

# Activity of hydroethanolic extract from *Kielmeyera coriacea* stems on central nervous system in rats

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**ABSTRACT.** The aim of the present study was to investigate psychotropic properties of the crude lyophilized hydroethanolic extract (HE) from *Kielmeyera coriacea* stems after acute or chronic administration by oral route (gavage) in rats. Anxiolytic, antidepressive and psychomotor stimulant effects were evaluated using Plus-Maze (PMT), Forced Swimming (FST) and Open Field (OFT) test experimental models, respectively. Evaluation of the extract stems (60.0 mg/kg) revealed decreased immobility time in the forced swimming test (FST), without increasing locomotor activity in OFT, after chronic administration. This effect of the extract was compared with imipramine (20.0 mg/kg). Acute administration of 120.0 mg/kg of extract stems increased the open arm entries percentage in the PMT. According to the results, *Kielmeyera coriacea* stems extract is effective as anxiolytic as well as an anti-depressive-like drugs.

**Key words:** *Kielmeyera coriacea*, depression, open-field, forced swimming test, plus-maze test.

**RESUMO:** Atividade farmacológica do extrato hidroetanólico liofilizado de caule de *Kielmeyera coriacea* em ratos. O objetivo deste estudo foi investigar as propriedades psicotrópicas do extrato hidroetanólico liofilizado obtido de caule de *Kielmeyera coriacea* após tratamento agudo ou crônico por via oral (gavagem) em ratos. As atividades ansiolítica, antidepressiva e estimulante de sistema nervoso central, foram avaliadas através da utilização dos testes do labirinto em cruz elevado (LCE), nado forçado (NF) e campo-aberto (TCA), respectivamente. A administração crônica de EH de caule de *Kielmeyera coriacea* (60.0 mg/kg) produziu redução no tempo de imobilidade no teste do NF, comparável a imipramina (20.0 mg/kg), sem aumentar a atividade locomotora no TCA. A dose de 120.0 mg/kg do mesmo extrato aumentou significativamente a percentagem de entradas nos braços abertos no teste do LCE após administração aguda. Estes resultados mostram que EH de caule de *Kielmeyera coriacea* apresenta perfil de composto ansiolítico e antidepressivo.

**Palavras-chave:** *Kielmeyera coriacea*, depressão, campo-aberto, teste do nado forçado, labirinto em cruz elevado.

## Introduction

*Kielmeyera coriacea* Mart. is a tree of the Clusiaceae (Guttiferae) family, popularly known in Brazil as *pau santo*. A decoction of the stems is used to treat various tropical diseases including schistosomiasis, leishmaniasis, malaria and fungal and bacterial infections, among others (Alves *et al.*, 2000). Previous analysis of the same hydroethanolic extract (HE) of the stems of *Kielmeyera coriacea* by HPLC with photodiode array detection (LC-UV) showed the presence of xanthenes, triterpenes and biphenyl derivatives, and exhibit antifungal activity against *Cladosporium cucumerinum* and *Candida*

*albicans*. Combined with HPLC using a thermospray mass spectrometry interface (TSP/LC-MS), it was possible to identify the xanthenes - 2, hydroxy - 1, methoxyxanthone - 3, hydroxy - 2 and 4, dimethoxyxanthone - 4, hydroxy - 2 and 3, dimethoxyxanthone, swertinin - 6, hydroxy-1, 3, 5, trimethoxyxanthone - 1, 3, 7, trihydroxy - 2, (3 - methylbut - 2, enyl) - xanthone - 1, 3, 5, trihydroxy - 2, (3 - methylbut - 2, enyl), xanthone - 1, 3, 7, trihydroxy - 2, (3 - hydroxy - 3 - methylbutyl) - xanthone and kielcorin (Bennet and Lee, 1989; Cortez *et al.*, 1998).

*Hypericum perforatum*, a plant from the same family (Guttiferae) is considered an effective alternative in the treatment of mild to moderate

depression (Muller *et al.*, 1997; Josey and Tackett, 1999; Muruganandam *et al.*, 2000).

