

AN INVESTIGATION OF CERTAIN PLANT EXTRACTS
FOR THE PRESENCE OF BACTERIAL INHIBITORY PROPERTIES

by

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A Thesis

submitted to the faculty of the

Department of Bacteriology

in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

in the Graduate College, University of Arizona

1951

Approved:

Mary E. Caldwell May 18, 1951
Director of Thesis Date



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ACKNOWLEDGMENTS

It is with deep appreciation that I acknowledge the supervision and direction of Dr. Mary E. Caldwell, whose enthusiasm and constant interest furthered this work. Also, I express deep appreciation to Dr. Kitty Parker, curator of the University of Arizona Herbarium, for her assistance in identifying the plants used in this thesis.

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INTRODUCTION

The discovery and isolation of antibiotics from the higher plants are similar in many respects to their detection and separation from the microorganisms. Although the problems involved are basically analogous, to date relatively few workers have undertaken such studies with the plant extracts. As yet, the latter have not attained the dramatic success that has rewarded the efforts of many workers who have found antibiotics produced by certain microorganisms. The utilization of higher plants in various ways for the treatment of disease is older than medical history. No part of the world, new or old, is devoid of its herbs. It is no wonder then that modern science has turned to investigate this possible source for substances which may be inhibitive to bacteria. Florey et al (1949) give an excellent chronological account of medicinal plant history including much of the recent work with antibiotics from the higher plants. Baron (1950) includes in his Handbook of Antibiotics those antibiotics derived from plants that have been crystallized.

As far as the author is aware, the only search for antibacterial substances associated with higher plants growing in the semi-arid region of the Southwest has been carried on by Mary E. Caldwell and several students whose work she has directed.

EXPERIMENTAL

The preliminary work of screening certain plants native to this area for bacterial inhibitive properties has involved the testing of extracts prepared by the use of different solvents; sometimes the entire plant and in other instances only certain plant organs such as the leaves, stems, roots, or fruits were employed. Each extract was tested individually against two gram negative bacilli, one gram positive bacillus, one gram positive coccus, and an acid fast bacterium, all of which were obtained from the American Type Culture Collection:

Bacillus subtilis #102Escherichia coli #6790Micrococcus pyogenes var. aureus #6538Pseudomonas aeruginosa #97Mycobacterium tuberculosis
#607

A. Plants collected and tested:

- | | |
|--|--|
| 1. <u>Solanum eleagnifolium</u> Cav.
(Horse Nettle) | 10. <u>Sorghum halepense</u> (L) Pers.
(Johnson grass) |
| 2. <u>Malva parviflora</u> L.
(Mallow) | 11. <u>Atriplex canescens</u> (Pursch)
Nutt (Saltweed) |
| 3. <u>Amaranthus palmeri</u> S. Wats.
(Careless Weed) | 12. <u>Cynodon dactylon</u> L. Pers.
(Bermuda grass) |
| 4. <u>Rumex hymenosepalus</u> Torr.
(Canaigre) | 13. <u>Erythrina flabelligormis</u>
Kearney (Coralbean) |
| 5. <u>Cyperus fendlerianus</u> Boeckl.
(Nut Grass) | 14. <u>Agave schottii</u> Engelm |
| 6. <u>Prosopis juliflora</u> (Swartz)
DC. (Velvet Mesquite) | 15. <u>Tribulus terrestris</u> L.
(Bull head) |
| 7. <u>Caesalpinia gilliesii</u> Wall.
(Bird-of-paradise) | 16. <u>Trianthema portulacastrum</u> L.
(Rabbit weed) |
| 8. <u>Arctostaphylos pungens</u> HBK
(Manzanita) | 17. <u>Cirsium arizonicum</u> (A. Gray)
Petraek (Thistle) |
| 9. <u>Verbesina encelioides</u> (Cav.)
Benth and Hook, ex A. Gray
(Desert Daisy) | 18. <u>Brickellia californica</u> (Torr
and Gray) A. Gray (Pachaba) |

