Behavioral effects of *Kielmeyera coriacea* extract in rats

*Kielmeyera coriacea* Mart. is a tree that belongs to the family *Clusiaceae*, popularly known in Brazil as “pau santo”. *Hypericum perforatum*, a plant from the same family, is considered an effective alternative treatment for moderate depression.\(^{[1]}\) Earlier we have reported that chronic administration of the ethanolic stem extract of *Kielmeyera coriacea* in rats reduced immobility time in the forced-swimming test (FST) without altering locomotor activity in the open-field test (OFT), and this effect was mediated through serotonergic mechanism.\(^{[2]}\) The ethanolic extract of *Kielmeyera coriacea* stem was purified by vacuum chromatography on silica gel eluted with hexane and dichloromethane (DcM) to yield a semi-pure DcM fraction with high degree of purity, and the effect of chronic administration of DcM on FST or OFT was investigated in the present study.

The plant collected from Mogi-Guaçu (SP, Brazil) in July 1999 was authenticated by an expert. A voucher specimen (SP298-463) was deposited with the Herbarium of the State Botanical Institute, SP, Brazil. The extract and the semi-pure DcM fraction (patent application # 001342 with the National Patents Institute (INPI) on October 9, 2002.) were analysed by HPLC-UV and spectroscopy (NMR), to identify probable active substances such as: (1) kielcorin; (2) swertinin; (3) 1,3,2-trihydroxy-2-(3-methylbut-2-enyl)-xanthone; (4) 1,3,5-trihydroxy-2-(3-methylbut-2-enyl)-xanthone and (5) and (6) mixture of complex triterpenes. [Figure 1](#)

Male Wistar rats (55 days old, 240-270 g) were housed in groups of four per cage and maintained on a 12:12 h light:dark cycle (lights on at 7:00 h) in controlled temperature (22±1ºC), with food and water freely available, and were acclimated for 3 days before the start of the treatment. All experiments were carried out between 8:00 or 12:00 h. The animals were treated with different doses (4.0, 5.0 and 6.0 mg/kg) of the semi-pure DcM fraction, nortriptyline (both dissolved in saline containing 0.2% Tween 80 used as a vehicle), or vehicle (control group). All treatments were given i.g. for 45 days. The drug doses and treatment period were based on the references drawn from pilot studies.

In the FST\(^{[3]}\) the animals were placed individually in an open cylindrical container (diameter 30 cm, height 60 cm, containing 45–50 cm of water at 25±1ºC) for 15 min (pre-test), followed 24 h later by a 5-min test (on day 44). After 30 s for acclimatization, the test session was videotaped for subsequent measurement of the time of immobility by a trained observer. After 24 h, each animal was placed in the OFT (on day 45). During a 5-min period, the number of squares visited was recorded using Royce’s validation criteria.\(^{[10]}\) The experimental procedures adopted were approved by the UEM Ethics Committee (# 084-02/COBEA), and follow the norms recommended as international guiding principles for Biomedical Research Involving Animals (CIMS), Geneva, 1985.

The data were analysed using one-way ANOVA followed by Dunnett’s test. A value of P≤0.05 was considered statistically significant. The results are expressed as mean±SEM.

The semi-pure DcM fraction did not induce a consistent and significant decrease in immobility time in the FST when administered by i.g. route in sub-acute (24, 12 and 1 h before the test) or chronic periods (15 or 30 days) at doses of 2.0, 4.0, 6.0 or 8.0 mg/kg (results not shown). After 45 days of i.g. treatment, at dose of 6.0 mg/kg, but not at dose of 8.0 mg/kg, DcM fraction produced a significant antiimmobility effect in the FST, without altering the crossings number in the OFT. For this reason, the doses of 4.0, 5.0 and 6.0 mg/kg were selected for our study. We observed that the lower effective dose of DcM fraction to produce antiimmobility effect in the FST (F\(^{[1,33]}\) = 5.074, *P* = 0.0027) after 45 days treatment was 5.0 mg/kg. The crossings number in the OFT was not altered by different doses used (F\(^{[1,33]}\) = 2.833, *P* = 0.0812). [Table 1] The inactivity of DcM in dose of 8.0 mg/kg in FST could probably be due to a mixture of unspecific activity or due to active substances present in this fraction but detected in the higher dose used.

The effect of the semi-pure fraction in reducing immobility time in the FST at doses of 5.0 and 6.0 mg/kg is comparable to the *Kielmeyera coriacea* ethanolic extract at a dose of 60.0 mg/kg.\(^{[2]}\) These results suggest that the semi-pure DcM fraction possesses an antidepressant-like drug profile, and contains the component or components responsible for antiimmobility effect detected with the *Kielmeyera coriacea* ethanolic extract.

In accordance with this suggestion, the analysis of the extract and the semi-pure DcM fraction by HPLC-UV\(^{[3]}\) revealed the presence of several xanthones (1–4). [Figure 1] These substances such as: (1) kielcorin; (2) swertinin; (3) 1,3,2-trihydroxy-2-(3-methylbut-2-enyl)-xanthone; (4) 1,3,5-trihydroxy-2-(3-methylbut-2-enyl)-xanthone and (5) and (6) mixture of complex triterpenes.

### Table 1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Forced swimming test Immobility time (s)</th>
<th>Open field test Crossings number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (2% Tween 80 in saline)</td>
<td>4.0</td>
<td>187.0±17.2</td>
<td>62.9±14.2</td>
</tr>
<tr>
<td>DcM</td>
<td>4.0</td>
<td>199±12.3</td>
<td>70.8±10.0</td>
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<tr>
<td></td>
<td>5.0</td>
<td>122.8±18.3**</td>
<td>100.0±9.8</td>
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<tr>
<td></td>
<td>6.0</td>
<td>126.9±18.7*</td>
<td>96.8±15.1</td>
</tr>
<tr>
<td>Nortriptyline</td>
<td>15.0</td>
<td>123.7±16.6*</td>
<td>58.1±12.3</td>
</tr>
</tbody>
</table>

Values are mean±SEM. n=6-8 in each group. All changes were administered i.g.; for 45 days, ANOVA followed by Dunnett’s test showed *P*<0.05, **P<0.01 when compared to control.
Figure 1. High-performance liquid chromatography chromatogram of standardised ethanolic extract stems (upper panel) and of DcM fraction stems (lower panel) of *Kielmeyera coriacea*; xanthones (1) kielcorin; (2) swertinin; (3) 1,3,7-trihydroxy-2-(3-methylbut-2-enyl)-xanthone; (4) 1,3,5-trihydroxy-2-(3-methylbut-2-enyl)-xanthone and mixture of complex triterpenes (5) and (6). Conditions: Metasil ODS column (5 µm, 150 x 4.6 mm); step gradient of acetonitrile-water (containing 0.05% TFA): 10% → 100% CH₃CN over 40 min; flow rate 1 ml/min; detection UV 254 nm.

In conclusion, the present study shows that the semi-pure DcM fraction is active orally and suggests an antidepressant-like drug profile. The xanthones may be responsible for the antidepressant-like action detected in the semi-pure DcM fraction in this study. Further studies are in progress to identify the mechanisms underlying the pharmacological activity observed.

Acknowledgments

The authors are thankful to Maria Claudia Young of the Instituto Botânico de São Paulo for analysis and identification of *Kielmeyera coriacea*. This work is supported by CNPq.

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References


