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A Research Note

Antimicrobial Activity of Compounds from *Artemisia campestris*

SALEM M. THARIB[a], SAID O. GNAN[2] and G. BRYAN A. VEITCH[3*]

Department of Pharmacognosy, Faculty of Pharmacy and Department of Food Science, Faculty of Agriculture, Al-Faateh University, P.O. Box 13645, Tripoli, Libya and Department of Pharmacy, University of Aston in Birmingham, Gosta Green, Birmingham B4 7ET, England

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ABSTRACT

The antimicrobial activities of several compounds isolated from *Artemisia campestris* were studied using an agar diffusion technique. At a concentration of 125 μg/ml, six of the extracted compounds inhibited growth of *Staphylococcus aureus*, three of the compounds inhibited *Escherichia coli* and two inhibited growth of *Proteus vulgaris*. *Pseudomonas pyocyanea* was resistant to all extracted compounds.

During a continuing search for antimicrobial compounds from plants, an investigation was initiated on the leaves of *Artemisia campestris* (Family *Compositae*). The *Compositae* is the largest family of flowering plants and contains about 900 genera and some 1300 species. Compared with some other families such as the *Leguminosae*, the number of important economic products derived from it is relatively small. Chemical research in recent years has increased due to medical interest in the family, e.g. some plants in this family have been found to possess antitumor or antimicrobial activity while some are used commercially for their latex (*8*).

Many of the species of *Artemisia* have found their way into folklore medicine (*1*). In Libya, the genus *Artemisia* is represented by seven species (*5*), namely *A. arborescens* L, *A. campestris* L, *A. glutinosa*, *A. herba-alba*, *A. judaica*, *A. monosperma* and *A. variabilis*. Several workers have reported on the antimicrobial activities of plant extracts from the genus *Artemisia*. Imai et al. (*4*) studied the antimicrobial effect of *A. capillaris* and found that the active principle, Capillin, was guite effective in vitro against dermatophytes. The volatile oil of the same plant proved to be active against pathogenic dermatophytes. Hyuk et al. (*3*) investigated the antifungal effects of different extracts of *A. japonica*. Of the extracts examined, only the alcohol and the acetone extracts inhibited fungal growth. In 1966, Milova et al. (*6*) reported that the sesquiterpene γ-lactone isolated from *A. sieveiana* was active against *Oidium lactis*, *Saccharomyces cerevisiae* and *Helminthosporum sativum*. Two water-soluble compounds containing two active peptides were isolated from *A. tridenata* by Ramirez et al. (*7*). These compounds were shown to possess minimum inhibitory concentrations (MIC) of 30 μg/ml against a number of *Bacillus* species as well as *Staphylococcus aureus*. However, the MIC against *Streptococcus faecalis* was 70 μg/ml while that against *Escherichia coli*, *Aeromonas hydrophilla*, *Proteus vulgaris*, *Salmonella gallinarum* and *Pseudomonas aeruginosa* was reported to be as high as 200 μg/ml.

This study was undertaken to investigate the potential antimicrobial activity of the crystalline compounds isolated from *A. campestris*.

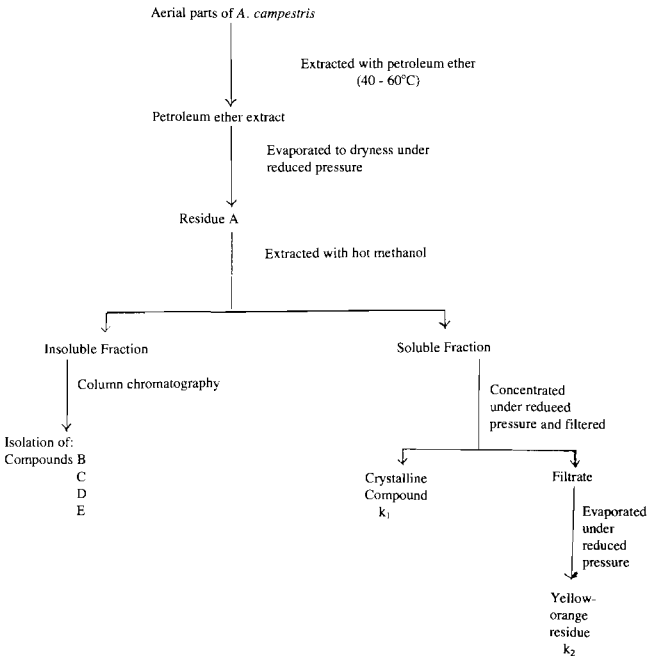

Figure 1. *Extraction scheme for* A. campestris.

[1]*Department of Pharmacognosy, Al-Faateh University.*
[2]*Department of Food Science, Al-Faateh University.*
[3]*University of Aston in Birmingham.*

TABLE 1. *Antimicrobial activity of the compounds isolated from the aerial parts of* A. campestris *tested in the petri plate diffusion assay test.*

Compounds (125 μg/ml)	*S. aureus*	*E. coli*	*P. pyocyanea*	*P. vulgaris*
A	+++[a]	++	−	++
B	+++	++	−	++
C	++[b]	−	−	−
D	+++	++	−	−
E	−[d]	−	−	−
k_1	+[c]	−	−	−
k_2	+	−	−	−

[a] + + + Inhibition zone 10-15 mm.
[b] + + Inhibition zone 5-10 mm.
[c] + Inhibition zone 2-5 mm.
[d] − No inhibition.

