Anti-inflammatory potential of flavone glycoside from ethanol extract of the aerial parts of the plant *Leucas lavandulaefolia*

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Abstract

Chrysoeriol-4′-O-α-L-rhamnopyranosyl (1→2)β-D-glucopyranoside. has been isolated from *Leucas lavandulaefolia* Rees aerial parts and its structure has been determined by UV, IR, ¹H NMR and ¹³C NMR spectroscopic methods. This compound has been isolated for the first time. Ethanol extract from which the flavone glycoside isolated from of aerial parts of plant *Leucas lavandulaefolia* was tested for anti-inflammatory activity using technique of carageenan induced paw edema in albino rats. The ethanol extract showed significant anti-inflammatory activity compared to the reference standard Diclofenac. Acute toxicity study in mice revealed the nontoxic nature of crude extract.

INTRODUCTION

*Leucas lavandulaefolia* Rees (family Labiatae) is a herbaceous annual weed found in pastures and waste land throughout India. It has a strong flavour and is reputed for its use as sedative, vermifuge, stomachic, dermatosis and is also useful in the treatment of migraine[1-3]. A literature survey revealed that the presence of Acacetin and Chrysoeriol from this plant has been reported[4]. In continuation of our research[5], we report in this paper, the anti-inflammatory activity of the aerial parts of *Leucas lavandulaefolia* to justify its folkloric use.

MATERIALS AND METHODS

The aerial parts (including leaves, flowers, stem and branches) of *L.lavandulaefolia* were collected from Udupi, Karnataka, India and its botanical identity was confirmed by Dr.Gopalkrishna Bhat, Department of Botany, Poornaprajna college, Udupi. A voucher specimen
Experimental

The aerial parts of *L. lavandulaefolia* were dried under shade and powdered. 1 kg of dry plant material was defatted with petroleum ether in soxhlet apparatus. The defatted plant material was extracted with hot ethanol to give a solid residue (10 g) which was extract with ethyl acetate. The ethyl acetate fraction on silica gel column chromatography eluting with EtoAc-MeOH (7:4, v/v) yielded compound 1 (200 mg). This was further purified by preparative TLC on silica gel with the same solvent system to get 175 mg of pure compound 1. IR spectra (KBr) were recorded on a Shimadzu 8201 PC FT spectrometer. ¹H NMR spectra were obtained on a Bruker WM 400 instrument at 90.56 MHz in DMSO-d₆ using TMS as internal standard (chemical shift in δ, ppm). TLC were run on silica gel G (Merck). UV-vis spectra (MeOH) were obtained on a Hitachi 320 spectrophotometer. Melting point of compound 1 was found to be 175-176°C. UV-vis (nm): MeOH 239, 269, 288 (sh), 334; (MeOH-NaOMe): 223 (sh), 231, 278, 309 (sh), 369; (MeOH-AlCl₃): 257(sh), 279, 291(sh),345, 381; (MeOH-AlCl₃-HCl): 257, 280, 291(sh), 342, 381; (MeOH-NaOAc) 232, 275, 310 350 ; IR(KBr): 1651, 335 5, 1208, 1256, 1033, 1103, 3081, 2907 cm⁻¹; ¹H NMR (DMSO-d₆): δ6.54(1H; s, H-3), 3.33-4.50 (18 H, m, sugar-H), 3.89 (3H, s, 1XOCH₃), 5.05(1H, d, J=7.5 Hz, rha H-1), 5.45 (1H, d, J=7.5 Hz, glu H-1), 6.42 (1H, d, J=2.5 Hz, H-6), 6.53 (1H, d, J=2.5Hz, H-8), 7.18 (1H, d,J=2.5 Hz, H-2΄), 7.28 (1H, dd, J=2.5 and 9.5 Hz, H-6΄), 7.39(1H, d, J=9.5 Hz, H-5΄), 13.17(1H, s, OH-5); ¹³C NMR (DMSO-d₆) : δ164.1 (C-2), 103.0 (C-3), 196.01 (C-4), 103.03 (C-4a), 163.98 (C-5), 97.33 (C-6), 163.74 (C-7), 106.28 (C-8), 166.03(C-8a), 119.44(C-1΄), 156.95 (C-2΄), 98.58 (C-3΄), 148.66(C-4΄), 104.5 (C-5΄), 126.43(C-6΄);glucose 101.3 (C-1), 74.3 (C-2), 76.7 (C-3), 70.2 (C-4), 77.2 (C-5), 67.8 (C-6); rhamnose 100.6 (C-1), 71.8 (C-2), 72.2 (C-3), 73.7 (C-4), 68.1 (C-5), 18.1 (-CH₃); EIMS: m/z 622 [M+H]⁺, 591 [M+H-OmE]⁺, 444 [M+H-rha].

