Evaluation of analgesic activity of *Evodia lunu-ankenda* (Gaertn) Merr. bark

K.G. Lalitha1,*, T.Venkatachalam2, G.Rathinavel3, V. Kishor Kumar2 and P. Kalaiselvi2

1Department of Pharmaceutical Chemistry, Ultra College of Pharmacy, Madurai, Tamil Nadu, India
2Department of Pharmaceutical Chemistry, JKKMMRF College of Pharmacy, B. Komarapalayam, Tamil Nadu, India
3Prist University, Thanjavur, Tamilnadu, India

**ABSTRACT**

Ethylacetate extract of the bark of *evodia lunu ankenda* were evaluated for analgesic activity using Eddys Hot plate method and heat conduction method on swiss albino mice. The findings support the use of this drug, *evodia.lunuankenda* in the treatment of pain both the extracts were not toxic up to 3000mg/kg body weight.

**Key words:** Evodia lunukanda, analgesic activity, eddys hot plate method, heat conduction method.

**INTRODUCTION**

*Evodia lunu-ankenda* (Gaertn) Merr. bark (Rutaceae) available throughout central and south India, in most dry stony hills and black cotton soil. Along the margin of evergreen forests upto 1400 m. Trees ca.10m tall. Bark grey, corky when mature; blaze Brownish. Leaves compound, trifoliate, opposite, decussate; rachis 3.5-11 cm long, minutely pubescent when young, pulvinate; petiolule 0.6-11 cm long, canaliculate, slightly pubescent; leaflets 7-20X3-8.5 cm (usually larger in saplings) elliptic to obovate, apex acuminae, base assymetric, or slightly attenuate, argin entire chartaceous, glandular unctate, glabrous shining above; midrib slightly canaliculat; secondary nerves 7-16 pairs, straight or gradually curved; tertiary nerves slender, broadly reticulate. Inflorescence spreading paniced cyme. Flowers greenish white, sessile. Follicles, 4-valved; 1-seeded cocci, black[1-2]. In the present study, we report the analgesic activities of ethyl acetate extract of *Evodia Lunu-ankenda*. A review of the literature revealed that the analgesic activities of the plant.
MATERIALS AND METHODS

The plant was collected from Tiruvandapuram, Kerala, India, in the month of March 2009. The collected plants were identified by Department of Botany the American college Madurai.

Extraction procedure
The barks of *evodia lunu-ankenda* were shade dried under shade and then made in to a coarse powder with a mechanical grinder. The passed through sieve no 40 and stored in air tight container for further use. The bark (500mg) was first extracted with ethyl acetate in a soxhlet apparatus (48 hrs) the extract was concentrated by distillation under reduced pressure using rotary flash evaporator to yield (5.12%w/w) a solid residue.

Preliminary Phytochemical Screening
Preliminary phytochemical screening of the ethyl acetate of *evodia lunu-ankenda* showed the presence of Glycosides, Proteins, Phytosterols, Flavonoids, Phenolic Compounds, and Saponins[3-6].

Animal
Swiss albino mice weighing 20-30 g of either sex were maintained under controlled conditions of light (12hr) and temperature 25±1ºC in the animal house, two weeks prior to the experiment for acclimatization. Animals had access to food and water ad libitum. All pharmacological activities were carried out as per CPCSEA (Committee for the purpose of control and supervision of experiment on animal) norms, after obtaining the approval from the institutional animal ethical committee.

Acute toxicity studies
Acute toxicity studies were carried out on Swiss albino mice according to method proposed by Ghosh. Ethyl acetate extract at doses of 30, 100, 300, 1000 and 3000 mg/kg body weight were administrated to separate group of mice (n=6), after overnight fasting. Subsequent to administered of drug extracts, the animals were manifestations, like increaser motor activity, salivation, clonic, convulsions, coma and death. Subsequent observation was made at regular interval for 24 hr. the animals were observed for further one week.

