ANTIBACTERIAL SUBSTANCES FROM PLANTS COLLECTED IN INDIANA

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The possibility that naturally occurring compounds showing pronounced antibiotic activity may be discovered in common plants offers an intriguing possibility for economical production of substances of interest and importance. During the summer of 1944 it was possible to collect and test substances from a number of plants in Indiana. It is the purpose of this report to give the details of this preliminary survey.

Osborn (1943) reported the results of examination of 2,300 samples from English plants and concluded that members of the Ranunculaceae offered most promise. Pederson and Fisher (1944) studied substances, in the juice of cabbage and other vegetables, which were active against gram-negative bacteria. Cavallito and Bailey (1944) demonstrated cysteine inactivation of active principles of various plant species. Lucas and Lewis (1944) reported preliminary results of a systematic survey of members of families of higher plants.

EXPERIMENTAL

One or more samples of approximately 120 plant species were collected, mostly in Monroe County, Indiana, and transported to the laboratory in the usual ecological collection boxes. For the majority of specimens, the testing was done immediately or within 24 hours. When not used at once, the samples were refrigerated until time for testing. The exact list of species will be given later, but in the survey an attempt was made to include as great a variety of samples within the classification system as possible, yet some thought was given to the availability or ease of production of the species to be tested. Certain specimens were included because of previous interest, folklore or otherwise, in the medicinal value of the species. Deam (1940) was used as the authority for assignment of names of species, etc., except for a few cultivated varieties.

For testing, the juice of the specimen was expressed, without use of solvents, from the plant tissue by means of a Carver hydraulic press. Even in plants which were not especially succulent sufficient juice could be obtained by this method to supply the small amount needed for assay. The expressed juice was placed immediately, by means of a clean Wright pipette, into glass cylinders, as in the familiar Oxford cup (Abraham *et al.*, 1941) for assay of penicillin. The samples were placed in duplicate cups; one cup was on agar seeded with spores of *Bacillus subtilis* in approximately the concentration specified by Foster and Woodruff (1944). The second cup was on agar seeded with an 18-hour nutrient broth culture of *Escherichia coli*. The medium was the nutrient agar suggested by Schmidt and Moyer (1944) and the incubation temperature was

30 C. The results were usually observed at the end of 14 to 16 hours; a Quebec counter magnifying lens was used to improve accuracy in the measurement of the zones of inhibition or stimulation.

RESULTS

It is evident that many of the species showed some antibacterial substances for one or both of the test organisms. An additional group of plants, in contrast to these, produced a marked stimulation of the bacterial cultures as shown by a zone of more luxuriant growth in the region of the cup in comparison with that on the edge of the plate. Other samples showed no activity. For convenience, the exact values have been converted to the following symbols:

S	= zone of stimulation
0	= no activity
+	= inhibitory zone, 12.0 to 15.0 mm
++	= inhibitory zone, 15.1 to 19.9 mm
+++	= inhibitory zone, 20.0 to 24.9 mm
++++	= inhibitory zone, 25.0 to 30.0 mm
*	= zone definite but inhibition incomplete

The symbol or symbols before the slant line (/) in the list indicate the results using *B. subtilis*; and the symbols after this mark refer to activity for *E. coli*. In the majority of cases the values for duplicate samples (specimens collected at different dates or several specimens of the same species collected on the same date at different stations) confirmed the value obtained for the first sample tested. These have not been indicated, but in the cases of disagreement between duplicate samples the range of values is shown. Similarly, when various portions of the plant were tested separately, the results have been combined unless different values were obtained.

