FRACTIONATION OF EXTRACTS OF LITHOSPERMUM ARVENSE L. AND THEIR ACTIVITY AGAINST MOSQUITO LARVAE

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ABSTRACT. Methanol extracts of corn gromwell (Lithospermum arvense L.) are toxic to larvae of mosquitoes (Aedes aegypti). The active principle of this extract was concentrated by a fractionation sequence that included solvent partitioning, countercurrent distribution and preparative thin-layer chromatography. Fractionation at each stage was monitored by bioassays with mosquito larvae. Activity was concentrated in the hexane-soluble portion of the extract. After further fractionation by 10-stage countercurrent distribution followed by 200-tube countercurrent distribution, one form of activity was concentrated between tubes 80 and 90, as shown by treating larvae at 10 ppm. Other active components were concentrated at tubes 1 and 190.

INTRODUCTION

Several Lithospermum species have been used as herbal medicines in various parts of the world. Natural product chemists have given considerable attention to these species, particularly emphasizing the constituents responsible for antifeertility activities. These investigations have resulted in the identification of lithospheric acid and rosmarinic acid (Kelley et al. 1975; Wagner and Wittman 1974). Recently Sosa et al. (1977) isolated and characterized a new cyanoglucoside from L. purpureo-caeruleum.

Restrictions on the use of chemicals for insect control have stimulated investigations of the insecticidal properties of plant materials. In their studies of extracts of 36 plant species, Supavarn et al. (1974) tested extracts on larvae of Aedes aegypti L. mosquitoes. They found significant activity (survival rate, 22.5% or less) in 5 extracts, including those from Lithos-

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permum arvense L., or corn gromwell. L. arvense was chosen for the present study because its crude extract appeared to contain the most toxic substances.

L. arvense is a native of Eurasia and is a member of the Boraginaceae. This weed, of minor importance, occurs in isolated areas over a wide area. It is found in Kentucky in disturbed areas, in fall-sown small grains, and in pastures and hay fields. Although designated as either an annual or a biennial in Gray's Manual of Botany (1950), the plant acts as a winter annual in Kentucky. It germinates in early to late fall and forms a low rosette. In early spring upright growth takes place and one to five stems develop which are from 20 to 70 cm in length. Small white to bluish blooms appear from April to June with seeds ripening over a corresponding period. Plants mature and die by the middle of July.

In this paper, the fractionation of the extracts of L. arvense are described. Each step of solvent partitioning, countercurrent distribution and chromatographic separation was followed by a bioassay of the resulting fractions for mosquito larvicidal activity.

MATERIALS AND METHODS

PLANT COLLECTION. Specimens of the above ground parts of L. arvense for extraction and bioassay were harvested at a stage near maximum growth. The oldest
leaves were beginning to senesce and the
1st seeds were brown to black in color.
Harvest dates over a 5-year period
ranged from May 8 to June 2. Plants were
dried at 70°C for 24 hr and ground to
pass through a 40-mesh screen.

Extractions. The dried and finely
ground whole plant material (1,364 gm)
was placed in a glass percolator and
covered with methanol. After overnight
contact at ambient temperature,
the methanol solution was drained and the
solid material was covered again with
fresh methanol; this procedure was re-
peated 5 times. Combined extracts were
concentrated with a rotating evaporator
to a thick syrup, which was partitioned
between water and hexane (2 liters of
each). The hexane solution was not
washed with water, but was dried imme-
diately over sodium sulfate. Both phases
were taken to dryness with a rotating
 evaporator.

Countercurrent Distributions.
Hexane solubles (56.0 gm) were sepa-
rated into a series of fractions by countercurrent distribution (CCD) (Litchfield
1972) in 10 stages with 500 ml of each
phase per “tube” (1 of 10 Erlenmeyer
flasks). The mobile layer was the upper
phase of a solvent system prepared by
mixing hexane (b.p. 68-70°C) with an
equal volume of methanol-water (85:15,
v/v). A rotating evaporator was used to
isolate the resulting 10 fractions. CCD
also was conducted with a 200-tube
Craig-Post apparatus (Dutton 1954).5
Selected fractions (13.1 gm) from 10-tube
CCD were subjected to further fractiona-
tion by 200-tube CCD with the solvent
system hexane-chloroform-ethanol-water
(35:15:40:10, v/v/v/v) (Wetterau et al.
1964). Throughout the distribution,
tubes contained 40 ml of each phase.
After the 200 fundamental transfers,
contents of every 10th tube were evapo-
rated and weighed to establish a distribu-
tion curve.

