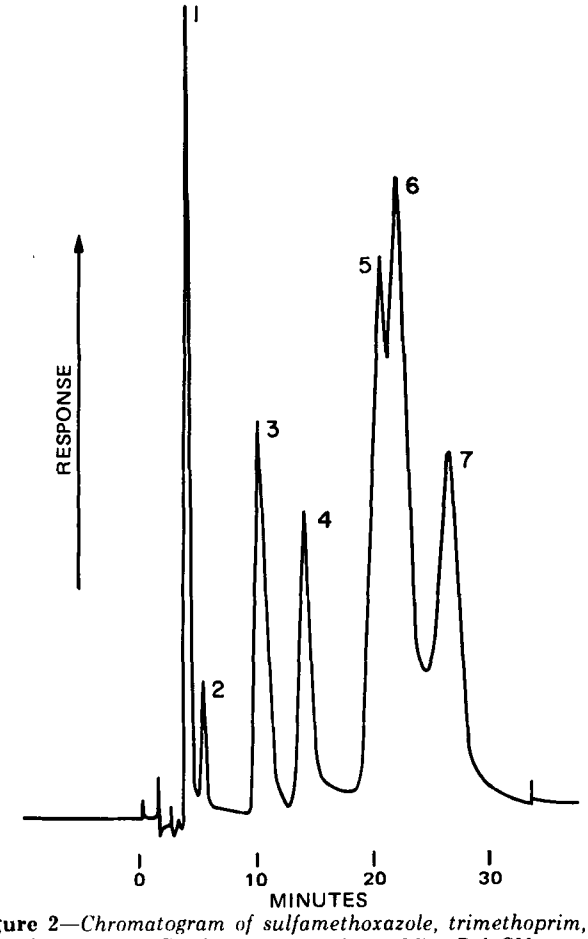

Figure 2—*Chromatogram of sulfamethoxazole, trimethoprim, and related compounds. Conditions were: column, MicroPak CN-10; mobile phase, methanol–ethyl acetate–diethylamine–heptane (16.8:1:75); and flow rate, 1.0 ml/min. Key: 1, trimethoprim precursor A; 2, trimethoprim precursor B; 3, trimethoprim; 4, N'-acetylsulfamethoxazole; 5, sulfanilamide; 6, sulfamethoxazole; and 7, isosulfamethoxazole.*

from the compounds of interest using the system without diethylamine; sulfanilic acid was eluted after all of the other sulfa compounds.

In some instances, 0.1 *N* HCl in methanol was chosen as the extraction solvent to ensure complete extraction of the drug substances from the matrix. Comparison of the HPLC results with results obtained using wet chemical procedures is made in Table I. Peak height responses *versus* amounts injected were linear for the formulation ranges for all components; however, deviations from linearity were found for many constituents when very high amounts were injected. Using the procedures described in this report, detection limits in the nanogram range were obtained. The determination of any compound tested in Table I, alone or in combination with another active drug substance, had a relative standard deviation of ±1.2%. An exception was found for the determination of trimethoprim and sulfamethoxazole formulated together, where the relative standard deviation was ±2.5%.

To ensure column-to-column duplication of the separation, three columns from the same supplier were evaluated. Differences in component retention times were noted with these columns, but only a slight modification in the mobile phase ratio was needed to effect resolution within a reasonable time.

In summary, the HPLC method presented was reliable, reproducible, rapid, and specific and should be useful for sulfa drug determinations in tablets, solutions, creams, and suspensions.

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Antibacterial Activity of *Artemisia herba-alba*

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Abstract □ The antibacterial activity of *Artemisia herba-alba* was investigated. Only its essential oil was active against some Gram-positive and Gram-negative bacteria. The essential oil was fractionated by column chromatography, and these fractions were tested for antibacterial activity. The principal component of the most active fraction was santolina alcohol.

Keyphrases □ *Artemisia herba-alba*—antibacterial activity, essential oil, column chromatography □ Antibacterial activity—evaluated for *Artemisia herba-alba* essential oil, column chromatography □ Folk medicine—*Artemisia herba-alba*, antibacterial activity of essential oil

Artemisia herba-alba Asso.[1], known as the desert wormwood, is a dwarf shrub growing in North Africa and the Middle East. This plant is used by the local population as an anthelmintic and for other purposes in folk medicine such as relief of coughing, intestinal disturbances, colds, measles, and muscle weakness.

Recently, several compounds were isolated from the aerial parts of this plant and their structures were established. These compounds were the sesquiterpene lactones

[1] Plant material was collected and identified as *Artemisia herba-alba* Asso. (Compositae) by Dr. Avinoam Danin, Department of Botany, The Hebrew University, Jerusalem, Israel. A voucher specimen (AHA-1) representing material collected for this investigation is available for inspection at the Department of Pharmacognosy (Dr. R. Segal), School of Pharmacy, The Hebrew University, Jerusalem, Israel.

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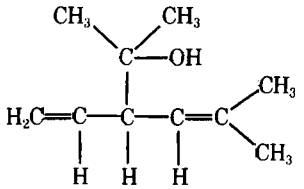

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santonin (2), herbolides A, B, and C (3), and a flavone derivative (4). Santonin is well known as a "vermifuge" (5) and is probably responsible for the anthelmintic activity of *A. herba-alba*. No other biological activities of *A. herba-alba* or of compounds isolated from it have been reported.

Because the plant extracts were never contaminated with microorganisms, even after staying at room temperature for very long periods, the antibiotic activity of *A. herba-alba* was investigated.

