

and Ar)  $\text{cm}^{-1}$ .  $^1\text{H NMR}$   $\delta$  (300 MHz,  $\text{Me}_2\text{CO}-d_6$ ): 7.36 (2H, m, H-2, 6A<sub>1</sub>), 6.89 (2H, m, H-3, 5A<sub>1</sub>), 7.99 (1H, d, J=15.5 Hz, H- $\beta$ ), 7.79 (1H, d, J=15.5 Hz, H- $\alpha_1$ ), 5.97 (1H, s, H-5'B<sub>1</sub>), 7.48 (2H, m, H-2, 6A<sub>2</sub>), 6.84 (2H, m, H-3, 5A<sub>2</sub>), 6.05 (1H, d, J=5.7 Hz, H- $\beta_2$ ), 5.30 (1H, d, J=5.7 Hz, H- $\alpha_2$ ), 6.35 (1H, d, J=2.3 Hz, H-3, B<sub>2</sub>), 6.38 (1H, dd, J=8.8; 2.3 Hz, H-5'B<sub>2</sub>), 7.77 (1H, d, J=8.8 Hz, H-6'B<sub>2</sub>), 14.13 (1H, s, OH), 12.03 (1H, s, OH). The spectral data suggested it to be trans -  $\alpha_2, \beta_2$  - dihydroderivative of calodenin B<sup>8</sup>.

#### ACKNOWLEDGEMENTS

We are very grateful to Professor Bernard Bodo, Director of the Laboratoire de Chimie du Museum - CNRS, ESA 8041 for the structural informations. We thank the botanist Nole for harvesting and identification plant material. We are also indebted to the Yaounde I University Grants Committee for financial assistance.

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### Antiinflammatory Activity of Various extracts of *Pergularia extensa* N. E. Br. (Asclepiadaceae)

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Accepted 12 July 2001

Revised 25 June 2001

Received 29 August 2000

Crude ethanol extract of *Pergularia extensa* leaves was successively fractionated with petroleum ether, solvent ether, ethyl acetate, butanol and butanone. The ethanolic extract and various fractions were investigated for antiinflammatory activity in rats at a dose of 100 mg/kg intraperitoneally. Ethanol extract and its butanol fraction exhibited significant antiinflammatory activity when compared with respective controls and were comparable with that of standard drug aspirin.

Even in this modern era, a large extent of Indian population still relies on the traditional systems of medicine, which are mostly plant based. Hence it is considered necessary to experimental evidence to validate, the traditional use of one such plant *Pergularia*

*extensa* (Asclepiadaceae) syn.: *Pergularia daemia* (Forsk.); *Daemia extensa* R. Br. is a perennial twining herb, foetid when bruised and with much milky juice, stems clothed with spreading hairs<sup>1</sup>. Traditionally the plant is used as a pungent, coolant, anthelmintic, laxative and antipyretic. It is also known to cure biliousness, asthma, ulcers, leucoderma, uterine complaints, facilitates

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parturition and useful in eye troubles. Juice of the leaves is squeezed into sore eyes and is applied to inflammatory swellings due to rheumatic fever and rheumatoid arthritis in combination with lime and ginger<sup>2</sup>.

A literature survey reveals that no scientific study has been made with respect to anti-inflammatory properties of *Pergularia extensa*. Therefore in the present communication, we report the antiinflammatory activity of the leaf extracts of *Pergularia extensa*.

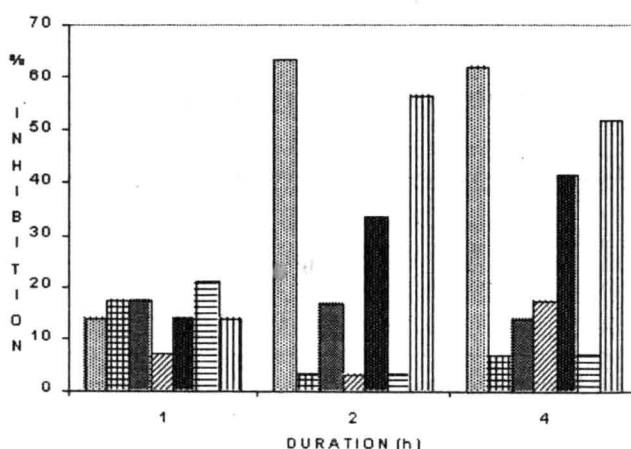
The leaves of *Pergularia extensa* (Asclepiadaceae) were collected from Hattargi (Dist. Belgaum) during December 1998 and authenticated at the Department of Biological sciences, K.L.E.S's College of Pharmacy, Belgaum. The shade dried leaves were pulverized to a fine powder and subjected to soxhlet extraction with ethanol (95%). The extract was concentrated to dryness by heating on a steam bath at a controlled temperature of 40°-50°.

A part of extract was kept aside for pharmacological studies and the remaining dispersed in distilled water and was successively fractionated with petroleum ether, solvent ether, ethyl acetate, butanol and butanone using a separating funnel. The separated organic fractions were washed with water to eliminate water soluble impurities, then dried over anhydrous sodium sulphite and finally reduced to dryness on a steam bath. All the extracts were kept in a desiccator and stored in a refrigerator for pharmacological studies. The test doses were prepared in propylene glycol (1%) suspension in distilled water of each extract to get the desired concentration of the extract.