The following list shows the plants collected and the results obtained:(1) ACANTHACEAE: *Dianthera americana* (dense-flowered water willow)—0/0. (2) ACERACEAE: *Acer negundo* (box elder)—0/0. (3) ALISMACEAE: *Sagittaria* sp. (arrowleaf)—0/S, 0. (4) ANACARDIACEAE: *Rhus copallina* (shining sumac)—0/S; *R. glabra* (smooth sumac)—0/0; *R. typhina* (staghorn sumac)—S/S. (5) ANONACEAE: *Asimina triloba* (papaw)—0/0. (6) APOCYNACEAE: *Vinca minor* (periwinkle)—0/0. (7) ARACEAE: *Arisaema triphyllum* (jack-in-the-pulpit) leaves, stem, green berries—S/S; *Symplocarpus foetidus* (skunk cabbage) leaves—0/0, green fruit—0/+. (8) ASCLEPIADACEAE: *Ampelamus albidus* (bluevine)—S/S; *Asclepias verticillata* (horsetail milkweed)—0/0. (9) BALSAMINACEAE: *Impatiens pallida* (touch-me-not)—S/S. (10) BIGNONIACEAE: *Campsis radicans* (trumpet creeper)—0/0. (11) CAPRIFOLIACEAE: *Sambucus canadensis* (elderberry)—0/0. (12) CELASTRACEAE: *Celastrus scandens* (bittersweet)—0/S, 0; *Evonymus atropurpureus* (wahoo)—0/0. (13) COMPOSITAE: *Ambrosia elatior* (common ragweed)—+++,++,0/+++,0,S; *A. trifida* (giant ragweed)—0/0; *Arctium minus* (burdock) leaves, flowers, stem—+++, ++/S,++; *Caclia suaveolens* (Indian plantain)—S/+,+++; *Cichorium intybus* (chicory) —+/+,0; *Cirsium arvense* (Canada thistle)—0/0; *C. discolor*

