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Flavonoids of *Lonchocarpus speciosus*

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Lonchocarpus speciosus Bolus (Fabiaceae, Papilinoideae) was previously examined and exhibited 5 flavonoids, namely lonchocarpin, chalcone, 5,7-dihydroxyaurone, taxifolin, astilbin, fustin and fustin 3-O glucoside. The stem, leaf and bark showed marked insecticidal effects against *Drosophila melonogaster* Meig flies (1). We report here 7 known and one new flavonoid, namely apigenin 6-C[β-D glucopyranosyl (1→6) O-β-D-glucoside] 8-C[β-D-glucopyranosyl (1→6) O-β-D-glucoside]. The known compounds are apigenin 7-O neohesperidoside, apigenin 7-O glucoside, vicenin-2, luteolin 7-O glucoside, kaempferol, ferulic and caffeic acids. Extracts of different polarities were examined for fungicidal activities.

INTRODUCTION

The genus Lonchocarpus (Fabiaceae, Papilionideae, Lonchocarpinae) is widely distributed in tropical regions. Members of this genus are known by a wide variety of common names including loncepod bitter wood, turtle bone, cabbage bark, water wood and tree liliac[1]. Previous work showed the presence of some known flavonoids, sterols, alkaloids and tannins. Various parts of the plant exhibited insecticidal activities against *Drosophila melongawter* Maig[2]. We describe here more flavonoids of this plant including one new natural product. Plant extracts of different polarities were tested against various mycotoxins-producing fungi and the minimum inhibition concentration, MIC, was compared to the known fungicide Benlate 50.

EXPERIMENTAL

^{1}H-NMR and ^{13}C-NMR were run in DMSO with TMS as an internal standard using Bruker AMX-500 and Unity + 300 spectrometers with (δ) reported in ppm. The E1, CI and FABMS were measured on a Finigan MAT TSQ 70 system spectrometer. UV spectra were obtained on a Shimadzu 400 spectrophotometer. For column chromatography, polyamide DC 66 100 mesh was used using water and then increasing proportions of water/ethyl alcohol for flavonoid separation. Whatmann No. 1 papers were used for chromatographic analysis while 3 mm papers were used for preparative separations, using Baw and 15 % HOAc as

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developing systems. Final purification was done with Sephadex LH-20 and MeOH/H_2O 1 : 1 as eluent.

Different amounts of the examined plant extracts were thoroughly mixed with potato dextrose agar medium to prepare concs. of 0, 500, 1000 and 2000 ppm plant extract. The melted sterilized medium with each extract concentrations were poured (15 mL) into a set of four dishes (replicates), which after solidification were inoculated by streaking with an overnight suspension of the *Aspergillus flavus, A. paraziticus, Alternaria tenius; Fusarium culmorum, F. graminearum; Penicillium citrinum* and *Penicillium sp.*, then inoculated at 28 ± 2°C for 24–48 h. The lowest concentrations of extract required to inhibit the growth of the tested fungi was designated as MIC (Table-1).

TABLE-1
MINIMUM INHIBITORY CONCENTRATIONS OF SOME PLANT EXTRACTS ON THE GROWTH OF SOME MYCOTOXIN PRODUCING FUNGI

Fungi / Extracts	**Asper-gillus flavus**	**Aspergills flavus**	Alternaria tenuis	**Fusarium culmorum**	**Fusarium orgamine-arum**	**Penicillium citrinum**	**Penici-llium sp.**
Chloroform extract ($CHCl_3$)	500	500	1000	500	500	1000	1000
Ether extract	1000	1000	>2000	1000	1000	1000	1000
Ether succ.	1000	1000	>2000	500	500	>2000	>2000
Methanol extract	1000	1000	>2000	1000	1000	>2000	>2000
Methanol succ.	1000	1000	>2000	500	500	>2000	>2000
Water extract	1000	1000	>2000	>2000	>2000	>2000	>2000
Water succ.	1000	1000	>2000	>2000	>2000	>2000	>2000
Benlate fungicide 6.5 ppm	*	†	†	*	*	†	†
Benlate fungicide 12.5 ppm	—	—	*	—	—	*	*
Benlate fungicide 25 ppm	—	—	—	—	—	—	—

Benlate 50 is a widely used fungicide. Its chemical structure is methyl-1-(butyl-carbamyl)-2-benziimidazole carbamate.