Dose fixation for anti-inflammatory activity was carried out by Stair-case[6] method on albino rats (Wistar strain 8-100 mg). The studies were conducted in accordance with the internationally accepter principles for laboratory animal use and care as found in US guidelines. The extract was homogenized in Tween-80 (1%) and dissolved in distilled water and was administered to rats orally by means of intra gastric catheter. It was observed that the extract was not found to be lethal even at the dose of 3000 mg/kg body weight. Hence 1/10th of 3000mg/kg i.e. 300 mg/kg body weight of the crude extract was fixed as the standard dose. The anti-inflammatory activity
was evaluated by carageenan induced rat paw edema method[7]. Colony bred young albino rats (Wistar strain), weighing about 150-250 gm were used for the study. They were fed with standard diet (Pranav Agro Industries Ltd., Sangli, Maharashtra) and ad libitium. They were housed in polypropylene cages maintained under standard condition (12 hour light/ 12 hour dark cycle; 25 ± 3°C, 35-60% humidity). The animals were divided into three groups of six animals each. First group serving as control received Tween 80 (1%, 10 ml/kg p.o), second and third groups were receiving the ethanolic extract 300 mg/kg. The third group served as positive control and received diclofenac sodium 25 mg/kg body weight. Food was withdrawn overnight, but adequate supply of water was given to the rats before the experiment. The drugs were given orally. After one hour, a subplantar injection of 0.1 of 1% solution carageenan was administered in the left hind paw to all the three groups. The paw volume was measured with the help of plathysmograph immediately after injection. The paw volume was again measured after 3 hours[11]. The average paw of swelling in a group of extract treated rats were compared with control group (treated with vehicle) and the standard (Diclofenac sodium) and shown in Table 1. The statistical analysis was done to determine significant difference of results between treated and control groups using student’s t-test as described by Kulkarni[8].

Table 1: Antiinflammatory activity of ethanol extract of the aerial parts of L.lavandulaefolia on carageenan induced rat hind paw edema

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg p.o.)</th>
<th>Mean paw volume (ml) ± SEM 3hr.</th>
<th>Inhibition (1%) (after 3 hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tween 80 (1%)</td>
<td>10 ml/kg p.o.</td>
<td>0.15 ± 0.005</td>
<td>---</td>
</tr>
<tr>
<td>Ethanol extract</td>
<td>300</td>
<td>0.05 ± 0.006*</td>
<td>62.5</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>25</td>
<td>0.04 ± 0.006*</td>
<td>68.75</td>
</tr>
</tbody>
</table>

Mean ± S.E n=6, *p<0.001 when compared to control

RESULTS AND DISCUSSION

The compound was found to be a flavone glycoside by its spectral analysis and Molisch test. Upon acid hydrolysis (7% H₂SO₄), the compound yielded D-glucose, L-rhamnose and aglycone. The aglycone was characterized on the basis of UV-Vis, ¹H and ¹³CNMR and co-TLC[9]. When UV-Vis spectrum (NaOMe) was compared with that of Chrysoeriol, no shift was observed indicating the glycosylation site at C₄. The absence of signal by ¹H and ¹³CNMR at δ9-9.42 for C₄-OH in Chrysoeriol clearly indicated the glycosylation at C₄. The aglycone gave characteristic colour reactions of flavonoids. It was confirmed from the red colour with magnesium and hydrochloric acid (Shinoda test)¹⁰. The inter sugar configurations were deduced from ¹H and ¹³CNMR spectra which confirmed β-D-pyranosyl configuration for glucose and α-L-pyranosyl configuration for rhamnose. The glycoside completely methylated, was hydrolysed and the resulting methylated sugars were identified as 2,3,4-tri-o-methyl- L-glucose and 2,3,4-tri-o-methyl-L-rhamnose. Hydrolysis of glucose with takadiastase liberated rhamnose. After complete takadiastase hydrolysis, the glycoside was hydrolysed with β-glucosidase. The ¹H NMR spectrum of the compound showed two anomeric proton signals at 4.5 (1H,d,J=7.1Hz) assignable to 1-H-β-glucoside proton and δ5.05 (1H, d,J=2.0Hz) assignable to 1-H-α-rhamnopyranosyl proton. The remaining sugar protons resonated between δ3.33-4.50. CH₃ of rhamnose appeared at δ1.25 (3H,d, J=6.0Hz). The ¹³CNMR of the compound matched with the reported values for Chrysoeriol¹⁰. Anomeric carbons of glucose and rhamnose appeared at

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δ101.3 and 100.6 respectively. 13CNMR signals of sugar were similar to their reported values except for a 5.9ppm downfield shift of C-6° of glucose. This downfield shift established 1→2 linkage between rhamnose and glucose. Further, the C-4° site of glycoside resonated at higher field (148.66) as compared to C-5 and C-7. Thus C-4° of the glycoside exhibited a shielding effect which confirmed that the attachment of sugar was at C-4° of the glycoside via a C-O-C linkage. Based on the evidences, the structure of the compound was assigned as chrysoeriol-4°-O-α-L-rhamnopyranosyl (1→2)β-D-glucopyranoside. The isolation and characterization of this flavonoid glycoside has been reported for the first time.

The ethanol extract of the aerial part of *L.lavandulafolia* showed significant reduction in the edema volume at a dose of 300 mg/kg body weight, which is comparable to the standard (Table 1) drug Diclofenac.

Indigenous drug system can be source of variety of new drug which can provide relief in inflammation, but their claimed reputation has to be verified on a scientific basis. In some cases indigenous drugs may be the only the answer.

The activity may be attributed due to the presence of flavonoid glycoside present in the ethanol extract of aerial part of *L.lavandulafolia*.

REFERENCES