

COMPREHENSIVE SURVEY OF INDIGENOUS IRAQI PLANTS FOR POTENTIAL ECONOMIC VALUE. 1. SCREENING RESULTS OF 327 SPECIES FOR ALKALOIDS AND ANTIMICROBIAL AGENTS

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ABSTRACT.—Three hundred and twenty-seven indigenous Iraqi plant species were screened for their antimicrobial activities and for the presence of alkaloids. The plants represent 221 genera and 49 families. Ethanolic extracts were tested for the presence of alkaloids with both Mayer's and Dragendorff's reagents. Of these, 146 species, belonging to 109 genera distributed among 32 families, showed positive alkaloid tests to both reagents. Of these, 26 genera have not been previously reported in the literature as containing alkaloid-bearing plants.

The extracts were also tested for their *in vitro* antimicrobial activity by an agar dilution-streak method against six economically significant microbes. Five species were highly active, while another 90 species showed weak activity against one or more microorganisms.

The importance of screening little-studied plant species for the presence of substances having potential medicinal significance is widely understood. Despite intensive study in this area over the last two centuries, a surprising number of plants, indeed genera, remain relatively untouched except, perhaps, in the anti-tumor area where support funds have been relatively more available. Some sense of urgency is brought to these considerations because of the inroads increasing urbanization and intensification of cultivation are making upon the indigenous flora of many areas. Having available to us a relatively substantial and relatively untouched collection of the flora of Iraq, including some plants well known to be toxic to cattle and some of which are used in herbal medicine, we have begun a comprehensive, systematic study of these plants with the ultimate objective of identifying and isolating the active constituents from the most promising species.

Alcohol extracts of the plants have been tested for antibacterial and antifungal activity against six indicator organisms following the procedures outlined previously in this journal (2). Because of widespread continuing interest in their properties and the frequency with which they are found to have significant biological activity, we have also screened the plants for the presence of alkaloids. The results of our work on the first 327 species are presented herein.

EXPERIMENTAL

PLANT MATERIAL.—The plant materials for screening were authenticated, duplicate pressed specimens of reference materials obtained from the National Herbarium of Iraq, Botany Directorate of Abu-Ghrabi, about 15 km west of Baghdad.

EXTRACTION PROCEDURE.—The available dried plant materials were ground into coarse powders and extracted at room temperature with 80% ethanol. The solvent was removed, *in vacuo*, below 40° to produce the crude extracts.

ANTIMICROBIAL SCREENING.—The crude extracts were tested by an agar dilution method at concentrations of 1000 mcg/ml and 100 mcg/ml against the organisms listed in table 1, by the

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general procedure reported by Mitscher *et al.* (2). The antimicrobial activities of the extracts were expressed by (-) for no effect, (=) for partial growth inhibition, and (+) for complete growth inhibition.

PRELIMINARY ALKALOID TEST.—The crude extracts were extracted with 5% aqueous HCl. The acidic extract was filtered. A portion of the filtrate was tested with Mayer's reagent (3). The other portion was basified with aqueous ammonia and extracted with chloroform. The organic layer was tested on filter paper with Dragendorff's spraying reagent (4).

TABLE 1. Organisms used in screening of the plants for antimicrobial activity.

Number	Organism	ATCC no.	Classification
1	<i>Staphylococcus aureus</i> Smith strain	13709	Gram positive
2	<i>Escherichia coli</i>	9637	Gram negative
3	<i>Salmonella gallinarum</i>	9184	Gram negative
4	<i>Klebsiella pneumoniae</i> AD	10031	Gram negative
5	<i>Mycobacterium smegmatis</i> 607B	607	Acid fast
6	<i>Candida albicans</i>	10231	Yeast

Both tests were required to be positive in order to confirm the presence of alkaloids. Otherwise, positive results with only Dragendorff's reagent, but not with Mayer's reagent, were considered negative for our purposes (5). Furthermore, depending on Mayer's reagent, if the reaction produced slight opaqueness, it was recorded as +; if the reaction produced a definite turbidity but no precipitate, it was recorded as ++; the production of a definite heavy precipitate or heavy flocculation was recorded as +++. No attempt was made to make the results more quantitative.

RESULTS AND DISCUSSION

We have evaluated 334 extracts, representing 221 genera belonging to 49 families. These are presented in table 2 arranged alphabetically according to their families with their botanical names, plant parts and results of the screening tests. The results were encouraging as 28.4% of the plants showed antimicrobial activity, and 44.6% showed positive preliminary alkaloidal tests.

Ninety-three extracts showed positive results of at least 1000 mcg/ml against at least one microorganism. Of these, five were very promising plants, as they were active at 100 mcg/ml level. They are *Silene coniflora* Otth., *Lotus gebelia* Vent., *Allium dilutum* Stapf., *Linaria kurdica* Boiss et Hoh., and *Peganum harmala* L. (the seeds). More detailed fractionation work has been started on these plants in order to isolate and characterize the active constituents, and the results will be published in due course. Of the remainder (table 2), 73 extracts were active against only one organism; 16 against two organisms; and only 4 showed a wider range of activity. The latter, which are potentially more interesting, are: *Aleuropus lagopoides* (L.) Trin, and *Tamarix macro-carpa* (Ehrenb) Bge, each active against organisms 1, 5 and 6, *Ammannia baccifera* L.; active against 1, 3, 5 and 6; and *Peganum harmala* L. (the seeds), active against all of the test organisms.

It was also noted that *Mycobacterium smegmatis* was the most susceptible microorganism, being inhibited by most of the families found to possess activity (Caryophyllaceae, Compositae, Euphorbiaceae, Graminae, Hypericaceae, Labiatae, Leguminosae, Potamogetonaceae, Rutaceae, Umbelliferae, and Zygophyllaceae), followed by *Candida albicans*, inhibited by plants of the Euphorbiaceae, Geraniaceae, Labiatae, Leguminosae, Lythraceae and Polygonaceae. On the other hand, *Escherichia coli* and *Klebsiella pneumoniae* were very rarely inhibited by the tested plant extracts. These findings parallel closely our experience with indigenous United States plants.

TABLE 2. Iraqi plants tested for alkaloids and antimicrobial activity.

