Journal of Natural Products
Vol. 46, No. 6, pp. 874-875, Nov-Dec 1983

ANTIBACTERIAL ACTIVITY STUDIES OF FLAVONOIDS FROM *SALVIA PALAESTINA*

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ABSTRACT.—Ten aglycones and six glycosides of luteolin and apigenin were identified from the leaves of *Salvia palaestina* Bentham (Labiatae). Among them cirsimaritin showed a high activity against *Staphylococcus aureus, Staphylococcus epidermitis, Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris,* and *Pseudomonas aeruginosa,* while the others have little or no activity against the same bacterial strains.

As a part of our continuing investigation of the genus *Salvia* (Labiatae) (1-10), we report here the isolation and identification of 16 known flavonoids from 500 g of leaves of *Salvia palaestina* Bentham: salvigenin (25 mg), eupatilin (5 mg), apigenin 7,4′-dimethyl ether (12 mg), luteolin 7,4′-dimethyl ether (9 mg), genkwanin (6 mg), 6,7,3′,4′-tetramethoxyflavone (30 mg), cirsimaritin (30 mg), chrysoeriol (18 mg), apigenin (21 mg), luteolin (20 mg), apigenin 7-glucoside (18 mg), apigenin 7-glucuronide (46 mg), luteolin 7-glucoside (14 mg), luteolin 7-glucuronide (42 mg), chrysoeriol 7-glucoside (7 mg), and chrysoeriol 7-glucuronide (28 mg).

Salvia palaestina is widespread in southeastern Turkey, where an ointment made from its leaves is used to heal wounds. Because of its use as a folk medicine, we investigated the antibacterial activity of some of the isolated flavonoids against the standard strains *Staphylococcus aureus, Staphylococcus epidermis, Escherichia coli, Pseudomonas aeruginosa, Proteus vulgaris,* and *Klebsiella pneumoniae*; cirsimaritin gave the only significant activity (table 1).

This is the first chemical and pharmacological investigation of *Salvia palaestina*.

TABLE 1. The antibacterial activity of the flavonoids with standard bacterial strains.

Flavonoid	Bacteria											
	S. aureus ATCC 6538		*S. epidermidis* ATCC 12228		*E. coli* ATCC 8739		*K. pneumoniae* UC 57		*P. vulgaris* ATCC 8427		*P. aeruginosa* ATCC 1539	
	MIC[a]	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
6,7,3′,4′-Tetramethoxyflavone	[b] —	—	—	—	—	—	—	—	—	—	125	250
Cirsimaritin	31.25	125	62.5	125	45	90	45	90	31.25	125	31.25	125
Luteolin	—	—	—	—	125	500	125	500	—	—	125	500
Luteolin 7-glucoside	—	—	—	—	250	500	—	—	—	—	—	—

[a]μg/ml sample.

[b]Tube dilution studies were not conducted for those flavonoids having < 7 mm of inhibition in the disc diffusion experiments.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Spectra were recorded on the following instruments: uv, Varian Techtron model 635; ir, Perkin-Elmer 577 model; pmr, Varian 90 MHz; and ms, DuPont 21-491. Adsorbants used for tlc and cc were from E. Merck and Sephadex LH-20 from Pharmacia. Nutrient agar was from Difco.

PLANT MATERIAL.—The plant material was collected from Gaziantep in southeastern Turkey in June 1979 by the senior author and identified by Prof. Dr. A. Baytop (Istanbul). A voucher specimen (ISTE 42444) is deposited in the Herbarium of the Faculty of Pharmacy, University of Istanbul.

EXTRACTION AND ISOLATION OF FLAVONOIDS.—Powdered leaf material (500 g) was extracted in a Soxhlet by previously described methods (8). The benzene concentrate was fractioned on a silica gel column (4×50 cm) to yield salvigenin, 6,7,3′,4′-tetramethoxyflavone, cirsimaritin, eupatilin, apigenin and luteolin 7,4′-dimethyl ethers, apigenin 7-methyl ether (genkwanin), chrysoeriol, apigenin, and luteolin. The concentrate from the alcohol extract was chromatographed on a Polyclar column to yield apigenin, luteolin, and chrysoeriol 7-glucosides and apigenin, luteolin, and chrysoeriol 7-glucuronides.

The aglycones were identified by uv, pmr, ms, and color reactions, as well as by standard sample comparisons.

Acid hydrolysis of the glycosides yielded apigenin, luteolin and chrysoeriol, and glucose for the first three compounds and glucuronic acid for the latter three compounds; uv and pmr spectra of the glycosides showed their identities. In the case of all three glucuronides, ir showed an extra carbonyl band at 1745 cm^{-1} showing the lactonization of the glucuronic acid. The acetyl methyl ester of apigenin 7-glucuronide was the same by tlc and ir as a standard sample.[1]

ANTIBACTERIAL ACTIVITY.—The disc-diffusion method (11, 12) was used to measure the antibacterial activity; paper discs impregnated with sample solutions were placed on the nutrient agar, which were inoculated with test organisms. The plates were kept overnight in the incubator at 37°, and the sizes of the inhibition zones were measured. Compounds that had more than 7-mm inhibition zones were selected for the tube-dilution test (13) to determine the antibacterial activity more precisely. In order to determine the minimum inhibition concentration (MIC) and minimum bactericidal concentration (MBC) by the tube dilution method, twofold dilutions of the samples were made in a series of tubes containing nutrient broth.

A suspension of an overnight culture of each test organism containing approximately 10^6 cells/ml was added to the tubes; control tubes (without compounds) were used to observe the normal growth. After an overnight incubation, each tube was examined visually for growth. The lowest concentration of the sample required to inhibit the growth of the test organisms was designated as the MIC. A subculture was made from each of the clear tubes to the agar slants to determine the MBC. After an overnight incubation, the lowest concentration of the sample at which the subculture from the test dilution yielded no viable organisms was recorded as the MBC. The compounds were dissolved in a mixture of EtOH-H_2O (1:1); as a control, this same solution showed no inhibitory effect when tested against bacteria. The results are given in table 1; cirsimaritin was found to be the most active compound against both gram negative and gram positive bacteria.

ACKNOWLEDGMENTS

The work at The University of Texas was supported by NIH (Grant HDO 4488) and by the Robert A. Welch Foundation (Grant F-130). The work in Turkey was supported by the Faculty of Pharmacy, University of Istanbul.

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Received 10 January 1983

[1]Standard sample was supplied by Prof. Dr. H. Wagner (Munich).