(field thistle)—S/0; *Eupatorium perfoliatum* (boneset)—0/0; *E. purpureum* (joe-pye weed)—+*/+*; *Galinsoga ciliata* (quickweed)—0/0; *Helianthus mollis* (ashy sunflower)—++/0; *H. tuberosus* (Jerusalem artichoke)—0/0; *Heliopsis helianthoides* (sunflower heliopsis) all but root—0/0; *Ratibida pinnata* (cone-flower)—S/0; *Rudbeckia subtomentosa* (sweet coneflower)—0/0; *R. triloba* (brown-eyed Susan)—S/0; *Silphium integrifolium* (entire-leaf rosinweed)—S/S; *S. laciniatum* (compass plant)—S/S; *S. perfoliatum* (cup rosinweed)—0/0; *S. terebinthinaceum* (dock rosinweed)—0, S/S; *Tanacetum vulgare* (common tansy) —0/0; *Taraxacum palustre* (dandelion)—0/0; *Xanthium pennsylvanicum* (cockle-bur)—+/0. (14) CONVOLVULACEAE: *Cuscuta* sp. (dodder)—+/0. (15) ERICACEAE: *Gaultheria procumbens* (wintergreen)—0/0; *Gaylussacia baccata* (black huckleberry)—S/S; *Vaccinium vacillans* (dry-land blueberry)—0/0. (16) EUPHORBIACEAE: *Acalypha virginica* (three-seeded mercury)—0/0, S; *Euphorbia maculata* (nodding spurge)—S/S; *E. marginata* (snow-on-the-mountain)—0/0; *E. supina* (spurge)—+*/+++*. (17) GRAMINEAE: *Digitaria sanguinalis* (crab grass)—S/S; *Eleusine indica* (goose grass)—S/S. (18) IRIDACEAE: *Iris* sp. (Iris)—0/0. (19) JUGLANDACEAE: *Carya* sp. (hickory)—0/0; *C. laciniosa* (big-leaf shagbark hickory)—S/0; *Juglans nigra* (black walnut)—+/0. (20) LABIATAE: *Lycopus americanus* (American bugleweed)—0/0; *Monarda fistulosa* (bergamot)—S/S; *Nepeta cataria* (catnip)—S/0; *Physostegia virginiana* (Virginia false dragonhead)—0/0; *Prunella vulgaris* (selfheal)—0/0; *Teucrium canadense* (American germander)—0/0. (21) LAURACEAE: *Sassafras albidum* (sassafras)—0/0. (22) LEGUMINOSAE: *Apios americana* (potato bean)—0/0; *Cassia nictitans* (small-flower sensitive plant)—S/0; *Robinia pseudoacacia* (black locust)—0/S; *Strophostyles helvola* (trailing wild bean)—0/S; *Tephrosia virginiana* (Virginia goat's rue)—S/S. (23) LILIACEAE: *Allium cepa* (onion)—++, S/0; *A. sativum* (garlic)—+++/+++; *Asparagus officinalis* (garden asparagus)—0/0; *Convallaria majalis* (lily of the valley)—0/0; *Funkia subcordata* (day lily)—0/0; *Hemerocallis fulva* (day lily)—0/0; *Smilacina stellata* (starry false Solomon's-seal)—0/0. (24) MAGNOLIACEAE: *Liriodendron tulipifera* (tulip tree)—0/S. (25) MARTYNIACEAE: *Martynia louisianica* (unicorn plant)—0/+. (26) MENISPERMACEAE: *Menispermum canadense* (common moonseed)—S/S. (27) NYMPHAEACEAE: *Nymphaea odorata* (water lily)—0/0. (28) ONAGRACEAE: *Ludwigia alternifolia* (seedbox)—0/++*; *Oenothera pycnocarpa* (evening primrose)—0/S; (29) OSMUNDACEAE: *Osmunda cinnamomea* (cinnamon fern)—0/0; *O. regalis* (royal fern)—S/S. (30) OXALIDACEAE: *Oxalis europaea* (lady's sorrel)—+/++,+++; *O. grandia* (great yellow wood sorrel)—++/++. (31) PAPAYACEAE: *Carica papaya* (papaya)—S/S. (32) PHYTOLACCACEAE: *Phytolacca americana* (pokeberry) leaves, stems, flowers, greenberries—++,S/++, S. (33) PLANTAGINACEAE: *Plantago rugelii* (common plantain)—S/S. (34) POLYGONACEAE: *Polygonum arifolium* (halberd-leaf tearthumb)—0/0; *P. aviculare* (knot weed)—S/S. (35) POLYPODIACEAE: *Cystopteris fragilis* (brittle fern) —S/S; *Onoclea sensibilis* (sensitive fern)—S/S; *Pteridium latiusculum* (bracken) —0/0. (36) PRIMULACEAE: *Lysimachia nummularia* (moneywort)—0/0. (37) RANUNCULACEAE: *Berberis thunbergii* (Japanese barberry)—++,+/++,+;

Jeffersonia diphylla (twinleaf) root—0/S; *Ranunculus repens* (buttercup)—S/S. (38) ROSACEAE: *Agrimonia parviflora* (small flower agrimony)—0/0; *Paeonia* sp. (peony)—S/S; *Prunus serotina* (Black cherry)—0/0; *Spiraea tomentosa* (hardhack)—0/+++*. (39) RUBIACEAE: *Cephalanthus occidentalis* (hairy buttonbush)—+++/+++*. (40) SALICACEAE: *Salix babylonica* (weeping willow)—0/0. (41) SAXIFRAGACEAE: *Heuchera americana* (alumroot)—0/0; *Hydrangea arborescens* (smooth hydrangea)—+*/0. (42) SCROPHULARIACEAE: *Mimulus alatus* (monkey flower)—0/0. (43) SIMARUBIACEAE: *Ailanthus altissima* (tree of heaven) male and female plants—S/S. (44) SOLANACEAE: *Datura stramonium* (Jimson weed) leaves and stem—S/S; *Physalis subglabrata* (smooth ground cherry)—++/++, S; *Solanum carolinense* (horse nettle)—++/0; *S. dulcamara* (bittersweet nightshade)—0,S/0,S; *S. nigrum* (common nightshade)—S,0/S,0. (45) TILIACEAE: *Tilia heterophylla* (white basswood)—0/0. (46) TYPHACEAE: *Typha latifolia* (common cattail)—0/0. (47) ULMACEAE: *Ulmus americana* (American elm)—0/0. (48) UMBELLIFERAE: *Cicuta maculata* (water hemlock)—0/0; *Daucus carota* (Queen Anne's lace; wild carrot)—0/S. (49) URTICACEAE: *Boehmeria cylindrica* (false nettle)—0/0. (50) VIOLACEAE: *Viola* sp. (Violet) leaves and petioles—0/0. (51) VITACEAE: *Parthenocissus quinquefolia* (Virginia creeper)—0/0.