Family Botanical name ^a	Plant part(s) ^b	Preliminary alkaloid test ^c	Minimum inhibitory conc. (mcg/ml) vs. organisms no. ^d					
			1	2	3	4	5	6
Amaranthaceae								
<i>Alternanthera sessilis</i> (L.) R.Br.	Ap	-	-	-	-	-	-	-
<i>Amaranthus albus</i> L.	Ap	-	-	-	-	-	-	-
<i>Amaranthus graecizans</i> L.	Ap	-	-	-	-	-	-	-
Berberidaceae								
<i>Bongardia chrysopogon</i> (L.) Boiss.	Ap	-	-	-	-	-	-	-
<i>Bongardia chrysopogon</i> (L.) Boiss.	R	+	-	-	-	-	+1000	-
Boraginaceae								
<i>Anchusa italica</i> Retz.	Ap	-	-	-	=1000	-	-	-
** <i>Arnebia decumbens</i> (Vent.) Coss et								
Kral.	Ap	+	-	-	+1000	-	-	-
<i>Arnebia linearifolia</i> DC.	Wpl	-	-	-	=1000	-	-	-
<i>Asperugo procumbens</i> L.	Ap	+	-	-	-	-	-	-
** <i>Gastrocotyle hispida</i> (Forssk.) Bge.	Wpl	+	-	-	-	-	-	-
<i>Heliotropium bacciferum</i> Forssk.	Wpl	++	-	-	-	-	-	-
<i>Lappula spinocarpos</i> (Forssk.) Aschers.	Wpl	-	-	-	-	-	-	-
<i>Moltkiopsis ciliata</i> (Forssk.) Johnst. (Syn. <i>Lithospermum callosum</i> Vahl., <i>Moltkia callosa</i> [Vahl.] Wettst.)	Wpl	+	-	-	-	-	-	-
<i>Trichodesma molle</i> DC.	Ap	+++	-	-	-	-	-	-
Campanulaceae								
<i>Campanula flaccidula</i> Vatke.	Ap	-	-	-	-	-	-	-
<i>Campanula radula</i> Fisch.	Wpl	-	-	-	-	-	-	-
<i>Campanula sypsiensis</i> C. Koch.	Ap	+	-	-	-	-	-	=1000
<i>Mitaxia nuda</i> DC.	Ap	-	-	-	-	-	-	-
Capparidaceae								
<i>Cleome arabica</i> L.	Ap	-	-	-	-	-	-	-
<i>Cleome arabica</i> L.	R	---	-	-	-	-	=1000	-
<i>Cleome glaucescens</i> DC.	Ap	-	-	-	-	-	-	-
Caryophyllaceae								
<i>Dianthus orientalis</i> Adam.	Wpl	-	-	-	=1000	-	-	-
<i>Dianthus strictus</i> Banks et Soland.	Wpl	+	-	-	-	-	-	-
<i>Gypsophila capillaris</i> Forssk.	Ap	-	-	-	-	-	-	-
<i>Gypsophila ruscifolia</i> Boiss.	Ap	-	-	-	-	-	-	-
<i>Silene arabica</i> Boiss.	Ap	-	-	-	-	-	-	-
<i>Silene chlorifolia</i> Sm.	Wpl	-	-	-	-	-	-	-
<i>Silene conifera</i> Otth.	Wpl	-	-	-	-	-	+100	-
<i>Silene conoidea</i> L.	Wpl	-	-	-	-	-	-	-
<i>Silene dichotoma</i> Ehrh.	Wpl	-	-	-	-	-	-	-
<i>Silene linearis</i> Decne.	Ap	-	-	-	-	-	+1000	-
<i>Silene longipetala</i> Vent.	Wpl	-	-	-	-	-	-	-
<i>Silene pungens</i> Boiss.	Ap	-	-	-	-	-	=1000	-
<i>Stellaria</i> spp.	Ap	-	-	-	-	-	-	-
<i>Vaccaria pyramidata</i> Medik.	Ap	-	-	-	-	-	=1000	=1000
Chenopodiaceae								
<i>Aellenia subaphylla</i> (C. A. Mey.) Botsch.	Ap	+++	-	-	-	-	-	-
<i>Anabasis articulata</i> (Forssk.) Moq-Tand.	Wpl	+++	-	-	-	-	-	-
<i>Anabasis setifera</i> Moq-Tand.	Wpl	+	-	-	-	-	-	-
<i>Atriplex leucoclada</i> Boiss.	Wpl	-	-	-	-	-	-	-
<i>Bassia eriophora</i> (Schrad.) Aschers.	Wpl	+	-	-	-	-	-	-
<i>Bienertia cycloptera</i> Bge.	Ap	-	-	-	-	-	-	-
<i>Chenopodium album</i> L.	Ap	-	-	-	-	-	-	-
<i>Haloenemum strobilaceum</i> (Pall.) M.B.	Ap	-	-	-	-	-	-	-
<i>Haloxylon salicornicum</i> (MoQ.) Bge.	Wpl	+++	-	-	-	-	-	-
<i>Noaea mucronata</i> Forssk.	Ap	-	-	-	-	-	-	-

TABLE 2. Continued.