The present work aimed to investigate whether the acute or chronic administration of the HE from *Kielmeyera coriacea* stems induces anxiolytic, antidepressant, or motor stimulant effects in rats. The experimental models chosen were the elevated plus maze (PMT), forced swimming (FST) and open field tests (OFT), respectively.

## Material and methods

### Plant material and extract

*Kielmeyera coriacea* was collected near Mogi-Guaçu (São Paulo, Brazil) in July, 1999. A voucher specimen (#SP298463) was deposited with the Herbarium of the São Paulo State Botanical Institute, São Paulo, Brazil. Species identification was performed by Dr. Maria Claudia Young of the same institution.

The dried and crushed stems (1.0 kg) of *Kielmeyera coriacea* were exhaustively extracted with 38 liters of ethanol/water (9:1) at room temperature for 7 days, yielding 167.3 g of extract after evaporation of the solvents and lyophilization. The resulting compound was registered by State University of Maringá under patent application # 001342 with the National Patents Institute (INPI) on October 9, 2002.

### Animals

The experiment utilized male Wistar rats (50-55 days old, 220-250 g) provided by the Animal Housing, Facility of State University of Maringá, housed 5 per cage, in constant room temperature (22-23°C), under a 12-h light-dark cycle, with free access to food and water.

### Treatment

The animals were treated once daily for one (acute treatment) or 40-45 (chronic treatment) day(s), with different doses of the HE from *Kielmeyera coriacea* stems administered by gavage in rats or the reference drugs, imipramine (20.0 mg/kg) or diazepam (2.0 mg/kg) by intraperitoneal (i.p.) route. HE from *Kielmeyera coriacea* stems and Imipramine HCl (20.0 mg/kg, Crystalia Chemical, i.p.) were dissolved in a vehicle that consisted of 0.9% NaCl (saline) containing 0.2% Tween 80. Diazepam was used directly from the ampoule in solution *União Química*. The procedures adopted were approved by the UEM Ethical Committee (# 084-02/COBEA) and followed according to the norms recommended by international guiding principles for Biomedical Research Involving Animals (CIMS), Geneva, 1985.

### Forced swimming test (FST)

The FST was similar to that described by Porsolt *et al.* (1977; 1978). Duration of immobility per a 5-min period was measured. An animal was considered immobile when it ceased struggling and swimming, remaining floating in the water, making only necessary movements to keep its head above water.

### Open-field test (OFT)

After 24 hours, each animal was placed in the OFT. During a 5min period, the number of squares visited (four feet placed in the same square) was registered using Royce's validation criteria (1977).

### Elevated plus-maze test (PMT)

The procedure for the PMT was performed using the original method described by Pellow *et al.* (1986). The number of entries and time spent in the open and closed arms of the maze were recorded for 5min. For statistical analyses, the percentage of open arm entries (100 x open/total entries) and time spent in open arms (100 x open/open + closed) and number of closed arm entries were calculated for each rat.

### Chronic effect of HE from *Kielmeyera coriacea* stems on body weight increase and mortality

The same experimental groups used in the above experiments were assessed for body weight increase and mortality over the course of up to chronic treatment. After that period, the rats were killed and the organs were removed for observation and weighing. Body weight was measure every week.