Chromatographic Methods. Pre-
parative thin-layer chromatography
(TLC) of various fractions was carried out
with chloroform-methanol solvent sys-
tems on 2-mm layers of Silica Gel G F 254
(Merck). The separated bands were
located under ultraviolet light and were re-
covered from the adsorbent by conven-
tional procedures.

Biological Activity. Bioassays of
various fractions from L. arvensis extract
were tested against 3rd instar A. aegypti
L. as described by Supavarn et al. (1974).
Because the presently used extracts had
been further purified, only 10 ppm on a
dry weight basis were used in this study,
instead of 100, 500 and 1,000 ppm as
used in the previous study.

Results and Discussion
Methanol extraction of the dried plant
material (1,364 gm) provided 185.5 gm of
product which, through solvent parti-
tioning, afforded 56.3 gm of hexane-
soluble and 129.2 gm of water-soluble

5 The mention of firm names or trade prod-
ucts does not imply that they are endorsed or
recommended by the U.S. Department of Ag-
iculture over other firms or similar products
not mentioned.

Figure 1. Ten-stage countercurrent distri-
bution of hexane-soluble portion of gromwell
extract.
matter. According to bioassay results, the active principle was concentrated mainly in the hexane-soluble portion. Ten-stage CCD of this hexane-soluble portion (13.1 gm) produced the weight distribution indicated in Figure 1, with the active principle concentrated in fractions 3 through 8. A 200-tube CCD of combined fractions 3–8 (2.8 gm) gave the weight distribution shown in Figure 2. Bioassay of samples from every 10th tube—the same ones used for establishment of the weight distribution curve (Figure 2)—indicated that activity was concentrated around tubes 80 and 90.

A summary of selected day counts of larvae, pupae and adults is presented in Table 1. These results give a partial summary of data of the bioassay of the materials from the 200-tube CCD. In conducting these screening bioassays, counts of larvae were made at the beginning of the test and each day thereafter for 15 days or longer if significant numbers of larvae or pupae were still present. The 10 ppm concentration proved to be satisfactory in locating the presence of active ingredients.

Although larval mortality was over 50% in tube 1 and over 90% in tube 190 by day 5, these fractions were not subjected to further study because of the availability of

Figure 2. Further fractionation of gromwell extract by 200-tube countercurrent distribution.
Table 1. Bioassay results for gromwell extract fractions from 200-tube countercurrent distribution with *Aedes aegypti*.\(^a\)

<table>
<thead>
<tr>
<th>Tube Number</th>
<th>Larvae</th>
<th>Pupae</th>
<th>Adults</th>
<th>Dead</th>
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<td>Day 1</td>
<td>Day 5</td>
<td>Day 9</td>
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<td>4.8 c</td>
<td>3.0 c-f</td>
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<td>10.0 ab</td>
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</table>

\(^a\) Concentration of each extract was 10 ppm on a dry weight basis.
\(^b\) Numbers are from the average of 4 observations per treatment with approximately 10 3rd instar larvae each.
\(^c\) Means within a column followed by the same letter are not significantly different (P=0.05) (Duncan, 1955).
the extract. Tubes 100 through 180 contained substances which delayed pupation.

Bioassays were run on each of the 4 fractionation materials but in the interest of brevity only the 200-tube CCD assay is given.

After pooling of residues from CCD tubes 81–119, further fractionation by preparative TLC (solvent system = chloroform-methanol, 75:25, v/v) yielded the following fractions in order of ascending Rf (fraction number, weight percent recovered): 1, 8%; 2, 14%; 3, 44%; 4, 34%. Fraction 4, the most active of this series according to bioassay, was further resolved by TLC (solvent system = chloroform-methanol, 95:5, v/v) into nine bands. In order of ascending Rf these were grouped as follows (fraction, weight percent recovered): A, 23%; B, 28%; C, 16%; D, 16%; E, 17%. Fraction D was the most active of this series according to the mosquito larva assay; some activity was observed in Fraction E as well. Fractions D and E were not obtained in sufficient quantities to facilitate further separation or characterization.

CONCLUSION

It would appear that more than one biologically active material is present in the methanol extract of L. arvense. This was illustrated in the 200-tube CCD fractionation where tubes 1, 80, 90 and 190 had definite larvicultural properties. A 2nd group which retarded larval growth and subsequent pupal and adult development was found in tubes 100–180.

The active components in L. arvense have not been identified. On the basis of solubilities, these active ingredients do not appear to be any of the compounds characterized by Kelley et al. (1975), Wagner and Wittmann (1974) or Sosa et al. (1977).

ACKNOWLEDGMENTS

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Literature Cited