- | | |
|---|---|
| 19. <u>Encelia farinosa</u> A. Gray
ex Torr in Emory
(Brittle Bush) | 28. <u>Datura meteloides</u> DC.
(Jimson weed) |
| 20. <u>Xanthium saccharatum</u> Wallr
(Cockleburr) | 29. <u>Euphorbia albomarginata</u>
Torr and Gray (La Golondrina) |
| 21. <u>Gnaphalium wrightii</u> A. Gray | 30. <u>Chilopsis lenearis</u> (Cav.)
Sweet (Desert Willow) |
| 22. <u>Gossypium hirsutum</u>
(Cotton) | 31. <u>Perezia thurberi</u> A. Gray |
| 23. <u>Gossypium thurberi</u> Todardo
(Wild Cotton) | 32. <u>Baccharus sarothroides</u> A.
Gray (Desert Broom) |
| 24. <u>Dalea</u>
(Indigobush) | 33. <u>Oenothera hookeri</u> Torr and
Gray (Primrose) |
| 25. <u>Cirsium wheeleri</u> (A. Gray)
Petrak (Thistle) | 34. <u>Humulus americanus</u> Nutt
(Wild hop) |
| 26. <u>Haploppapus fruticosus</u>
(Green) Blake in Benson
(Rayless Goldenrod) | 35. <u>Artemisia dracunculoides</u> Pursch |
| 27. <u>Haplopappus laricifolius</u> A.
Gray (Turpentine Bush) | |

As often as was possible fresh material was used for the preparation of the extracts. Collection of twenty-four plants was made just prior to use; others, gathered before the cold weather affected them, were stored and later extracted in the dried state.

B. Method of extraction:

The plant or organ of the plant was macerated in an ordinary meat grinder. Weighed amounts of the ground plant were placed in covered jars, an equal amount of the solvent was added, and after the mixture had stood for an hour at room temperature it was placed in a refrigerator at 5 C for 18 to 20 hours. With a few modifications this was the procedure of extraction described by Carlson and Douglas (1946). Due

to the dryness of some of the desert plants and also because of the fact that some of the plants were desiccated when tested, it was not always possible to obtain an extract using a volume of the solvent equal to the weight of the plant. Therefore, it was sometimes necessary to add an additional amount of the solvent; this increase in the solvent varied with the nature or condition of the plant. Furthermore, the addition of distilled water to the ground plant material one hour prior to the addition of the solvent was omitted due to the possibility of rendering those constituents which were insoluble in water inaccessible to the solvents.

Five extracts of each plant or plant organ were tested. These five extracts according to Carlson and Douglas (1947) could contain some of the chemical substances which are mentioned with each extract.

1. An aqueous extract buffered at pH 7.0 was prepared by combining 61.1 ml of 1/15M. sodium hydrogen phosphate and 38.9 ml of 1/15 M. potassium dehydrogen phosphate. This aqueous extract would yield the following substances from plants: inorganic compounds (metal salts), a few enzymes, albumin, histones, protamines, proteoses, peptones, and similar amino acids. In this extract and the other extracts used tests for the presence of these chemical compounds were not carried out in the work herein reported.

2. An aqueous extract buffered at pH 4.0 was prepared by combining 50 ml of 0.200 M. potassium hydrogen phthalate with 0.40 ml of 0.200 M. sodium hydroxide and diluting to 200.0 ml. In the results this extract will be referred to as the acid extract. This extract could yield the following substances from the plant: glutelins, several enzymes, possible metaproteins, possible albuminoids.

3. An aqueous extract buffered at pH 9.0 was prepared by combining 50.00 ml of 0.200 M. boric acid with 21.40 ml of 0.200 M. sodium hydroxide and diluting with distilled water to 200.0 ml. In the results this will be referred to as the basic extract. This extract could yield acidic compounds and glucosides.