Family Botanical name ^a	Plant part(s) ^b	Preliminary alkaloid test ^c	Minimum inhibitory conc. (mcg/ml) vs. organisms no. ^d						
			1	2	3	4	5	6	
<i>Salsola crassa</i> M.B.	Ap	—	—	—	—	—	—	≠1000	
<i>Salsola incanescens</i> C. A. Mey.	Ap	++	—	—	—	—	—	—	
<i>Salsola jordaniicola</i> Eig.	Ap	+++	—	—	—	—	—	—	
<i>Salsola subaphylla</i> L.	Ap	+++	—	—	—	—	—	—	
<i>Seidlitzia rosmarinus</i> (Ehrenb.) Solms.-Laub.	Ap	+	—	—	—	—	—	—	
<i>Suaeda vermiculata</i> Forssk.	Ap	+++	—	—	—	—	—	—	
Cistaceae									
<i>Helianthemum leditolium</i> (L.) Mill.	Ap	—	—	—	—	—	—	—	
<i>Helianthemum salicifolium</i> (L.) Mill.	Ap	—	—	—	—	—	—	+1000	
<i>Helianthemum salicifolium</i> (L.) Mill.	R	—	—	—	—	—	—	—	
Compositae									
<i>Achillea conferta</i> DC.	Wpl	—	—	—	—	—	—	≠1000	
<i>Achillea fragrantissima</i> (Forssk.) Sch.-Bip.	Ap	+++	—	—	—	—	—	≠1000	
<i>Achillea micrantha</i> M.B.	Wpl	+	—	—	—	—	—	—	
<i>Achillea santolina</i> L.	Ap	—	—	—	—	—	—	—	
<i>Achillea vermicularis</i> Trin.	Ap	—	—	—	—	—	—	—	
<i>Anthemis pseudocotula</i> Boiss.	Ap	—	—	—	—	—	—	—	
** <i>Anvillea garcini</i> (Burm.) DC.	Wpl	++	—	—	—	—	—	—	
<i>Artemisia herba-alba</i> Asso.	Ap	—	—	—	—	—	—	≠1000	
<i>Artemisia scoparia</i> Waldst et Kit.	Wpl	—	—	—	—	—	—	≠1000	
** <i>Atractylis fava</i> Desf.	Wpl	++	—	—	—	—	—	—	
<i>Bidens tripartita</i> L.	Ap	—	—	—	—	—	—	—	
<i>Calendula aegyptiaca</i> Desf.	Ap	—	—	—	—	—	—	—	
<i>Calendula persica</i> C. A. Mey.	Ap	—	—	—	—	—	—	—	
<i>Carduus pycnocephalus</i> L.	Ap	+++	—	—	—	—	—	—	
<i>Carthamus</i> spp.	Ap	+++	—	—	—	—	—	—	
<i>Centurea ammoncyanus</i> Boiss.	Ap	++	—	—	—	—	—	—	
<i>Centurea behen</i> L.	Ap	+++	—	—	—	—	—	—	
<i>Centurea cana</i> Sibth et Sm.	Wpl	+++	—	—	—	—	—	—	
<i>Centurea rigida</i> Banks et Soland.	Wpl	++	—	—	—	—	—	—	
<i>Centurea sinatica</i> DC.	Ap	+++	≠1000	—	—	—	—	—	
<i>Centurea virgata</i> Lam.	Ap	+++	—	—	—	—	—	—	
<i>Chardinia orientalis</i> (Mill.) O. Ktze. (Syn. <i>Xeranthemum orientale</i> Mill.)	Wpl	—	—	—	—	—	—	—	
** <i>Chrysophthalmum montanum</i> (DC.) Boiss.	Wpl	+	—	—	—	—	—	—	
<i>Cirsium acarna</i> (L.) Moench.	Ap	++	—	—	—	—	—	—	
<i>Crepis partiflora</i> Desf.	Ap	+	—	—	—	—	—	—	
<i>Crupina crupinastrum</i> (Moris) Vis.	Ap	—	—	—	—	—	—	—	
<i>Echinops</i> spp.	Wpl	—	—	—	—	—	—	—	
<i>Eclipta alba</i> (L.) Hausskn.	Ap	—	—	—	—	—	—	≠1000	
<i>Filago spatulata</i> Presl.	Ap, F	—	—	—	—	—	—	—	
<i>Francoeuria crispa</i> (Forssk.) Cass.	Ap	—	—	—	—	—	—	—	
** <i>Gymnarrhena micrantha</i> Desf.	Ap	+	—	—	—	—	—	—	
<i>Helichrysum Aucheri</i> Boiss.	Ap, F	+	—	—	—	—	—	—	
<i>Koelpinia linearis</i> Pall.	Ap	—	—	—	—	—	—	—	
<i>Lactuca orientalis</i> Boiss. et Kotschy.	Ap	+	—	—	—	—	—	—	
<i>Launaea nudicaulis</i> (L.) Hook. F.	Ap	—	—	—	—	—	—	—	
** <i>Leontodon laciniatus</i> (Bertol) Widd.	Ap	+	—	—	—	—	—	—	
<i>Matricaria aurea</i> (Loefl.) Sch.-Bip.	Ap	—	—	—	—	—	—	—	
<i>Phagnalon rupestre</i> (L.) DC.	Wpl	—	—	—	—	—	—	—	
<i>Picris babylonica</i> Hand-Mzt.	Ap	—	—	—	—	—	—	—	
<i>Pluchea tomentosa</i> DC.	Ap	+	+1000	—	—	+1000	—	—	
<i>Pluicaria guestii</i> Rech.-F. et Rawi.	Ap	—	—	—	—	—	—	—	
<i>Rhanterium epapposum</i> Oliv.	Ap, F	—	—	—	—	—	—	—	
<i>Scorzonera papposa</i> DC.	Wpl	—	—	—	—	—	—	—	
<i>Scorzonera Rawi</i> Rech. f. et Guest.	Wpl	—	—	—	—	—	—	—	
<i>Senecio Desfontainei</i> Druce.	Wpl	+	—	—	≠1000	—	—	—	

TABLE 2. Continued.