DISCUSSION

From the foregoing list it can be seen that, although the juice of several species showed antibacterial activity, no specimen was encountered which gave exceptionally high values. It must be remembered, however, that with this method of assay it would be necessary for the substance to be of such a nature that it would diffuse easily through the agar. It is possible that samples which show some activity by this method would be found to have a greater potency when tested by another method. Similarly, the method of preparation of the sample juice did not include extraction of substances by organic solvents, and it seems probable that compounds might be present which would appear only under such conditions. In a few samples, particularly with *Cephalanthus occidentalis*, two zones of inhibitory activity against *B. subtilis* were pronounced; this would indicate the probability of more than one compound. No information is available yet on the separation of these.

It is believed that the inhibitory activity of some samples may be explained easily by the presence of well-known substances which are known to be toxic (such as oxalic acid, etc.) and may not be due to some new substance. This might apply to *Allium*, *Oxalis*, etc. The two species of *Ambrosia* which were tested gave different results, and this was true of several repeat samples; *A. trifida* consistently failed to show any activity against either test organism whereas *A. elatior* gave strong inhibition in almost every sample tested.

The high percentage of samples showing stimulation of the test organisms is perhaps worthy of mention. When it occurred, the phenomenon was usually so striking as to argue against the probability of a simple nutrient being the only stimulatory factor. The basal medium used for seeding is fairly adequate in

nutrients; thus the plant juices may have supplied growth factors or similar compounds. It would seem that an investigation of this phenomenon might yield fruitful results.

SUMMARY

The results are reported of a preliminary survey of antibacterial substances in a series of 120 or more plant samples collected in Indiana during the summer of 1944. The juice of the plants, or particular portions of them, obtained by a Carver hydraulic press, was tested for inhibitory activity against *Bacillus subtilis* and *Escherichia coli* with the Oxford cup technique.

Representatives (1 to 50 specimens) of the following families were included: Acanthaceae, Aceraceae, Alismaceae, Anacardiaceae, Anonaceae, Apocynaceae, Araceae, Asclepiadaceae, Balsaminaceae, Bignoniaceae, Caprifoliaceae, Celastraceae, Compositae, Convolvulaceae, Ericaceae, Euphorbiaceae, Gramineae, Iridaceae, Juglandaceae, Labiatae, Lauraceae, Leguminosae, Liliaceae, Magnoliaceae, Martyniaceae, Menispermaceae, Nymphaeaceae, Onagraceae, Osmundaceae, Oxalidaceae, Papayaceae, Phytolaccaceae, Plantaginaceae, Polygonaceae, Polypodiaceae, Primulaceae, Ranunculaceae, Rosaceae, Rubiaceae, Salicaceae, Saxifragaceae, Scrophulariaceae, Simarubiaceae, Solanaceae, Tiliaceae, Typhaceae, Ulmaceae, Umbelliferae, Urticaceae, Violaceae, Vitaceae.

Although about one tenth of the specimens showed some degree of inhibitory activity against one or both test organisms, no sample was encountered which gave exceptionally high values. A marked stimulation of growth of the test organism was evident with many samples.

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