4. A weak solution of a strong acid was prepared by neutralizing 3 per cent sulfuric acid with 1.0 M. sodium hydroxide just prior to use. Mineral acid as used in this report refers to this extract. Alkaloids and similar substances of a basic nature could be obtained by this extract.

5. An ethyl ether extract was used to yield primarily chlorophyll, waxes, steroles, and denatured proteins and enzymes.

C. Methods of Assay:

In order to quickly screen a number of plants for the possible presence of antibacterial substances, the disc-plate method was used. Difco Penassay base agar was prepared, tubed in 20 ml amounts, autoclaved, and the contents of each tube poured aseptically into sterile petri plates and allowed to harden. This composed the base agar. Difco Penassay base agar, also used as seed agar, was prepared in 5 ml amounts, autoclaved, and stored for future use. When needed, the latter medium was melted, cooled to 42 to 45 C, inoculated using a sterile loop 3 mm in diameter with a 24 hour old nutrient broth culture of all bacteria with the exception of Myco. tuberculosis which required 48 hours incubation for sufficient growth. The tube was then vigorously rolled to insure equal dispersion of the organism throughout the medium and poured aseptically from the tube onto the surface of the solidified base agar plate. After the seed agar had solidified, sterile assay discs were

placed upon this surface with flamed forceps. The plant extract which had been passed through cheesecloth to remove the solid portion of the macerated plant, was then added by means of a sterile pipette to the discs in 0.065 ml amounts. For each disc with extract, a second one impregnated with an equal amount of the corresponding solvent served as a control. The controls were always placed on the same plate as the extract under test so as to eliminate any possibility that a difference in the growth of the organism from one plate to the next might influence interpretation of the results. Four to six discs were arranged on each plate. A duplicate set of plates was prepared for every extract. Incubation at 37 C followed with 24 and 48 hour readings; however, because of the slower growth of the Mycobacterium tuberculosis these observations were made at 48 and 72 hour intervals. The diameter of the cleared zone measured in millimeters was indicative of the degree of inhibition. With two plants it was found impossible to obtain accurate measurements due to the growth of extraneous organisms present in the extract; therefore, the experiment was repeated using extracts that were passed through a Seitz filter.

If the results of the above method warranted further investigation of a given plant, the cylinder-plate technic was employed as a second method of testing. The preparation of the medium, cultures, extracts and their respective controls as well as the periods of observation, and the measurement of the zones of inhibition were the same as with the disc-plate method. Each cylinder received 0.25 ml of an extract or its corresponding solvent.

The third method employed for determining antibiotic activity was the agar-streak method described by Waksman and Reilly (1947). Only

when unusually favorable results were obtained in the former two techniques was the agar-streak determination used. This method has proven to be particularly useful for obtaining a rapid and fairly accurate determination of the potency of an extract. A Seitz filtrate of the extract was evaporated to dryness in air, the residue weighed and dissolved in a known volume of the solvent. Serial increments of such a solution were then added aseptically to a series of 10 test tubes, each containing 10 ml of melted agar cooled to 45 to 50 C. The contents of each tube were poured into a sterile petri plate, which had been marked off in quarters with a wax pencil and numbered consecutively, rotated to thoroughly mix the extract with the medium and allowed to solidify. Four standard loopfuls of bacterial growth from agar slant cultures incubated 24 hours at 37 C were suspended uniformly in 10 ml of nutrient broth. Whenever cultures of the mycobacteria were tested, a 48 hour old growth on agar slants was used. Because of the usual growth characteristics of the latter genus, to obviate the difficulty of obtaining directly a uniform suspension, the culture on an agar slant was flooded with nutrient broth, the growth scraped off with an inoculating needle, the supernatant withdrawn through a sterile pipette the tip of which was imbedded in sterile cotton previously placed aseptically in the tube below the cotton plug. Thus, all suspensions that were used in this method were free from aggregates of cells that would preclude accurate observations. Suspensions of test organisms were then streaked in the appropriate sector with a flamed wire triangle that would produce a streak 1.0 cm wide. These plates were then incubated at 37 C and readings were made at 24 and 48 hours or in the case of the mycobacteria, at 48 and 72 hours. An estimate of the potency of the extract was made

by noting which plate completely, or nearly completely, inhibited the growth of the organisms.