EXPERIMENTAL

Extraction of Plant Material—*A. herba-alba* was collected near Sde-Boker (Negev desert), Israel. The dried flowers, leaves, and small stems (5 g) were crushed and extracted with methanol in a soxhlet apparatus. The waxy materials, which precipitated after cooling, were filtered and discarded. The methanol was evaporated, and the dark-green residue was dissolved in methanol to a defined concentration.

Steam Distillation of Plant Material—The dried flowers, leaves, and small stems (100 g) of *Artemisia* were crushed and steam distilled. The oil yield was about 1%. The plant material left after steam distillation was filtered, dried at 40°, and extracted with methanol as described.

Column Chromatography of Distillate—The volatile oil (200 mg) was chromatographed on a 10-g silica gel column and eluted with methylene chloride. The column was monitored by TLC, and five crude fractions were collected. These fractions were screened for antibiotic activities without further purification.

Bacterial Strains—The bacterial strains[2] used were: *Staphylococcus aureus*[3], *Escherichia coli*[4], *Salmonella typhosa*[5], *Shigella sonnei*[6], and *Streptococcus hemolyticus*[7].

Antibacterial Activity Detection by Disk Method—The essential oil and its fractions were diluted with methanol to a concentration (per milliliter) that was 100-fold the amount applied on the disks. Paper disks[8] of 6-mm diameter were impregnated with 0.01 ml of these solutions. The tested bacterial strains (0.1 ml of a log phase suspension of 10^9 cells/ml) were spread on petri plates containing nutrient agar[9]. The dried impregnated disks were placed on the inoculated plates. The plates were incubated at 37° for 24 hr, and the inhibition zones were measured.

Antibacterial Activity Assay in Liquid Cultures—The preparations were dispersed in 2% dimethyl sulfoxide (5 mg/ml), and 15 ml of this solution was added to 7.5 ml of sterile nutrient broth[9]. Further dilutions were made by the transfer of 4.5 ml of the solution to 4.5 ml of sterile nutrient broth. Similar dilutions were made from 2% dimethyl sulfoxide as a control. The suspension (0.1 ml) of the tested bacterial strains (10^4 log phase cells/ml) was added to each tube, and the cultures were incubated at 37° for 24 hr. Turbidity was measured by a photometer[10] at 420 nm. The absorption of the uninoculated control was subtracted.

[2] Taken from the strain collection of the Department of Clinical Bacteriology, The Hebrew University-Hadassah Medical School, Jerusalem, Israel.
[3] Identified as Gram-positive cocci, which fermented mannitol and coagulated plasma.
[4] Identified as motile Gram-negative rods, which fermented lactose, produced acid from glucose (methyl red test) and indole from tryptophan, utilized citrate, and did not produce acetoin (Voges-Prosqauer test).
[5] Identified as motile Gram-negative rods, which did not ferment lactose, produced acid but no gas from glucose and mannose, and produced hydrogen sulfide from thiosulfate.
[6] Identified as nonmotile Gram-negative rods, which fermented mannitol and lactose.
[7] Identified as Gram-positive cocci, which produced β-hemolysis on blood agar.
[8] Whatman, 3M.
[9] Difco.
[10] Klett-Summerson photoelectric colorimeter, Klett Manufacturing Co., New York, N.Y.

Table I—Antibacterial Activity of Various Plant Fractions Tested by the Disk Method

Fraction	Diameter of Growth Inhibition[a], mm	
	S. aureus	*E. coli*
Whole plant extract	14[b]	11
Essential oil (2.5 mg)	18	13
Residue extract[c] (2.5 mg)	6[d]	6

[a] Including 6-mm diameter of the disk. [b] By an amount extracted from 1 g of dry plant material as described. [c] Extract of the residual material after steam distillation. [d] Lack of activity.

Table II—Inhibition[a] of Growth of Various Bacterial Strains in Liquid Cultures by the Essential Oil

Essential Oil Concentration, mg/ml	*S. aureus*	*E. coli*	*Sal. typhosa*	*Sh. sonnei*	*St. hemolyticus*
0.8	100	50	100	78	100
0.4	40	26	73	67	100
0.2	0	16	64	55	73
0.1	0	6	45	45	46
0.05	0	0	45	45	36

[a] Inhibition was determined by the dilution method described and is expressed as percentage of decrease in culture turbidity from the turbidity of the control culture.

Table III—Antibacterial Activity of Essential Oil Fractions against *S. aureus* Tested by the Disk Method

Fraction	Diameter of Growth Inhibition[a], mm
1	6[b]
2	14
3	56
4	16
5	16

[a] Including 6-mm diameter of the disk. Each disk contained 1 mg of material. [b] Lack of activity.

RESULTS AND DISCUSSION

The aerial parts of *A. herba-alba* were tested for antibacterial activities. The antibiotic activity observed for the whole plant extract was concentrated in the essential oil (Table I). The oil inhibited the growth of five bacterial strains (Table II), both Gram positive (*S. aureus* and *St. hemolyticus*) and Gram negative (*E. coli, Sal. typhosa,* and *Sh. sonnei*). *St. hemolyticus* was slightly more sensitive to the oil than were the other tested organisms.

For the detection of the active antibacterial principle, the oil was subjected to column chromatography. Five fractions were collected according to their polarity and were tested for antibiotic activity by the disk method. *E. coli* and *S. aureus* were selected as representatives from Gram-negative and Gram-positive bacteria. The results (Table III) indicate that only fraction 3 possessed significant antibiotic activity, so the chemical composition of this fraction was investigated. Repeated column chromatography yielded a pure compound, which was identified as santolina alcohol (I) by its optical rotation value and IR and NMR spectra (6).

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