Family	Botanical name ^a	Plant part(s) ^b	Preliminary alkaloid test ^c	Minimum inhibitory conc. (mcg/ml) vs. organisms no. ^d					
				1	2	3	4	5	6
	<i>Serratula cerinthefolia</i> Sibth. et Sm.	Ap	—	±1000	—	—	—	—	—
	<i>Tragopogon major</i> Jacq.	Wpl	—	—	—	—	—	—	±1000
	<i>Zoegea leptocrea</i> L.	Ap, F	—	—	—	—	—	—	—
Convolvulaceae									
	<i>Convolvulus oxyphyllus</i> Boiss.	Ap	+	—	—	—	—	—	—
	<i>Convolvulus reticulatus</i> Chosy.	Ap	+	—	—	—	—	—	—
	<i>Convolvulus stachydifolius</i> Choisy.	Ap	±	—	—	±1000	—	—	—
Cruciferae									
	<i>Aethionema grandiflorum</i> Boiss. et Hoh.	Wpl	—	—	—	—	—	—	—
	** <i>Anchonium elychnisifolium</i> (DC) Boiss.	Wpl	++	—	—	—	—	—	—
	<i>Arabis caucasica</i> Willd.	Wpl	++	—	—	—	—	—	—
	** <i>Aubrieta parviflora</i> Boiss.	Wpl	++	—	—	—	—	—	—
	<i>Capsella bursa-pastoris</i> (L.) Moench								
	S.I.	Ap	—	—	—	—	—	—	—
	<i>Cardaria draba</i> (L.) Desv.	Ap	++	—	—	—	—	—	—
	** <i>Carrichtera annua</i> (L.) Aschers.	Ap	+++	—	—	—	—	—	—
	** <i>Diplotaxis acris</i> (Forssk.) Boiss.	Ap	++	—	—	—	—	—	—
	** <i>Diplotaxis erucoides</i> (L.) DC.	Ap	±	—	—	—	—	—	—
	** <i>Diplotaxis harra</i> (Forssk.) Boiss.	Wpl	++	—	—	—	—	—	—
	** <i>Erucaria cakiloides</i> (DC.) O. E. Schulz.	Ap	++	—	—	—	—	—	—
	<i>Erysimum aciphyllum</i> Boiss.	Wpl	+	—	—	—	—	—	—
	<i>Farselia aegyptiaca</i> Turra.	Wpl	—	—	—	—	—	—	—
	<i>Horwoodia Dicksoniae</i> Turrill.	Ap	—	—	—	—	—	—	—
	<i>Isatis aegyptiaca</i> Scop.	Ap	+++	—	—	—	—	—	—
	<i>Lepidium Aucheri</i> Boiss.	Wpl	—	—	±1000	—	—	—	—
	** <i>Malcolmia africana</i> (L.) R. Br.	Ap	—	—	—	—	—	—	—
	** <i>Malcolmia grandiflora</i> (Bge.) O. Ktze.	Ap	±	—	—	—	—	—	—
	<i>Matthiola oxyceras</i> DC.	Wpl	—	—	±1000	—	—	—	—
	<i>Nasturtium officinale</i> R. Br.	Ap	±	—	—	—	—	—	—
	** <i>Physolychnis gnaphalodes</i> (DC.) Boiss.	Wpl	+	—	—	—	—	—	—
	** <i>Sauignya parviflora</i> (Del.) Webb.	Wpl	+	—	—	—	—	—	—
	** <i>Schimpera arabica</i> Hochst et Staud.	Ap	++	—	—	—	—	±1000	—
	<i>Sisymbrium septulatum</i> DC.	Ap	+	—	—	—	—	—	—
	** <i>Zilla spinosa</i> (L.) Prantl.	Ap	+++	—	—	—	—	—	—
Cucurbitaceae									
	<i>Colocynthis citrullus</i> (L.) O. Kunze.	Ap	—	—	—	—	—	—	—
	<i>Cucumis melo</i> var. <i>flexuosus</i> L.	Ap	—	—	—	—	—	—	—
	<i>Cucumis sativus</i> L.	Ap, F	—	—	—	—	—	—	—
	** <i>Lagenaria leucantha</i> (Duch) Rusby.	Ap	+	—	—	—	—	—	—
	<i>Luffa cylindrica</i> (L.) Roem.	Ap	—	—	—	—	—	+1000	—
Cyperaceae									
	<i>Cyperus conglomeratus</i> Rottb.	Wpl	—	—	—	—	—	—	±1000
	<i>Cyperus difformis</i> L.	Wpl	—	—	—	—	—	—	—
	<i>Cyperus longus</i> L.	Wpl	—	—	—	—	—	—	—
	<i>Cyperus michelianus</i> (L.) Linkb.	Ap	—	—	—	—	—	—	—
	<i>Fimbristylis dichotoma</i> (L.) Vahl.	Ap	—	—	—	—	—	—	—
	<i>Scripus maritimus</i> L.	Wpl	—	—	—	—	—	—	—
Dipsacaceae									
	<i>Cephalaria syriaca</i> (L.) Schrad.	Ap	+	—	—	—	—	—	—
	<i>Pteroccephalus plumosus</i> (L.) Goult.	Ap	—	—	—	—	—	—	—
	<i>Scabiosa olivieri</i> Coult.	Wpl	—	—	—	—	—	±1000	—
	<i>Scabiosa palaestina</i> L.	Ap	—	—	—	—	—	—	—
Euphorbiaceae									
	<i>Andrachne telephioides</i> L.	Ap	—	—	—	—	—	—	—
	<i>Chrozophora tinctoria</i> (L.) Raf.	Ap	—	—	—	—	—	±1000	±1000
	<i>Euphorbia chesneyi</i> (Klotzsch et Garcke) Boiss.	Ap	—	—	—	—	—	±1000	±1000
	<i>Euphorbia denticulata</i> Lam.	Ap	—	—	—	—	—	—	—

TABLE 2. Continued.