RESULTS

In the organization of these results, the plants have been arranged in three groups; the first, plants which show neither inhibitory or stimulatory properties; the second, plants which show stimulatory properties; and the third, plants which show inhibition. It will be noted, however, that in some instances the results of one group encroach upon those of another; this is not uncommon in tests of this sort. All the plants were assayed with the disc-plate method. When other methods were used it will be mentioned in the text. The arabic numerals assigned plants where they are first listed in this dissertation will apply consistently to the tabulations of results. Detailed results of the present findings will be compared with those of Atterbury (1949) wherever such observations make for greater clarity even though there may be some repetition of such in the discussion of this thesis.

Group A. Plants which show neither inhibitory nor stimulatory properties.

1. Solanum elaeagnifolium: (Horse nettle)

Each extract was prepared from 15 grams of the ground stems and leaves, freshly collected, to which 15 ml of each solvent were added. No response either inhibitory or stimulatory was observed in the disc-plates. Because of these negative results no other methods of investigation were used with this plant. Prior work by Atterbury (1947) with this plant differed from these findings in that stimulation of M. pyogenes var. aureus was found with the ethyl ether extract; however, the fruit of the plant as well as the stems and leaves was used, whereas only the stems and leaves were used in this assay. Results are indicated

TABLE #1

RESULTS OF THE DISC-PLATE ASSAY METHOD

Plant: Solanum eleagnifolium Cav. (Horse Nettle)

Plant Extract	<u>B. subtilis</u>		<u>M. pyogenes</u>		<u>Ps. aeruginosa</u>		<u>E. coli</u>		<u>Myco. tuberculosis</u>	
	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr
Buffered Aqueous: pH 7.0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0
Ethyl Ether	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0
Buffered Aqueous: pH 9.0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0
Buffered Aqueous: pH 4.0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0
Mineral Acid	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0
Controls	0	0	0	0	0	0	0	0	0	0

Results with each extract were obtained from duplicate plates.
"0" denotes characteristic growth of the organism.

in Table 1.

2. Malva parviflora: (Mallow)

Each extract was prepared from 10 grams of the ground stems, leaves and flowers, collected five days previously, to which 10 ml of each solvent were added. This was then Seitz filtered in an effort to rid the extract of contaminating organisms that made impossible the readings of the first assay. The results are tabulated in Table 2. Two other tests by this method were made without difference in the outcome.

3. Amaranthus palmeri S. Wats. (Careless Weed)

Each extract was prepared from 15 grams of the ground dried stems, leaves, and fruit to which 15 ml of each solvent were added. At the time of the year in which this test was made, the imminence of cold weather necessitated the gathering of the plant some 10 days prior to use. Results of this test are shown in Table 3.

4. Rumex hymenosepalus Torr. (Canaigre)

Each extract was prepared from 10 grams of ground dried stems and leaves, collected five days previously, to which 20 ml of each solvent were added. The increase in the amount of solvent per gram of plant was needed because of the dehydrated condition of the plant. The results are tabulated in Table 4.

5. Cyperus fendlerianus Boeckl. (Nut Grass)

Each extract was prepared from 15 grams of ground dried stems, leaves, and roots, collected six weeks before testing, to which 30 ml of each solvent were added. Again the dehydrated condition of the plant required the addition of more solvent. The results are tabulated in Table 5.