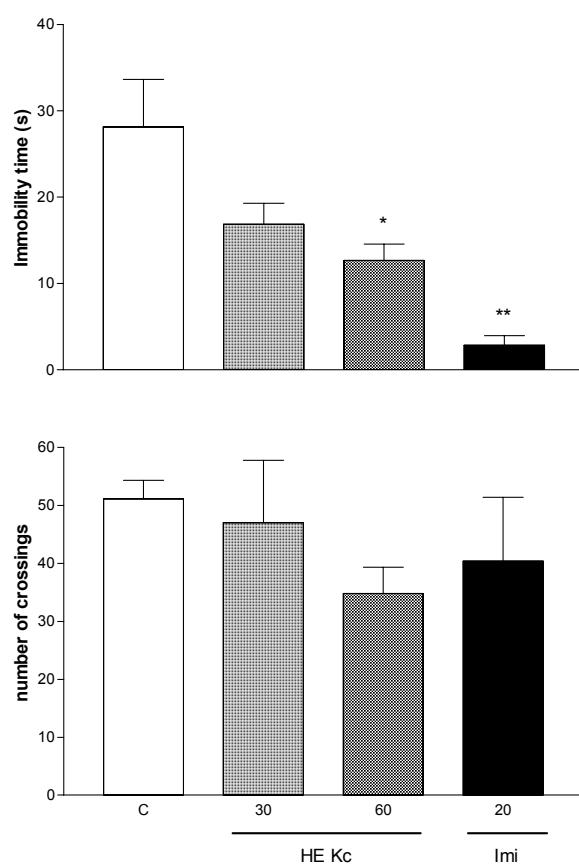
### Data Analysis

Results are expressed according to the mean  $\pm$  SEM for each group. A one-way analysis of variance (Anova) followed by Dunnett's test for multiple comparisons or the unpaired t-test was used. Effects or differences were considered significant at  $p=0.05$ .

## Results

Acute administration of different doses of HE from *Kielmeyera coriacea* stems by gavage in rats did not affect any of the parameters analyzed in the FST or OFT when compared to control (saline + 2% Tween 80) treated group (data not shown).

Figure 1 shows the results observed after chronic administration (40-45 days) of HE from *Kielmeyera coriacea* stems (30.0 or 60.0 mg/kg) or imipramine (20.0 mg/kg) compared to control (Saline + 2% Tween 80) in rats submitted to the FST. The reference drug imipramine and 60.0 mg/kg of HE from *Kielmeyera coriacea* stems reduced immobility time significantly ( $F(3,24)=9.314$ ,  $p<0.0003$ ) compared to the control group in FST without an increase in the number of crossings in the OFT ( $F(3,24)=0.7623$ ,  $p=0.5261$ ).



**Figure 1.** Mean  $\pm$  SEM immobility time in FST (upper panel) and number of crossings in OFT (lower panel) after chronic treatment (45 days) of HE from *Kielmeyera coriacea* (HE Kc, 30.0 or 60.0 mg/kg) or imipramine (Imi, 20.0 mg/kg) compared to control (Saline + 2% Tween 80). All treatments were administered chronically by gavage. ANOVA and Dunnett's tests revealed significant differences \* $p < 0.05$  and \*\* $p < 0.01$ , ( $n = 6-8$ ).

Table 1 shows effects of the chronic treatment with 60.0 and 120.0 mg/kg doses of HE extracts from *Kielmeyera coriacea* stems or diazepam (2.0 mg/kg) compared to control (saline + 2% Tween 80) on spatial/temporal and ethological parameters behavior in PMT in rats. The percentage of entries into open arms ( $F(3,29) = 16.7$ ,  $p < 0.0001$ ) was increase by 120.0 mg/kg dose of HE from *Kielmeyera coriacea* stems ( $p < 0.001$ ). The time spent ( $F(3,29) = 20.94$ ,  $p < 0.0001$ ) in open arms and the closed arm entries ( $F(3,39) = 1.76$ ,  $p = 0.1751$ ) was not altered significantly by different doses of the extract. The

ethological parameters analyzed, risk-assessment ( $F(3,29) = 4.943$ ,  $p = 0.068$ ) and head-dipping ( $F(3,29) = 17.73$ ,  $p < 0.0001$ ) showed no significant effect with any dose of HE extracts from *Kielmeyera coriacea* stems. The reference drug, diazepam (2.0 mg/kg), increased significantly the spatio/temporal parameters, percentage of entries into ( $p < 0.01$ ) and time spent ( $p < 0.001$ ) in open arms, without alter the closed arm entries. The ethological parameters, risk-assessment ( $p < 0.01$ ) and head-dipping ( $p < 0.01$ ) were significantly altered compared to control, revealing its anxiolytic effect.

## Discussion

The present study showed that the HE from *Kielmeyera coriacea* stems was active orally and exhibits a significant, antidepressant-like effect in the FST model of depression in rats, only after chronic administration.

