

In order to evaluate the actual modifying effect of different amounts of damaged cells on the ADP-Fe³⁺ or CCl₄ induced lipid peroxidation, simultaneous monitoring of the production of TBA-reacting compounds was carried out in cell populations containing 10–20–30–40% damaged cells. The cells were incubated for 60 min in the presence of ADP-Fe³⁺ (2.5 mM–100 μM) or CCl₄ (129 μM). As shown in the figure, the relationship between the stimulation of lipid peroxidation due to ADP-iron and CCl₄ and the amount of damaged cells present in the suspension is linear.

In cell suspensions containing 40% damaged cells, the lipid peroxidation induced by ADP-iron and CCl_4 was respectively 1.7 and 3.3 times higher than that theoretically obtainable with 100% viable cells (see figure). In other words, with an increase by 10% in the damaged cell content of hepatocyte preparations, an enhancement of ADP- Fe^{3+} and CCl_4 induced lipid peroxidation by 18% and 56% followed. In conclusion, the data reported here emphasize the critical role exerted by the quality of liver cell preparations on 'in vitro' lipid peroxidation studies employing hepatocytes in single cell suspension.

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Further studies on the sensitivity of plant pathogenic microorganisms towards some naturally occurring chalcones and flavanones

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Summary. The antifungal and antibacterial activities of some quinochalcons, chalcones and flavanones isolated from *Didymocarpus pedicellata* have been assayed.

A survey of the literature revealed that certain simple quinones and their derivatives possess marked fungicidal properties². In view of the fact that the leaves of *Didymocarpus pedicellata* (Gesneriaceae) keep well on storage and are not attacked by fungi in a humid atmosphere³, we thought it worthwhile to undertake biological investigations on the compounds isolated from different extracts of the dried leaves of this plant. Interestingly, we were able to isolate 2 quinochalcones, chalcones and flavanones (figure, I) from the leaves of *D. pedicellata*⁴⁻⁹. We have previously described the antifungal activities¹⁰⁻¹² of a number of furocoumarins from *Selinum tenuifolium* (Umbelliferae) and pterocarpan, coumestans and chalcones from *Flemingia chappar* (Leguminosae). This communication reports the antifungal and antibacterial activities of the compounds isolated from the leaves of *D. pedicellata*.

Materials and methods. A number of plant pathogenic sporeforming fungi viz., *Helminthosporium oryzae* Breda de Haan, *Fusarium oxysporum* f. spp. *ciceri* (Padwick) Synd. &

Hans and *Rhizopus artocarpii*; 3 sclerotial pathogens, *Sclerotium rolfsii* Sacc., *Thanatephorus cucumeris* (Frank) Donk and *Rhizoctonia oryzae sativae* (Saw) Mordue, and 1 plant pathogenic bacterium, *Xanthomonas campestris* (Pammel) Dowson, were used as test organisms. Solutions of 8 test compounds I-VIII at 4 concentrations, viz., 500, 250, 100 and 50 ppm were prepared by dissolving them first in 1-2 ml of ethyl alcohol and making up the rest of the volume with distilled water. 3-day-old slant cultures (PDA) of *R. artocarpii* and 7-day-old cultures of *H. oryzae* and *F. O. ciceri* were used for preparation of spore suspensions with sterilized distilled water; these were filtered through a double layer of sterilized muslin cloth, maintaining a final strength of 0.25 million spores per ml of suspension (counted with a haemocytometer). For sclerotial germination, sclerotia from 7-day-old PDA cultures of *S. rolfsii*, *R. oryzae sativae* and *T. cucumeris* were taken. Sensitivity of fungi and bacteria to the compounds I+VIII were tested following standard methods of spore germina-

Table 1. Sensitivity of some plant pathogenic spore forming fungi towards naturally occurring chalcones and flavanones (average of 5 replicates after 24 h)

[illegible]

Table 2. Sensitivity of some plant pathogenic sclerotial fungi towards naturally occurring chalcones and flavanones (average of 5 replicates after 24 h)