Family Botanical name ^a	Plant part(s) ^b	Preliminary alkaloid test ^c	Minimum inhibitory conc. (mcg/ml) vs. organisms no. ^d						
			1	2	3	4	5	6	
<i>Euphorbia macroclada</i> Boiss.....	Ap	—	—	—	—	—	—	—	
<i>Euphorbia petiolata</i> Banks et Soland.....	Ap	+++	—	—	—	—	≠1000	—	
<i>Euphorbia prostrata</i> Ait.....	Ap	—	—	—	—	—	—	+1000	
Gentianaceae									
<i>Centaurium spicatum</i> (L.) Frisch.....	Ap	—	—	—	—	—	—	≠1000	
<i>Centaurium tenuiflorum</i> (Hoffmgget Link) Fritsch.....	Wpl	—	—	—	—	—	—	—	
<i>Gentiana olivieri</i> Griseb.....	Wpl	—	—	—	—	—	—	—	
Geraniaceae									
<i>Ammonthamnus gibbosus</i> DC.....	Ap	+++	—	—	—	—	—	—	
<i>Biebersteinia multifida</i> DC.....	Ap	+++	—	—	—	—	—	—	
<i>Erodium ciconium</i> (L.) L'Her'.....	Wpl	—	—	—	—	—	—	—	
<i>Erodium glaucophyllum</i> (L.) L'Her'.....	Wpl	—	—	—	—	—	—	≠1000	
<i>Erodium laciniatum</i> (Cav.) Willd.....	Wpl	++	≠1000	—	—	—	—	≠1000	
<i>Geranium rotundifolium</i> L.....	Wpl	—	—	—	—	—	—	≠1000	
Gnetaceae									
<i>Ephedra alata</i> Decne.....	Ap	++	—	—	—	—	—	—	
Graminae									
<i>Aegilops speltoides</i> Tausch.....	Ap	—	—	—	—	—	—	—	
<i>Aegilops speltoides</i> Tausch.....	R	—	—	—	—	—	—	—	
** <i>Aegilops triuncialis</i> L.....	Wpl	+	—	—	—	—	—	—	
<i>Aeluropus lagopoides</i> (L.) Trin.....	Ap	++	≠1000	—	—	—	≠1000	≠1000	
<i>Alopecurus myosuroides</i> Huds.....	Wpl	—	—	—	—	—	—	—	
<i>Aristida ciliata</i> Desf.....	Wpl	—	—	—	—	—	—	—	
<i>Aristida plumosa</i> L.....	Wpl	—	—	—	—	—	—	—	
<i>Bromus danthoniae</i> Trin.....	Wpl	—	—	—	—	—	—	—	
<i>Bromus madritensis</i> L.....	Wpl	—	—	—	—	—	—	—	
<i>Cutandia memphitica</i> (Spreng.) Benth.....	Wpl	—	—	—	—	—	—	—	
<i>Cymbopogon parkeri</i> Stapf.....	Wpl	—	—	—	—	—	+1000	—	
<i>Cymbopogon schoenanthus</i> (L.) Spreng.....	Wpl	—	—	—	—	—	+1000	—	
<i>Cynodon dactylon</i> (L.) Pens.....	Wpl	—	—	—	—	—	—	—	
<i>Eremopoa persica</i> (Trin.) Roshev.....	Wpl	—	—	—	—	—	—	—	
** <i>Fragrostis diarrhena</i> (Schult.) Steud var. <i>Koenigii</i> (Kunth) Fischer.....	Wpl	+	—	—	—	—	—	—	
(Syn. <i>Poa diarrhena</i> Schult.)									
<i>Hordeum glaucum</i> Steud.....	Wpl	—	—	—	—	—	—	—	
<i>Lophochloa phleoides</i> (Vill) Reichenb.....	Wpl	—	—	—	—	—	—	—	
<i>Oryza sativa</i> L.....	Wpl	—	—	—	—	—	—	—	
<i>Phalaris minor</i> L.....	Ap	+	—	—	—	—	—	—	
<i>Poa bulbosa</i> L.....	Wpl	—	—	—	—	—	—	—	
<i>Polypogon monspeliensis</i> (L.) Desf.....	Wpl	—	—	—	—	—	—	—	
<i>Schismus arabicus</i> Nees.....	Wpl	—	—	—	—	—	—	—	
<i>Stipa barbata</i> Desf.....	Ap	—	—	—	—	—	—	—	
<i>Stipa capensis</i> Thumb.....	Ap	—	—	—	—	—	—	—	
<i>Stipagrostis plumosa</i> (L.) Munro..... (Syn. <i>Aristida plumosa</i> L.)	Wpl	—	—	—	—	—	—	—	
Hypericaceae									
<i>Hypericum crispum</i> L.....	Ap	—	—	—	—	—	≠1000	≠1000	
<i>Hypericum scabrum</i> L.....	Ap	—	+1000	—	—	—	+1000	—	
<i>Hypericum triquetrifolium</i> Turra.....	Ap	—	—	—	—	—	—	—	
Iridaceae									
<i>Glaucolus kotschyamus</i> Boiss.....	Ap	—	—	—	—	—	—	—	
<i>Iris sisyriuchium</i> L.....	Ap	—	—	—	—	—	≠1000	—	
Labiatae									
<i>Calamintha staminea</i> Boiss.....	Ap	—	—	—	—	—	—	—	
<i>Lallemantia Royleana</i> Benth.....	Wpl	—	—	—	—	—	—	—	
<i>Lycopus europaeus</i> L.....	Ap	—	—	—	—	—	—	—	
<i>Marrubium crassidens</i> Boiss.....	Ap	—	—	—	—	—	—	—	
<i>Marrubium cuneatum</i> Russ.....	Ap	+++	—	—	—	—	—	—	
<i>Micromeria myrtifolia</i> Boiss et Hoh.....	Wpl	—	—	—	—	—	—	—	

TABLE 2. Continued.