The therapeutic effect of antidepressant drugs after long-term use is known by popular medicine. Tricyclic or selective serotonin reuptake inhibitor antidepressant drugs shares the property of blocking serotonin (5HT), noradrenaline (NA) and/or dopamine (DA) transporters (Hyttel, 1994). This process takes place within a few hours, although the onset of the therapeutic effect ranges from 1 to 2 weeks of treatment, which is necessary for the development of specific adaptive changes (Blier and de Montigny, 1994; Harkin *et al.*, 1999). A reduction in immobility time in the FST has been reported for the main classes of antidepressant drugs frequently after long-term administration (Porsolt *et al.*, 1977, 1978; Borsine, 1995).

The results produced by HE from (2.0 mg/kg) *Kielmeyera coriacea* stems suggest that the increase in motor activity observed in the OFT is not related to the anti-immobility effect of the extract observed in FST, and confirms the specificity of their antidepressant-like effect. The efficacy of the 60.0 mg/kg dose of extract in reducing immobility time in the FST is comparable to the tricyclic antidepressive, imipramine, used as a reference drug. Imipramine significantly reduced immobility time without increasing locomotion.

**Table 1.** Mean ( $\pm$  SEM) effects of HE from *Kielmeyera coriacea* (Kc) stems (60.0 or 120.0 mg/kg), control (saline + 2% Tween 80) or diazepam (Dz, 2.0 mg/kg) on rats behavior in elevated plus-maze test after chronic treatment (45 days) by gavage.

Treatment	Dose (mg/kg)	% Open arm entries	% Time arm spent	Closed arm entries	Risk Assessment	Head Dipping
Control	-	26.6 $\pm$ 6.7	10.9 $\pm$ 6.3	8.3 $\pm$ 2.4	10.1 $\pm$ 1.6	8.5 $\pm$ 2.9
HE Kc	60	33.3 $\pm$ 3.7	11.4 $\pm$ 3.6	7.9 $\pm$ 1.8	9.0 $\pm$ 1.5	8.4 $\pm$ 2.0
	120	52.3 $\pm$ 3.1*	16.7 $\pm$ 4.1	9.8 $\pm$ 1.8	7.0 $\pm$ 1.1	12.4 $\pm$ 1.3
Dz	2.0	69.4 $\pm$ 4.9**	67.0 $\pm$ 8.2**	12.9 $\pm$ 1.3	3.9 $\pm$ 0.8**	28.7 $\pm$ 2.6**

Anova followed by Dunnett's test \* $p < 0.05$ , \*\* $p < 0.01$ , ( $n = 7-11$ ).

Anxiolytic effect of HE from *Kielmeyera coriacea* stems was observed by the percentage of entries into open arms parameter analyzed in PMT. The reference drug, diazepam, produced significant alteration in all traditional and ethological parameters. Nevertheless, the ethological analysis did not prove superior to traditional indexes (Cruz *et al.*, 1994).

The behavior on the PMT is supposed to be driven by the conflict between fear (open space and novelty) and exploration (novelty). As a consequence, rats at elevated plus-maze tend to avoid the open arms and stay longer in enclosed arms. The percentage of open to total arm entries has been used as indexes of anxiety. Anxiolytic drugs increase the percentage of entries number onto and/or time spent in open arms, whereas anxiogenic drugs do the opposite (Pellow *et al.*, 1986). Because changes in motor activity may also influence exploratory behavior in the PMT, the number of closed arm entries, that loads highly and exclusively on the factor associated with exploration, has been used to reflect this factor (Cruz *et al.*, 1994).

Different doses of HE from *Kielmeyera coriacea* stems did not alter the number in closed arm entries in the PMT and did not increase the number of crossings in OFT, showing that the extract has no stimulant effect in CNS (Wash and Cummins, 1976).

In conclusion, our results suggest that the extract from *Kielmeyera coriacea* stems exhibits anxiolytic and antidepressant-like effects in the PMT and in FST in rats. Further studies are in progress to identify the active constituent and the mechanisms underlying the pharmacological activities observed.

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