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SHORT COMMUNICATION

INSECTICIDAL PROPERTIES AND AN ACTIVE CONSTITUENT OF *LIMONIA ACIDISSIMA*

B.M.R. BANDARA, A.A.L. GUNATILAKE, S. SOTHEESWARAN AND E.M.K. WIJERATNE
Department of Chemistry, University of Peradeniya, Peradeniya, Sri Lanka.

AND

M.A.S.K. RANASINGHE
Department of Agricultural Biology, University of Peradeniya, Peradeniya, Sri Lanka.

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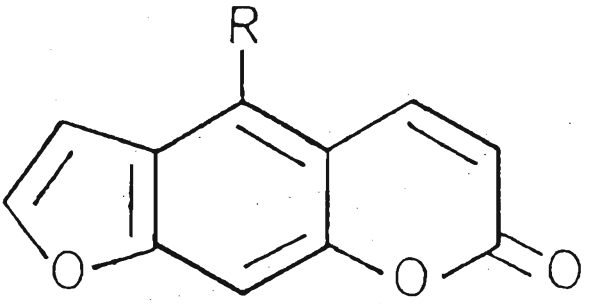
Limonia acidissima L. (Sinhala : Divul) (Rutaceae) is a medicinal plant widely distributed in tropical regions of India and Sri Lanka. The powdered stem bark of *L. acidissima* showed insecticidal properties against the brunchid, ***Callosobruchus chinensis*** L. The major active constituent of the stem bark was identified as bergapten (*1*).

The powdered stem bark was sequentially and exhaustively extracted with petroleum ether (b.p. 60—80^{0}C), dichloromethane and ethyl acetate under reflux conditions. Seeds of *Vigna radiata* L. Wilezek, mung bean, were coated with a thin film of the extractive using a concentrated solution of each extract in acetone, and the solvent removed by vacuum suction. After leaving the seeds in an open petri—dish overnight, 5 males and 5 females of active, one day old brunchids reared in the laboratory, were introduced to each petri—dish containing 50 treated mung beans. The dishes were kept in a dark incubator, insects were removed after 48 h and the number of eggs laid was recorded. The seeds were then returned to the petri—dish and the progeny which emerged from these was counted during the subsequent period of 21 days. The experiment was performed with four replicates for each extract. Mung bean seeds treated with acetone and untreated seeds were used as standard controls. The emergence of the adult brunchids was reduced by 100% with the petroleum ether extract and by 65% with the dichloromethane extract compared to the untreated control.

A portion of the petroleum ether extract was partitioned on preparative thin—layer chromatographic (PTLC) plates (eluant: dichloromethane). The two fractions obtained by dividing the developed plate symmetrically by an arbitrary line in the middle, were tested for insecticidal activity. The activity remained in the upper band. Similar partitioning of this fraction on TLC plates followed by bioassay of the resulting two fractions, furnished the

active component again in the upper band. Further purification of the active fraction by TLC gave two white crystalline compounds as major constituents The more polar compound ($C_{12}H_8O_4$), m.p. 189—190°C, was identified as bergapten (*1*) from spectroscopic data[3] (UV,IR, ^{1}H—NMR and Mass) The less polar compound, m.p. 162 — 163°C, was found to be psoralen (*2*) by comparison (UV,IR, ^{1}H—NMR and m.p.) with an authentic sample.[1]



1 R=OMe

2 R=H

Bergapten (*1*) and psoralen (*2*) were tested for insecticidal activity against *C. chinensis*. Bergapten (*1*) reduced the emergence of the adults by 100% (0.1 mg g^{-1} of seeds) while psoralen (*2*) was found to be inactive. From examination of the eggs under the microscope, it appears that the mode of action is ovicidal. Previous studies have shown that bergapten is phototoxic and photomutagenic in the green algae *Chlamydomonas reinhardii*[5], and toxic to toads, the fish *Lebiscus reticulatus*, and to the snail *Biomphalaria boissi*, an intermediate host in schistosomiasis.[4]

Bergapten has been found to occur in several species of the plant families, Umbelliferae (160 species), Rutaceae (40 species), Pittosporaceae (10 species) and Moraceae (7 species).[4] This compound has also been isolated from the leaves and the root bark of *L. acidissima*.[2,6]

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References

1. BANDARA, B. M.R., GUNATILAKA, A. A. L., WIJERATNE, E. M. K. & ADIKARAM, N. K. B. (1988) *Planta Med.*, **54** : 374.

2. CHAKRABORTY, D.P. (1959) *J. Sci. Ind. Res.*, **18B** : 90.

3. GUPTA, S.R., SESHADRI, T.R., SHARMA, C.S. & SHARMA, N.D. (1979) *Planta Med.*, **36** : 95.

4. MURRAY, R.D.H., MENDEZ, J. & BROWN, S.A. (1982) The Natural Coumarins, 471–594 pp, John Wiley & Sons Ltd., New York.

5. SCHIMMER, O., BECK, R. & DIETZ, U. (1980) *Planta Med.*, **40** : 68.

6. TALAPATRA, S.K., CHAUDHURI, M.K. & TALAPATRA, B. (1973) *Phytochemistry*, **12** : 236.