Family Botanical name ^a	Plant part(s) ^b	Preliminary alkaloid test ^c	Minimum inhibitory conc. (mcg/ml) vs. organisms no. ^d					
			1	2	3	4	5	6
<i>Phlomis armeniaca</i> Willd.	Wpl	-	-	-	-	-	-	-
<i>Phlomis bruguieri</i> Desf.	Wpl	-	-	-	-	-	-	-
<i>Phlomis praeterisa</i> Rech. F.	Wpl	-	-	-	-	-	-	-
<i>Phlomis rigida</i> Labill.	Ap	-	-	-	-	-	-	-
<i>Prunella vulgaris</i> L.	Wpl	-	-	-	-	-	-	-
<i>Salvia acetabulosa</i> L.	Ap	-	-	-	-	-	-	≠1000
<i>Salvia compressa</i> Vahl.	Ap	-	-	-	-	-	-	-
<i>Salvia lamigera</i> Poir.	Wpl	+	-	-	-	≠1000	-	-
<i>Salvia palaestina</i> Benth.	Ap	-	-	-	-	+1000	-	-
<i>Salvia spinosa</i> L.	Ap	+	-	-	-	-	-	-
<i>Salvia trichoclade</i> Benth.	Ap	-	-	-	-	-	-	-
<i>Scutellaria megalaspis</i> Rech. F.	Ap	-	-	-	-	-	-	-
<i>Teucrium divaricatum</i> (Celak) Rech. F.	Ap	-	-	-	-	-	-	≠1000
<i>Teucrium oliverianum</i> Ging.	Ap	++	-	-	-	-	-	-
<i>Teucrium oliverianum</i> Ging.	R	+	-	-	-	-	-	-
<i>Teucrium partiflorum</i> Schreb.	Ap	++	-	-	-	-	-	-
<i>Teucrium polium</i> L.	Ap	-	-	-	-	-	-	-
<i>Thymus kotschyanus</i> Boiss et Holdr.	Ap	-	-	-	-	-	-	-
Leguminosae								
<i>Astragalus kahiricus</i> DC.	Ap	++	-	-	-	-	-	-
<i>Astragalus platyraphis</i> Fisch.	Wpl	++	-	-	-	+1000	-	-
<i>Astragalus rugosus</i> Fisch.	Ap	++	-	-	-	-	-	-
<i>Astragalus spinosus</i> (Forsk.) Muschl.	Ap	-	-	-	-	-	-	-
** <i>Cicer arietinum</i> L.	Ap	+	-	-	-	-	-	-
<i>Colutea ciliata</i> Boiss et Bal.	Ap	+++	-	-	-	-	-	-
<i>Coronilla scorpioides</i> (L.) Koch.	Ap	-	-	-	-	-	-	-
<i>Glycyrrhiza glabra</i> L.	R	-	+1000	-	-	-	+1000	-
<i>Hedysarum kotschyi</i> Boiss.	Ap	++	-	-	-	-	-	-
<i>Hedysarum singarense</i> Boiss et Haussk.	Ap	+-	-	-	-	-	-	-
<i>Hedysarum varium</i> Willd.	Wpl	++	-	-	-	-	-	-
** <i>Hippocrepis bicontorta</i> Lois.	Wpl	+	-	-	-	-	-	-
<i>Hymenocarpus circinatus</i> (L.) Savi.	Wpl	-	-	-	-	-	-	-
<i>Lotus gebelia</i> Vent.	Ap	+	-	-	-	-	-	+ 100
<i>Lotus lanuginosus</i> Vent.	Ap	++	-	-	-	-	-	-
<i>Medicago laciniata</i> (L.) Mill.	Wpl	+	-	-	-	-	-	-
<i>Medicago polymorpha</i> L.	Ap	-	-	-	-	-	-	-
<i>Medicago radiata</i> L.	Wpl	+	-	-	-	-	-	-
<i>Melilotus indicus</i> (L.) All.	Ap	++	-	-	-	-	-	-
<i>Onobrychis Crista-galli</i> (L.) Lam.	Wpl	++	-	-	-	-	-	+1000
<i>Onobrychis ptolemaica</i> DC.	Wpl	-	-	-	-	-	-	-
<i>Onobrychis schahuensis</i> Bornm.	Ap	+++	-	-	-	-	-	-
<i>Prosopis fracta</i> (Banks et Sol.) Eig.	Ap, Fr.	-	-	-	-	-	-	-
<i>Scorpiurus muricatus</i> L. var. <i>subcilloso</i>	Ap	-	-	-	-	-	-	-
<i>Securigera securidaca</i> (L.) Deg. et Doerfl.	Ap	-	-	-	-	-	-	-
<i>Trifolium campestre</i> Schreb.	Ap	-	-	-	-	-	-	-
<i>Trifolium lappaceum</i> L.	Ap	-	-	-	-	-	-	-
<i>Trifolium purpureum</i> Lois.	Wpl	-	-	-	-	-	-	-
<i>Trifolium tomentosum</i> L.	Ap	-	-	-	-	-	-	-
<i>Trigonella filipes</i> Boiss.	Wpl	+-	-	-	-	-	-	-
<i>Trigonella Noeana</i> Boiss.	Wpl	+	-	-	-	-	-	-
<i>Vicia articulata</i> Hornem.	Wpl	++	-	-	-	-	-	-
<i>Vicia narbonesnsis</i> L.	Ap	+++	-	-	-	-	-	-
Liliaceae								
<i>Allium dilatatum</i> Stapf.	Ap	-	-	-	-	≠1000	+ 100	-
<i>Asphodelus tenuifolius</i> Cav.	Wpl	-	-	-	-	-	-	-
<i>Eremurus spectabilis</i> L.	Ap	-	-	-	-	-	-	-
Lythraceae								
<i>Ammannia baccifera</i> L.	Wpl	-	≠1000	-	≠1000	-	≠1000	≠1000

TABLE 2. Continued.

Family Botanical name ^a	Plant part(s) ^b	Preliminary alkaloid test ^c	Minimum inhibitory conc. (mcg/ml) vs. organisms no. ^d					
			1	2	3	4	5	6
<i>Lythrum salicaria</i> L.....	Ap, F	—	+1000	—	—	—	—	+1000
Malvaceae								
<i>Althea ludwigii</i> L.....	Ap	—	—	—	—	—	≠1000	—
<i>Malva aegyptiaca</i> L.....	Wpl	—	—	—	—	—	—	—
Meliaceae								
<i>Melia azedarach</i> L.....	S	+	—	—	—	—	—	—
Onagraceae								
<i>Jussiaea repens</i> L.....	Ap	—	—	—	—	—	—	—
<i>Ludwigia adscendens</i> L.....	Ap	—	—	—	—	—	≠1000	≠1000
Papaveraceae								
<i>Glaucium corniculatum</i> (L.) Rudolph.....	Ap, F	+++	—	—	—	—	+1000	—
<i>Hypocoum pendulum</i> L.....	Ap	+	—	—	—	—	—	—
<i>Papaver rhoeas</i> L.....	Ap, F	+	—	—	—	—	—	—
<i>Roemeria hybrida</i> (L.) DC.....	Ap	++	—	—	—	—	—	—
Plantaginaceae								
<i>Plantago Boissieri</i> Hausskn et Bornm.....	Wpl	—	—	—	—	—	—	—
<i>Plantago lagopus</i> L.....	Ap	—	—	—	—	—	—	—
<i>Plantago ovata</i> Forssk.....	Wpl	+	—	—	—	—	—	—
<i>Plantago psyllium</i> L.....	Wpl	—	—	—	—	—	≠1000	≠1000
Plumbaginaceae								
** <i>Limonium thouini</i> (Viv.) Kuntze.....	Ap	+	—	—	—	—	—	≠1000
<i>Psylliostachys spicata</i> (Willd.) Nevski..... (Syn. <i>Statice spicata</i> (Willd.) Kuntze	Ap	—	—	—	—	—	—	—
Polygonaceae								
<i>Calligonum comosum</i> L'Her.....	Ap	—	—	—	—	—	—	—
<i>Polygonum argyrocoleum</i> Steud in Kotschy.....	Wpl	—	—	—	≠1000	—	—	—
<i>Polygonum corrigioloides</i> Jaub et Spach.....	Ap	—	—	—	—	—	—	≠1000
<i>Polygonum salicifolium</i> Brouss et Willd.....	Ap	—	—	—	—	—	—	≠1000
<i>Rumex dentatus</i> L.....	Wpl	—	—	—	—	—	≠1000	+1000
<i>Rumex vesicarius</i> L.....	Ap	—	—	—	—	—	—	—
Potamogetonaceae								
<i>Potamogeton natans</i> L.....	Ap	—	—	—	—	—	≠1000	—
<i>Potamogeton pectinatus</i> L.....	Ap, S	—	—	—	—	—	≠1000	—
Primulaceae								
<i>Anagallis arvensis</i> L.....	Ap	+	—	—	—	—	—	+1000
Ranunculaceae								
<i>Adonis dentatus</i> Del.....	Ap	—	≠1000	—	—	—	—	—
<i>Delphinium brunonianum</i> Royle.....	Ap, F	+	—	—	—	—	≠1000	—
<i>Delphinium brunonianum</i> Royle.....	R	+++	—	—	—	—	≠1000	—
<i>Nigella arvensis</i> L.....	Wpl	++	—	—	—	—	—	—
<i>Ranunculus arvensis</i> L.....	Wpl	—	—	—	≠1000	—	—	—
<i>Ranunculus oxyspermus</i> M.B.....	Ap	—	—	—	—	—	—	—
<i>Ranunculus sericeus</i> Poir.....	Ap	—	—	—	—	—	≠1000	—
<i>Ranunculus sphaerospermus</i> Boiss et Blanche.....	Ap	—	—	—	—	—	—	—
<i>Ranunculus trichophyllus</i> Chaix in Vill.....	Ap	—	—	—	—	—	—	—
Resedaceae								
<i>Reseda arabica</i> Boiss.....	Wpl	++	—	—	—	—	—	—
<i>Reseda bracteata</i> Boiss.....	Ap	++	—	—	—	—	—	—
<i>Reseda decursiva</i> Forssk.....	Ap	+	—	—	—	—	—	—
Rhamnaceae								
<i>Paliurus spinachristi</i> Mill.....	Ap	—	—	—	—	—	—	—
Rosaceae								
<i>Amygdalus arabica</i> Olivier.....	Ap	—	—	—	—	—	—	—
<i>Poterium lasiocarpum</i> Boiss et Haussk.....	Ap	—	—	—	—	—	—	—
Rubiaceae								
<i>Galium coronatum</i> Sibth et Sm.....	Wpl	+	—	—	—	—	—	—
<i>Rubia tenuifolia</i> D'Urv.....	Ap	—	—	—	—	—	—	—