Analgesic activity
Eddy’s Hot Plate Method [7-8]
The animals were grouped into 6 groups of six animals each. Group I received distilled water, which served as control. Group II received Morphine sulfate (5 mg/kg, i.p.) (Astra Zeneca Pharma India Ltd., Bangalore), and served as the standard. Group III and IV received ethyl acetate extracts at doses of 500 and 1000 mg/kg respectively. All the extracts were administered orally.

Sixty min after oral administration of extract and 30 min after i.p. injection of morphine sulfate, animals were individually placed on the Hot plate (maintained at 55 ºC) and the response such as paw licking or jump response, whichever appeared first were noted. Cut off period of 15 sec was maintained.
Heat conduction method
The animals were grouped into 6 groups of six animals. Each group 1 received distilled water, which served as control group 2 received morphine sulphate (5 mg/kg, i.p) and served as the standard group 3 and 4 received ethyl acetate extracts at doses of 500 mg and 1000 mg/kg respectively. All the extracts were administered orally. Sixty minutes after oral administration of extracts and 30 min after i.p. injection of morphine sulfate, the tail tip of individual animals was dipped up to 5 cm into hot water (maintained at 58°C) and the response time was noted as the sudden withdrawal of the tail from the hot water. Cut off period of 10 sec was maintained.

Table 1: Analgesic activity of ethyl acetate extract of bark of Evodia lunu-ankenda by Eddy’s hot plate method

<table>
<thead>
<tr>
<th>Groups</th>
<th>Reaction time (seconds)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2 ± 0.2584</td>
</tr>
<tr>
<td>Morphine sulfate (5mg/kg)</td>
<td>9.4 ± 0.3334***</td>
</tr>
<tr>
<td>Ethyl acetate extract (500mg/kg)</td>
<td>4.2 ± 0.1668***</td>
</tr>
<tr>
<td>Ethyl acetate extract (1000mg/kg)</td>
<td>4.5 ± 0.3334***</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM; n = 6; ***p < 0.001

Table 2: Analgesic activity of ethyl acetate extract of bark of Evodia lunu-ankenda by heat conduction

<table>
<thead>
<tr>
<th>Groups</th>
<th>Reaction time (seconds)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2 ± 0.2584</td>
</tr>
<tr>
<td>Morphine sulfate (5mg/kg)</td>
<td>8.5 ± 0.2110 ***</td>
</tr>
<tr>
<td>Ethyl acetate extract (500mg/kg)</td>
<td>4.5 ± 0.2105***</td>
</tr>
<tr>
<td>Ethyl acetate extract (1000mg/kg)</td>
<td>4.8 ± 0.4285***</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM; n = 6; ***p < 0.001

Statistical analysis
Statistical analysis was performed, using one way analysis of variance (ANOVA) followed by Tukey-kramer Multiple comparison Test. All values were expressed as ±SEM.

RESULTS AND DISCUSSION

Acute toxicity studies did not reveal any toxic symptoms or death in any of the animals up to the dose of 3000 mg/kg body weight, were either extract.

Aqueous extract of Evodia lunu-ankenda showed significant analgesic activity as evidenced by the increase in reaction time to the pain stimulus. The results were significant at p <0.001 for both Eddy’s Hot plate method and Heat conduction method. The analgesic activity is presented in tables 1 and 2 and in Histogram 1 and 2. Analgesic activity of the drug in aqueous extract was comparable to the standard drug Morphine sulfate.
From this study, it can be concluded that aqueous extract of root tuber of Evodia lunu-ankenda marked analgesic activity and is equipotent to standard analgesic drugs. The present study establishes the effectiveness and pharmacological rationale for use of Evodia lunu-ankenda as an analgesic drug. The drug may be further explored for its phytochemical profile to identify the active constituents responsible for the analgesic activity. Also, the medical application of the drug of use in the treatment of pain in traditional systems of medicine is substantiated.

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REFERENCES