Ether succ. The plant was first extracted with chloroform by ether extraction.

Methanol succ. The plant was extracted with cholroform, ether and finally with MeOH.

Water succ. The plant was first extracted with chloroform, ether, methanol and finally with water.

Lonchocarpus speciosus Bolus, Fabiaceae (1.5 kg) was collected from the Botanical Garden in Cairo (1.5 kg) in July 1995. A voucher specimen has been deposited in the National Research Centre Herbarium, Cairo, Egypt.

Extraction and Isolation of Flavonoids and Phenolic Acids

The aerial parts were exhaustively extracted with 70% EtOH (3000 mL × 3), filtered and concentrated; then separation was done through column chromatography (C.C.) using polyamide DC 66 as adsorbent. Different fractions were subjected to preparative paper chromatography (P.P.C.) using BAW as eluent which allowed the separation of all the described compounds. Final purification was achieved by Sephadex LHO 20 and MeOH/H_2O 1 : 1 as eluent.

RESULTS AND DISCUSSION

The known compounds were identified by standard procedures[3]. The new apigenin derivative 1 exhibited a deep purple color under UV which changed to yellow upon exposure to ammonia vapours. Acid hydrolysis yielded the known di-C- glucoside vicenin-2 and glucose. UV spectral analysis in MeOH gave two main bands at 330, 272 nm. The addition of NaOMe produced a bathochromic shift with an increase in intensity, $\Delta\lambda = +58$ nm while NaOAc gave a +9 nm shift in band II and $AlCl_3$ gave + 54 nm shift thus establishing the presence of free OH groups at the 4, 7 and 5 positions.

^{1}H NMR of the new compound showed the absence of both H-6 and H-8 and the presence of signals at δ 4.7 (H-1″), 4.8 (H-1‴) characteristic for anomeric C-glucosides[4] and δ 5.1 (d, j = 8 Hz, H-1⁗), 5.2 (d, j = 8 Hz, H-1⁗′) both for the two anomeric O-glucosides. The remaining signals of apigenin were characteristic for the postulated structure.

^{13}C NMR showed C-6 and C-8 to be shifted downfield to δ108.0 and δ107.5 ppm thus proving C-glycoside nature of compound 1; in addition signals for C-1⁗ and C-1⁗′ at 102 and 101.0 respectively further confirming the presence of two O-glucoside. The two O-glucosides were considred to be linked ro C-6″

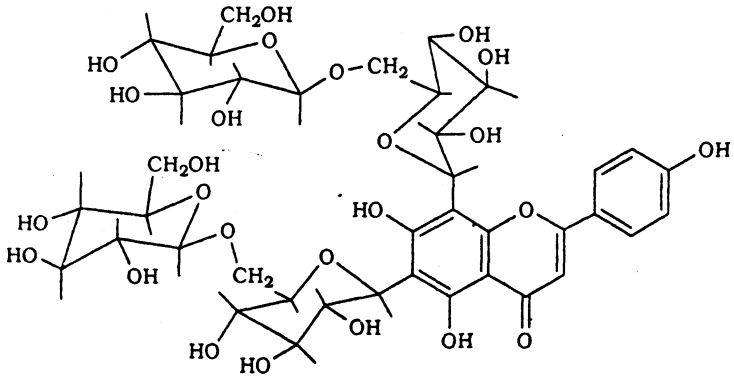

Fig. 1. Apigenin-6-C-[glucosyl-(1→6)-O-glucoside]-8-C-[glucosyl (1→6)-O-glucoside].

and C-6‴ of the glucose due to the downfield shift of the latter carbons to δ ppm 65.2 and 66.2 respectively, (Fig 1). CIMS showed M^+ at m/e 918, M-6H_2O at 811.

Table-1 indicates that chloroform followed by ether extracts gave the most effective extracts against the tested fungi, and that *A. flavus* and *A. paraziticus* were more tolerant to various extracts than other fungi, while Benlate 50 (a widely used fungicide) at 25 ppm stopped completely the growth of the different fungi.

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