TABLE #2

RESULTS OF THE DISC-PLATE ASSAY METHOD

Plant: Malva parviflora L. (Mallow)

Plant Extract	<u>B. subtilis</u>		<u>M. pyogenes</u>		<u>Ps. aeruginosa</u>		<u>E. coli</u>		<u>Myco. tuberculosis</u>	
	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr
Buffered Aqueous: pH 7.0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0
Ethyl Ether	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0
Buffered Aqueous: pH 9.0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0
Buffered Aqueous: pH 4.0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0
Mineral Acid	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0
Controls	0	0	0	0	0	0	0	0	0	0

Results with each extract were obtained from duplicate plates.

"0" denotes characteristic growth of organism.

TABLE #3

RESULTS OF THE DISC-PLATE ASSAY METHOD

Plant: Amaranthus palmeri S. Wats. (Careless weed)

Plant Extract	<u>B. subtilis</u>		<u>M. pyogenes</u>		<u>Ps. aeruginosa</u>		<u>E. coli</u>		<u>Myco. tuberculosis</u>	
	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr
Buffered Aqueous: pH 7.0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0
Ethyl Ether	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0
Buffered Aqueous: pH 9.0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0
Buffered Aqueous: pH 4.0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0
Mineral Acid	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0
Controls	0	0	0	0	0	0	0	0	0	0

Results with each extract were obtained from duplicate plates.

"0" denotes characteristic growth of the organism.

TABLE #4

RESULTS OF THE DISC-PLATE ASSAY METHOD

Plant: Rumex hymenosepalus Torr. (Canaigre)

Plant Extract	<u>B. subtilis</u>		<u>M. pyogenes</u>		<u>Ps. aeruginosa</u>		<u>E. coli</u>		<u>Myco. tuberculosis</u>	
	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr
Buffered Aqueous: pH 7.0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0
Ethyl Ether	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0
Buffered Aqueous: pH 9.0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0
Buffered Aqueous: pH 4.0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0
Mineral Acid	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0
Controls	0	0	0	0	0	0	0	0	0	0

Results with each extract were obtained from duplicate plates.
"0" denotes characteristic growth of the organism.

TABLE #5

RESULTS OF THE DISC-PLATE ASSAY METHOD

Plant: Cyperus fendlerianus Boeckl. (Nut Grass)

Plant Extract	<u>B. subtilis</u>		<u>M. pyogenes</u>		<u>Ps. aeruginosa</u>		<u>E. coli</u>		<u>Myco. tuberculosis</u>	
	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr
Buffered Aqueous: pH 7.0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0
Ethyl Ether	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0
Buffered Aqueous: pH 9.0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0
Buffered Aqueous: pH 4.0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0
Mineral Acid	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0
Controls	0	0	0	0	0	0	0	0	0	0

Results with each extract were obtained from duplicate plates.
"0" denotes characteristic growth of the organism.

Group B. Plants which show stimulation.

6. Prosopis juliflora (Swartz) DC. (Velvet Mesquite)

Each extract was prepared from 15 grams of the ground leaves, freshly collected, to which 15 ml of each solvent were added. Stimulation of B. subtilis only was evident with the aqueous extract, the acid extract, and the mineral acid extract. It is of interest that Atterbury (1949) found similar stimulation of B. subtilis with the aqueous extract, the acid extract, and the mineral acid extract, but differed in that B. subtilis was also stimulated by the basic extract and the ether extract. She also obtained stimulation of E. coli with all the extracts and partial inhibition of Ps. aeruginosa with the acid extract, whereas this author obtained neither inhibition nor stimulation with the same organisms and extracts. However, this prior work involved stem extracts only, whereas this author used the leaves.

7. Caesalpinia gilliesii Wall. (Bird-of-paradise)

Each extract was prepared from 15 grams of the ground dried stems, leaves, and flowers, collected one week prior to use, to which 15 ml of each solvent were added. Stimulation of all the test organisms was observed only with the aqueous extract and the mineral acid extract. Results, given in Table 7, show no other stimulation.

