td>
<td>4.4±3.16</td>
<td>0.00</td>
<td>58±5.29</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td>Positive control</td>
<td>100 mg/kg</td>
<td>4.7±2.41</td>
<td>47.73</td>
<td>22±1.00</td>
<td>63.79</td>
<td></td>
</tr>
<tr>
<td>Fraction A</td>
<td>100 mg/kg</td>
<td>21.5±1.56</td>
<td>52.27</td>
<td>20±2.70</td>
<td>65.52</td>
<td></td>
</tr>
<tr>
<td>Fraction B</td>
<td>100 mg/kg</td>
<td>31±4.99</td>
<td>29.55</td>
<td>20±3.96</td>
<td>65.52</td>
<td></td>
</tr>
<tr>
<td>Fraction C</td>
<td>100 mg/kg</td>
<td>40±2.31</td>
<td>9.09</td>
<td>60±4.82</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td>Fraction D</td>
<td>100 mg/kg</td>
<td>31±2.55</td>
<td>29.55</td>
<td>66±6.80</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td>Fraction E</td>
<td>100 mg/kg</td>
<td>37±2.74</td>
<td>15.91</td>
<td>66±2.88</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td>DCM Extract</td>
<td>100 mg/kg</td>
<td>37±4.78</td>
<td>15.91</td>
<td>24±3.51</td>
<td>56.82</td>
<td></td>
</tr>
</tbody>
</table>

One hour after test drug administration (p.o), 2.5% formalin was subcutaneously injected to a hindpaw in volume of 50µL. Each data represent the mean number of licking time ± SD from 5 rats in the early phase (0-5 min) and late phase (15-30 min) after formalin injection. *p<0.05, **p<0.01, ***p<0.001 compared with the control group.

**Formalin test**

The method used was similar to what has been described by Gonzales, 2007. To induce nociception, rat were injected with 50 µl of 2.5% formalin in 0.9 % of saline solution into the subplantar surface of the left hind paw, 1 hour after the administration of 0.9 % saline, 100mg/kg of Aspirin and 100mg/kg of each extracts. Rats were observed for 30 minutes and the time spent licking the paw was recorded in two phases. The data were express as total licking time in the early phase (0-5 min) and the late phase (15-30min) after formalin injection.

**Statistical Analysis**

The antinociceptive data were expressed as mean values ± standard deviation. The results were analyzed by one-way analysis of variance (ANOVA) and paired t-test using statistical package for social science (SPSS) computer program version 18. Values were considered statistically significant when p < 0.05, highly significant difference when p<0.01 and very highly significant difference when p<0.001.

**Phytochemical Analysis**

Simple chemical test were performed to identify the possible bioactive fractions present in the effective fraction of the LP. The bioactive fractions that were tested for are flavonoids, alkaloids, tannins, steroids and saponins

**RESULTS AND DISCUSSION**

It is believed that current analgesia-inducing drugs such as opioids and non-steroidal anti-inflammatory drugs are not useful in all cases because of their side effects and low potency. As a result, searches for other alternatives medicinal plant became necessary and beneficial. In this work we have studied the effect of all the partially purified fraction A-E and DCM crude extract from leaves of *LP* on two models of antinociceptive (hot plate and formalin). The hot-plate test is one of the widest used experimental methods to evaluate nociception in rats. It is based on the use of short duration nociceptive thermal stimulus. The nociceptive response in the hot plate model seems to results from direct activation of nociceptor such as opioid receptor and is inhibited by drugs which act mainly at central sites. The hot plate test was selected to investigate central antinociceptive activity because it had several advantages, particularly the sensitivity to strong antinociceptives and limited tissue damage. The second phase (tonic pain) appears immediately following formalin injection lasting only few minutes and is believed to be driven by primary afferent nociceptor activity. The second phase (tonic pain) is observed 15 min after formalin injection, lasts at least for 60 min, and is thought to arise from nociceptive spinal neuron hyperactivity. In this second phase various mediators operate in a sequence to produce an inflammatory response and has been...
correlated with the elevated production of prostaglandin (PG), induction of cyclo-oxygenase (COX) and release of nitric oxide (NO). Formalin (LP) extract given orally at 100 mg/kg inhibited both the early and late phases in the formalin test. The results showed that the partially purified fraction A-E and DCM crude extract from the plant LP has a potent antinociceptive (p<0.001) effect against the chemical stimuli provoked by the formalin subplantar injection when compared with the negative control. The partially purified fraction A shows a better percentage of inhibition in the early and late phase, 52.27 % and 65.52 % respectively and the percentage of inhibition in both phases are higher than that of aspirin. Thus, fraction A (p<0.001) has higher potency of pain inhibition when compared to aspirin and can be assumed to be mimicking the action of aspirin in the inhibition process. These results suggest that the partially purified fraction A-E and DCM crude extract exhibited centrally mediated effect and peripheral mediated effect. Preliminary phytochemical screening of the Labisia pumila extracts indicated the presence of flavonoids, tannins, saponin, alkaloids and steroids. Most of these of these phytochemical have been reported to have antinociceptive effect.

CONCLUSION
The partially purified fraction A-E and DCM crude extract of the leaves of Labisia pumila showed a significant antinociceptive effect, as observed through hot plate (thermal stimulus) and formalin test (chemical stimulus). From these result, the partially purified fraction A-E and DCM crude extract has a centrally mediated effect and peripherally mediated effect. The antinociceptive effect may also be due to the presence of the phytochemical such as flavonoids, steroids, saponin, alkaloids and tannins which have been reported to possess antinociceptive effect.

ACKNOWLEDGEMENT
The authors are grateful to the School of Applied Science of UCSI University for providing facilities to carry out the present study. The authors are also grateful to the Department of Pharmacology, School of Applied Science, UCSI University for providing facilities to carry out the present study.

REFERENCES