MATERIALS AND METHODS

Extraction and isolation of compounds

The plants were collected in November 1980 from the Zoarra region of Libya. Voucher specimens are held at the University of Al-Faateh herbarium. Air-dried, powdered, aerial parts (1.3 kg) were extracted with petroleum ether (40-60°C) for 24 h using the Soxhlet apparatus. The dark brown extract was evaporated to dryness under reduced pressure, yielding a brown residue (2.4% w/w) with a bitter taste and a pungent odor (A). This residue (Fig. 1) was exhaustively extracted with hot methanol which upon initial concentration yielded the crystalline compound (k_1) and on further concentration under reduced pressure gave a yellowish orange residue (k_2). The methanol-insoluble fraction was separated on an aluminum oxide column from which elution was effected with chloroform (30 drops/min). Altogether 120 fractions (20 ml) were collected and each fraction was analysed by thin layer chromatography using silica-gel GF_{254} as the stationary phase and toluene : ethyl acetate (9 : 1) as the mobile phase. Spots were located by anisaldehyde reagent. Those fractions yielding single spots running to the same R_f value were combined, evaporated to dryness and crystallized from an ethanol : methanol (1 : 1) mixture. Using this technique, four pure compounds (B, C, D, E) were isolated in small quantities.

Assay organisms

All the compounds were tested for activity against *S. aureus*, *E. coli*, *Pseudomonas pyocyanea* and *P. vulgaris*. The bacteria were grown on Brain Heart Infusion (BHI) agar (Difco) in Roux bottles for 1 week at 37°C and harvested with the use of distilled water and glass beads (4.5-mm diameter). The suspension was washed three times with sterile distilled water and finally re-suspended in water at a concentration of 3×10^{10} colony forming units per milliliter.

Antimicrobial assay

The quantitative evaluation of the antimicrobial activities of the pure compounds isolated from the aerial parts of *A. campestris* was accomplished using the agar diffusion technique. Twenty milliliters of base agar (regular melted agar) was overlaid with 5 ml of seed agar (base agar with inoculum of bacteria) previously inoculated with sufficient bacterial cells to yield a lawn of growth. After solidification of the seed agar, four wells, 15 mm in diameter and 3.0 mm deep were cut with a sterile brass cork borer and positioned one per quadrant of the 100-mm petri plates. Each plate of four wells received 0.3 ml of ethanol (negative control) in one well and 0.3 ml of each of three different isolated compounds dissolved in ethanol in each of the remaining wells. At least three replicate plates completed the test series for most assays. Plates were then incubated at 37°C until extensive and uniform colony growth was evident (24 - 48 h.)

Antibacterial activity was measured as zones of inhibition of colony growth around wells. Zone diameters were measured on a screen after × 10 magnification. Under the conditions of our assays the coefficient of variation of the diameter of a single zone was repeatedly found to be approximately 1%. Only the distinctly clear zones were measured. Data are expressed as diameter of zone corrected for well diameter.

RESULTS AND DISCUSSION

Table 1 summarizes the results of all the assays. A comparison of the isolated compounds expressed as MIC gives some indication of their antimicrobial activities. Residue A had a high level of activity against *S. aureus* and *E. coli* but a lower level of activity against *P. vulgaris* and none against *P. pyocyanea*. Growth of both *S. aureus* and *E. coli* was also inhibited by compounds B and D. The lower levels of activity against *Proteus* exhibited only by compounds A and B must closely reflect the true antimicrobial effect of B since A is a mixture of all the pure compounds isolated and all the remaining compounds are inactive against *Proteus*. Compound C was only moderately active against *S. aureus* and completely inactive at this concentration against the remaining test organisms. No activity against *P. pyocyanea* could be demonstrated by any of the isolated compounds.

Earlier observations (2) have led to a concept that infection can be halted when a specific interaction between a plant host and microorganisms leads to accumulation of certain chemical compounds. In the *Compositae,* these accumulating antimicrobial substances appear to be polyacetylenes. However, at any given time the plant may produce more than one chemical depending on the condition of attack.

In the past few years, it has become known that accumulation of these chemical compounds is not only dependent on a specific host-parasite interaction but may also occur by a wide range of biological, chemical and physical trauma. Some of the non-specific stimuli known to incite the host to produce these novel compounds are UV light, visible light, cut-injury, bruising, chilling injury, etc.

From the present results, one cannot be sure whether these compounds which were isolated from the aerial parts of *A. campestris* were the natural constituents of this plant or whether they were produced by exposure to adverse conditions. One possible theory is that these antimicrobial compounds were produced as stress metabolites due to high temperature exposure during growth. Another theory is that these compounds may have been produced when the plants were cut.

Further studies are to be carried out on *Artemisia* plants growing under different conditions to determine whether the effect of environmental stress does induce the production of these compounds. However, if these compounds are produced naturally in these species, then they may be an important source of new antimicrobial compounds.

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