8. Arctostaphylos pungens H.B.K. (Manzanita)

Each extract was prepared from 15 grams of the ground stems, and leaves, freshly collected, to which 15 ml of each solvent were added. Stimulation of B. subtilis, M. pyogenes var. aureus, and M. tuberculosis, but not of gram negative organisms, occurred with the aqueous extract. It is of interest to note that the ether extract had no effect on any of the organisms. Results are shown in Table 8.

TABLE #6

RESULTS OF THE DISC-PLATE ASSAY METHOD

Plant: Prosopis juliflora (Swartz) DC. (Velvet Mesquite)

Plant Extract	<u>B. subtilis</u>		<u>M. pyogenes</u>		<u>Ps. aeruginosa</u>		<u>E. coli</u>		<u>Myco. tuberculosis</u>	
	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr
Buffered Aqueous: pH 7.0	S	S	0	0	0	0	0	0	0	0
	S	S	0	0	0	0	0	0	0	0
Ethyl Ether	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0
Buffered Aqueous: pH 9.0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0
Buffered Aqueous: pH 4.0	S	S	0	0	S	S	0	0	0	0
	S	S	0	0	S	S	0	0	0	0
Mineral Acid	S	S	0	0	0	0	0	0	0	0
	S	S	0	0	0	0	0	0	0	0
Controls	0	0	0	0	0	0	0	0	0	0

Results with each extract were obtained from duplicate plates.

"0" denotes characteristic growth of organism.

"S" denotes stimulation.

TABLE #7

RESULTS OF THE DISC-PLATE ASSAY METHOD

Plant: Caesalpinia gilliesii Wall. (Bird-of-paradise)

Plant Extract	<u>B. subtilis</u>		<u>M. pyogenes</u>		<u>Ps. aeruginosa</u>		<u>E. coli</u>		<u>Myco. tuberculosis</u>	
	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr
Buffered Aqueous: pH 7.0	S	S	S	S	S	S	S	S	S	S
	S	S	S	S	S	S	S	S	S	S
Ethyl Ether	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0
Buffered Aqueous: pH 9.0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0
Buffered Aqueous: pH 4.0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0
Mineral Acid	S	S	S	S	S	S	S	S	S	S
	S	S	S	S	S	S	S	S	S	S
Controls	0	0	0	0	0	0	0	0	0	0

Results with each extract were obtained from duplicate plates.

"0" denotes characteristic growth of the organism. : "S" denotes stimulation.

TABLE #8

RESULTS OF THE DISC-PLATE ASSAY METHOD

Plant: Arctostaphylos pungens H.B.K. (Manzanita)

Plant Extract	<u>B. subtilis</u>		<u>M. pyogenes</u>		<u>Ps. aeruginosa</u>		<u>E. coli</u>		<u>Myco. tuberculosis</u>	
	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr
Buffered Aqueous: pH 7.0	S	S	S	S	0	0	0	0	S	S
	S	S	S	S	0	0	0	0	S	S
Ethyl Ether	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0
Buffered Aqueous: pH 9.0	S	S	S	S	0	0	0	0	S	S
	S	S	S	S	0	0	0	0	S	S
Buffered Aqueous: pH 4.0	S	S	S	S	0	0	0	0	S	S
	S	S	S	S	0	0	0	0	S	S
Mineral Acid	S	S	S	S	0	0	0	0	S	S
	S	S	S	S	0	0	0	0	S	S
Controls	0	0	0	0	0	0	0	0	0	0

Results with each extract were obtained from duplicate plates.

"0" denotes characteristic growth of the organism. : "S" denotes stimulation.