TABLE 2. Continued.

Family Botanical name ^a	Plant part(s) ^b	Preliminary alkaloid test ^c	Minimum inhibitory conc. (mcg/ml) vs. organisms no. ^d					
			1	2	3	4	5	6
Rutaceae								
<i>Haplophyllum mesopotamicum</i> Boiss.....	Wpl	++-	-	-	-	-	=1000	-
<i>Haplophyllum tuberculatum</i> (Forssk) ADR-Juss.....	Wpl	+++	-	-	-	-	=1000	-
Scrophulariaceae								
<i>Celsia heterophylla</i> Desf.....	Ap	-	=1000	-	-	-	=1000	-
<i>Celsia lanceolata</i> Vent.....	Wpl	+++	-	-	-	-	-	-
<i>Linaria chalapensis</i> (L.) Mill.....	Wpl	±	+1000	-	-	-	-	-
<i>Linaria kurdica</i> Boiss et Hoh.....	Ap, S	+	+100	-	-	-	-	-
<i>Scrophularia deserti</i> Del.....	Wpl	++	-	-	-	-	-	-
<i>Scrophularia gracilis</i> Blakelock.....	Wpl	++	-	-	-	-	-	-
<i>Scrophularia hypericifolia</i> Wydl.....	Wpl	+++±	-	-	-	-	-	-
<i>Scrophularia marginata</i> Boiss.....	Ap	-	=1000	-	-	-	-	-
<i>Scrophularia xanthoglossa</i> Boiss.....	Ap	-	-	-	-	-	=1000	=1000
<i>Veronica orientalis</i> Mill.....	Wpl	++	-	-	-	-	-	=1000
Solanaceae								
* <i>Lycium barbarum</i> L.....	Ap	+++	-	-	-	+1000	-	-
<i>Solanum nigrum</i> L.....	Ap	+++	-	-	-	-	-	+1000
<i>Withania somnifera</i> (L.) Dum.....	Ap	+++	-	-	-	-	+1000	+1000
Tamaricaceae								
<i>Tamarix macrocarpa</i> (Ehrenb) Bge.....	Ap	+	=1000	-	-	-	+1000	+1000
<i>Tamarix Meyeri</i> Boiss.....	Ap	-	-	-	-	-	+1000	-
Thymelaeaceae								
<i>Stellera lessertii</i> (Wikstr.) Boiss.....	Ap	-	-	-	-	-	-	-
Umbelliferae								
<i>Ammi majus</i> L.....	Ap	+++	-	-	-	-	-	-
<i>Ammi visnaga</i> (L.) Lam.....	Ap	+++	-	-	-	-	-	-
** <i>Anisosciadium isociadium</i> Bornm.....	Wpl	+++	-	-	-	-	-	-
** <i>Ducrosta anethifolia</i> (DC.) Boiss.....	Wpl	+	-	-	-	-	-	-
<i>Ferula acina</i> Boiss.....	Ap	+	-	-	-	-	=1000	-
<i>Ferula rubrans</i> Boiss.....	Ap	+++	-	-	-	-	-	-
** <i>Ferulago angulata</i> (Schlecht) Boiss.....	Ap	+++	-	-	-	-	-	-
<i>Hippomarathrum scabrum</i> (Fenzl) Boiss.....	Ap	++	-	-	-	-	-	=1000
<i>Pimpinella affinis</i> Ledeb.....	Wpl	+	-	-	-	-	+1000	-
<i>Pimpinella kotschyana</i> Boiss.....	Ap	++	-	-	-	-	-	-
<i>Pimpinella puberula</i> (DC.) Boiss.....	Ap	++	-	-	-	-	-	-
<i>Pimpinella tragiium</i> Vill.....	Ap	±	-	-	-	-	+1000	-
<i>Turgenis latifolia</i> (L.) Hoffm.....	Ap	-	-	-	-	-	-	-
<i>Zocimia absinthifolia</i> (Vent.) DC.....	Ap, S	-	-	-	-	-	-1000	-
Urticaceae								
<i>Parietaria judaica</i> L.....	Ap	-	-	-	-	-	-	=1000
Valerianaceae								
<i>Valeriana sisymbriifolia</i> Desf.....	Ap	+	-	-	-	-	-	-
Verbenaceae								
<i>Lippia nodiflora</i> (L.) L. C. Rich.....	Wpl	+	-	-	-	-	-	-
<i>Verbena officinalis</i> L.....	Ap	-	-	-	-	-	-	-
Zygophyllaceae								
<i>Fagonia Bruguieri</i> DC.....	Ap	+++	-	-	-	-	-	-
<i>Peganum harmala</i> L.....	Ap	+++	-	-	-	-	+1000	-
<i>Peganum harmala</i> L.....	S	+++	+1000	+1000	+1000	+1000	+100	+1000
<i>Zygophyllum coccineum</i> L.....	Ap	+++	-	-	-	-	-	-
<i>Zygophyllum fabago</i> L.....	Ap	---	-	-	-	-	-	-