Antiinflammatory activity was evaluated using carrageenan-induced rat hind paw oedema method of Winter *et al.*<sup>3</sup> Albino rats of either sex weighing between 150-200 g were divided into eight groups of six animals each. The first group served as control and received the vehicle only (propylene glycol 1%), second group of animals were administered with standard drug aspirin 100 mg/kg body weight, intraperitoneally. The animals of the third, fourth, fifth, sixth, seventh and eighth groups were treated with ethanol (95%) extract, petroleum ether, solvent ether, ethyl acetate, butanol and butanone fractions of *Pergularia extensa*. A dose of 100 mg/kg body weight was selected on basis of the acute toxicity studies and administered intraperitoneally. A mark was made on both the hind paws just below the tibio-tarsal junction so that every time the paw could be dipped in the mercury

column of plethysmograph up to the mark to ensure constant paw volume. After 30 min of above treatment an inflammatory oedema was induced in the left hind paw by injecting 0.05 ml of carrageenan 1% w/v in saline, in the planter tissue of all the animals. The paw volume was measured at 0 h and followed by every hour till the 6 h after administration of carrageenan to each group. The difference between the initial and subsequent reading gave the actual oedema volume.

Per cent inhibition of inflammation was calculated using the formula, % inhibition = 100 (1-vt/vc), where 'vc' represents oedema volume in control and 'vt,' oedema volume in group treated with test compound. The data were analysed using student's 't' test and the level of significance was set at P<0.01. The results represented as per cent inhibition of inflammation are presented in fig. 1.



**Fig. 1: Percent inhibition of carrageenan-induced rat paw oedema.**

The inhibitory effect of various extracts of *Pergularia extensa* on carrageenan-induced rat paw oedema was compared to that produced by standard drug aspirin (▤). Various extracts used were, petroleum ether (▣), solvent ether (▧), ethyl acetate (▨), butanol (■), butanone (▩) and ethanol (▩).

In carrageenan-induced rat paw oedema test, it was found that there was no significant reduction in the oedema in the groups treated with petroleum ether, solvent ether, and ethyl acetate and butanone fractions. In case of the groups treated with ethanol extract and its butanol fraction, no significant reduction was noticed after 1 h, but at the

end of 2 h up to 4 h, a significant reduction ( $P < 0.01$ ) was recorded, when compared to control. Thus, it can be concluded that, on preliminary screening of crude extracts of *Pergularia extensa*, the ethanol (95%) extract and its butanol fraction possessed significant antiinflammatory activity. These results indicate a need for a detailed photochemical investigation of *Pergularia extensa* to identify the active constituents.

#### ACKNOWLEDGEMENTS

We thank Dr. F. V. Manvi, Principal, K.L.E.S's College of Pharmacy, Belgaum for providing the facilities to carry out the research work. We wish to extend our thanks to

Late Shri A. P. Kore, Asst. Professor, Dept. of Biological Sciences, K.L.E.S's College of Pharmacy, Belgaum for authentication of the plant.

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## Production of Hypericin from Tissue Culture of *Hypericum perforatum*

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Accepted 24 July 2001

Revised 17 July 2001

Received 6 February 2001

Murashige and Skoog medium supplemented with 2,4-D (2 mg/l) and kinetin (1 mg/l) was found to be suitable for the establishment of callus cultures of *Hypericum perforatum*. The callus upon extraction and analysis by TLC/HPLC revealed the presence of hypericin. The content of hypericin in callus cultures of *H. perforatum* was significantly less than the intact plant. There is no significant effect of media or hormones on bioproduction of hypericin.

*H. perforatum* (Hypericaceae) is one of the top 15 selling popular herbs in USA and Europe<sup>1</sup>. It is a herbaceous perennial plant widely distributed in Europe, Northern Africa and it is naturalized in the USA. This plant is not described in Ayurvedic system of medicine. It grows widely in the Himalayan regions and in the hills of the central part of India. The local names of this plant are dendhu patta, basant (Hindi) and balasana (Urdu). It contains hypericin (0.05-0.3% w/w on dry weight basis) and hypericin-like substances, notably pseudohypericin, isohypericin, protohypericin, hyperforin and adhyperforin<sup>2</sup>. The hypericum herbal extract was reported to have

antiviral, wound healing and hepatoprotective properties. Hypericin was reported to have antidepressant activity<sup>3</sup>. It was recently reported that hyperforin a compound isolated from *Hypericum perforatum* known to inhibit protein Kinase C activity<sup>4</sup>.

Plant tissue culture is one of the alternative techniques for the production of phytopharmaceuticals. This technique has been already commercialized for the production of taxol, shikonin and rosmarinic acid<sup>5</sup>. Callus culture of *Hypericum perforatum* Var. *aungustifolium* was established from stem segments of *in vitro* shoot cultures on solid MS medium supplemented with NAA (4.5  $\mu$ M and kinetin (2.3  $\mu$ M)<sup>6</sup>. Cardoso<sup>7</sup> reported shoot multiplication and callus induction of *Hypericum*

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