Compounds	% of inhibition of sclerotial germination at concentration (ppm):									
	<i>S. rolfsii</i>		<i>T. cucumerinum</i>				<i>R. o. sativae</i>			
	100	250	500	100	250	500	50	100	250	500
Methylpedicinin (I)	0	0	0	0	40	40	25	40	80	100
Isomethyl pedicinin (II)	0	0	20	40	40	40	20	40	60	80
Pedicinin (III)	20	20	40	20	20	20	40	60	80	100
Pedicellin (IV)	0	0	0	0	0	20	50	80	80	100
Isodidymocarpin (V)	20	20	20	20	20	20	47	80	80	100
Didymocarpin-A (VI)	6	16	22	19	26	31	37	45	51	60
Didymocarpin (VII)	30	41	46	25	36	40	51	63	70	81
Despedicellin (VIII)	21	23	29	20	21	28	10	46	59	67
Control	0	0	0	0	0	0	0	0	0	0

tion inhibition¹³, sclerotial germination inhibition¹⁴, and inhibition zone based on the agar diffusion principle¹⁵ respectively. Fish spines were dipped in solutions in a watchglass, put aseptically on PDA plates seeded with the test bacterium, with sterilized forceps, incubated at $30 \pm 1^\circ\text{C}$ for 24 h and observed for determination of the diameter of the inhibition zone.

Air-dried finely powdered leaves of *D. pedicellata* were successively extracted with petroleum ether (60–80 °C), benzene and chloroform in a soxhlet apparatus. The petroleum ether extract on chromatographic resolution over silica gel (BDH, 60–120 mesh) followed by elution with solvents of increasing polarity furnished 2 new flavanones, didymocarpin⁴ (VII), didymocarpin-A⁵ (VI) and a new chalcone, isodidymocarpin⁶ (V) along with a known chalcone pedicellin⁷ (IV). The chloroform extract on chromatography over silica gel afforded 2 known quinochalcones⁷, methyl pedicinin (I) and pedicinin (III). 2',5'-Dimethoxy-4'-hydroxy-3',6'-quinochalcone⁸ (II) designated as isomethyl pedicinin is the oxidation product of isodidymocarpin (V) while despedicellin⁹ (VIII) is the demethylated product of pedicellin (IV).

Results and discussions. a) Inhibition of spore germination of 3 fungi. The results as presented in table 1 show that only 4 compounds (II, V, VI and VII) have been inhibitory to the germination of sporangiospores of *R. artocarpii* at 50 ppm concentration. All the others were inhibitory at 100 ppm. In the case of conidia of *H. oryzae* and *F. o. ciceri*, only 3, viz. didymocarpin-A (VI), didymocarpin (VII) and despedicellin (VIII) have been found to be inhibitory up to 250 ppm concentration. Isomethyl pedicinin (II) was found to be inhibitory only to *Fusarium* at 500 ppm concentration. b) Inhibition of sclerotial germination. The results in table 2 indicate that none of the compounds I–VIII showed satisfactory inhibitory properties against all the sclerotial

pathogens. In fact only 2 compounds, viz. pedicellin (IV) and didymocarpin (VII) were inhibitory to *R. oryzae sativae* upto 50 ppm concentration. 2 more, viz. pedicinin (III) and isodidymocarpin (V) showed similar inhibition at 100 ppm. The rest of the compounds (I, II, VI, VIII) were inhibitory only at concentrations as high as 250 ppm.

c) Antibacterial activity. All the compounds I–VIII appeared to have some degree of antibacterial activity against a plant pathogenic xanthomonad. Amongst them, pedicellin (IV), didymocarpin (VII) and pedicinin (III) were found to be good inhibitors at concentrations as low as 100 ppm. From the results, it is evident that the compounds in general have antibacterial activities against different types of plant pathogens even at dosages as low as 100 ppm. Amongst the fungi, the phycomycetous *R. artocarpii* appears to be the most sensitive one followed by *R. oryzae sativae*, the rice pathogen belonging to the mycelia-sterilia group of Deuteromycetes. The compounds seemed generally to be poor inhibitors of germination of both pigmented and hyaline conidia. Differential activities of the compounds towards different organisms were interesting. Antibacterial activities of all the compounds have opened up the possibility of carrying out tests against a large number of bacteria of a plant pathogenic nature. Thus, the chalcones (I–V) and flavanones (VI–VIII), now being reported for the first time, appear to offer possibilities against fungal and bacterial plant pathogens; further studies in this area are being carried out.

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