^aThe only cultivated (introduced) plant reported in this table.

^bThe nomenclature used follows Rechinger, K. H. (1), for species indigenous to Iraq.

^cAp, aerial parts; Wpl, whole plant; F, flowers; Fr., roots; S, seeds.

^d(-) negative, (+) positive.

^e(-) no effect, (±) partial growth inhibition, and (+) complete growth inhibition. The numbers refer to the concentration per ml of agar of the plant extract. Testing was carried out at 1000 and 100 mcg/ml. Each active extract was retested on a second occasion for confirmation of the activity before being assigned a + or = rating.

**These genera have not previously been reported to contain alkaloids (6-17).

With respect to the species most antibacterially active, there are no readily uncovered reports of such activity for *Lotus gebelia*, *Linaria kurdica*, *Peganum harmala*, *Aleuropus lagopoides*, *Tamarix macro-carpa*, and *Ammannia baccifera*. *Silene coniflora* has not been previously described to contain antimicrobial agents, but *S. stellata* has been reported to be active at 1000 mcg/ml *in vitro* against *M. smegmatis* (2). *Allium dilutum* is, likewise, newly reported to have antimicrobial activity; however, many reports can be found of antibacterial, antifungal and insecticidal activity of related *Allium* species, for example, *A. dilutum* (18), *A. cepa* (18), *A. sativum* (16, 19) and *A. saxatile* (20). Extracts of *Allium ursinum* have been patented in the Soviet Union for disinfection of wounds in the form of a bacteriocidal and protisticidal preparation ("Ursall") (21).

Preliminary alkaloidal tests show that 146 species belonging to 109 genera distributed among 32 families are positive to both Mayer's and Dragendorff's reagents. Of these, 26 genera have not been previously reported in the literature to include alkaloid-containing plants (6-17).

It is clear that detailed bioassay-directed and phytochemical fractionation of Iraqi higher plants will be scientifically fruitful.

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LITERATURE CITED

1. K. H. Rechinger, "Flora of Lowland Iraq," Ed. G. C. Townsend, Verlagron J. Cramer, West Germany, 1964.
2. L. A. Mitscher, R. Leu, M. S. Bathala, W.-N. Wu, J. L. Beal and R. White, *Lloydia*, **35**, 157(1972).
3. T. A. Gonzales, M. Vance, M. Helpern and C. J. Umberger, "Legal Medicine Pathology and Toxicology," Appleton Century-Crofts, Inc., New York, 2nd edition, 1954, p. 1260.
4. H. Thies and F. W. Reuther, *Naturwissenschaften*, **41**, 230 (1964).
5. N. R. Farnsworth, N. A. Pilewski and F. J. Draus, *Lloydia*, **25**, 312 (1962).
6. J. J. Willaman and B. G. Schubert, "Alkaloid-bearing plants and their contained alkaloids," Technical Bull. No. 1234, ARS, U.S.D.A., Washington, D.C. (1961).
7. J. J. Willaman and H.-L. Li, "Alkaloid-bearing plants and their contained alkaloids, 1957-1968", *Lloydia*, **33**, Supplement 3A (1970).
8. S. J. Smolenski, H. Silinis and N. R. Farnsworth, "Alkaloid screening I," *Lloydia*, **35**, 1 (1972).
9. H. H. S. Fong, M. Trojánková, J. Trojánek and N. R. Farnsworth, "Alkaloid screening II," *Lloydia*, **35**, 117 (1972).
10. S. J. Smolenski, H. Silinis and N. R. Farnsworth, "Alkaloid screening III," *Lloydia*, **36**, 359 (1973).
11. S. J. Smolenski, H. Silinis and N. R. Farnsworth, "Alkaloid screening IV," *Lloydia*, **37**, 30 (1973).
12. S. J. Smolenski, H. Silinis and N. R. Farnsworth, "Alkaloid screening V," *Lloydia*, **37**, 506 (1974).
13. S. J. Smolenski, H. Silinis and N. R. Farnsworth, "Alkaloid screening VI," *Lloydia*, **38**, 225 (1975).
14. S. J. Smolenski, H. Silinis and N. R. Farnsworth, "Alkaloid screening VII," *Lloydia*, **38**, 411 (1975).
15. S. J. Smolenski, H. Silinis and N. R. Farnsworth, "Alkaloid screening VIII," *Lloydia*, **38**, 497 (1975).
16. Chemical Abstract decennial and hemiannual indices.
17. R. F. Raffauf, "A Handbook of Alkaloids and Alkaloid-containing Plants," Wiley Interscience, New York (1969).
18. I. A. Abdou, A. A. Abou-Zeid, M. R. El-Sherbeeney and Z. H. Abou-El-Gheat, *Qual. Plant Mater. Veg.*, **22**(1), 29 (1972).
19. F. E. Barone and M. R. Tansey, *Mycologia*, **69**, 793 (1977).
20. R. K. Aliev, K. M. Safarov, A. Kh. Rakhimova and Z. N. Guseinova, *Vopr. Fiziol. Akad. Nauk. Azerb. SSR*, **6**, 91 (1963); *via Chem. Abstr.*, **60**, 2047 (1964).
21. G. Y. Tutaev, Russian Patent 157,464 (1963); *via Chem. Abstr.*, **60**: P9106C (1964).