9. Verbesina encelioides (Cav.) Benth and Hook, ex A. Gray
(Desert Daisy)

Each extract was prepared from 20 grams of ground stems, leaves, and flowers, freshly collected, to which 20 ml of the extract were added. Stimulation of B. subtilis and E. coli were evident with the aqueous extract, the ether extract, and the acid extract. M. pyogenes var. aureus was stimulated by the aqueous extract and the ether extract. Ps. aeruginosa was stimulated by the aqueous extract, the ether extract, the basic extract, and the acid extract. Myco. tuberculosis was stimulated by the ether extract and the acid extract. It is interesting to note that no stimulation was observed by the mineral acid extract. With this plant there was noted a difference between the 24 hour and 48 hour readings; B. subtilis was not affected by the acid extract during the first 24 hours, whereas stimulation was observed at the 48 hour reading. Similar results were observed with E. coli and Myco. tuberculosis with same extract. Results are shown in Table 9.

10. Sorghum halpense (L) Pers. (Johnson grass)

Each extract was prepared from 15 grams of ground stems and leaves, freshly collected, to which 15 ml of each solvent were added. Stimulation of B. subtilis was evidenced with the basic extract and acid extracts. M. pyogenes var. aureus was stimulated with all the extracts. Ps. aeruginosa was stimulated with the basic extract, acid extract, and the mineral acid extract. E. coli was stimulated with the aqueous extract, the basic extract, and the acid extract. Myco. tuberculosis was stimulated by the basic extract. Results are shown in Table 10.

11. Atriplex canescens (Pursch) Nutt (Saltweed)

Each extract was prepared from 15 grams of ground stems and leaves freshly collected, to which 15 ml of each solvent were added. Stimula-

TABLE #9

RESULTS OF THE DISC-PLATE ASSAY METHOD

Plant: Verbesina encelioides (Cav.) Benth and Hook, ex A.Gray (Desert Daisy)

Plant Extract	<u>B. subtilis</u>		<u>M. pyogenes</u>		<u>Ps. aeruginosa</u>		<u>E. coli</u>		<u>Myco. tuberculosis</u>	
	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr
Buffered Aqueous: pH 7.0	0	0	S	S	S	S	0	0	0	0
	S	S	S	S	S	S	S	S	0	0
Ethyl Ether	S	S	S	S	S	S	S	S	S	S
	S	S	S	S	S	S	S	S	S	S
Buffered Aqueous: pH 9.0	0	0	0	0	S	S	0	0	0	0
	0	0	0	0	S	S	0	0	0	0
Buffered Aqueous: pH 4.0	0	S	0	0	S	S	0	0	0	0
	0	S	0	0	S	S	0	S	0	S
Mineral Acid	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0
Controls	0	0	0	0	0	0	0	0	0	0

Results with each extract were obtained from duplicate plates.

"0" denotes characteristic growth of the organism. : "S" denotes stimulation.

TABLE #10

RESULTS OF THE DISC-PLATE ASSAY METHOD

Plant: Sorghum halepense (L) Pers. (Johnson grass)

Plant Extract	<u>B. subtilis</u>		<u>M. pyogenes</u>		<u>Ps. aeruginosa</u>		<u>E. coli</u>		<u>Myco. tuberculosis</u>	
	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr
Buffered Aqueous: pH 7.0	0	0	S	S	0	0	S	S	0	0
	0	0	S	S	0	0	S	S	0	0
Ethyl Ether	0	0	S	S	0	0	0	0	0	0
	0	0	S	S	0	0	0	0	0	0
Buffered Aqueous: pH 9.0	S	S	S	S	S	S	S	S	S	S
	S	S	S	S	S	S	S	S	S	S
Buffered Aqueous: pH 4.0	S	S	S	S	S	S	S	S	0	0
	S	S	S	S	S	S	S	S	0	0
Mineral Acid	0	0	S	S	S	S	0	0	0	0
	0	0	S	S	S	S	0	0	0	0
Controls	0	0	0	0	0	0	0	0	0	0

Results with each extract were obtained from duplicate plates.

"0" denotes characteristic growth of the organism. : "S" denotes stimulation.

tion of B. subtilis was evidenced with the basic extract, and the mineral acid extract; M. pyogenes var. aureus was stimulated by all the extracts with the exception of the acid extract; Ps. aeruginosa was stimulated by the aqueous extract, the basic extract, and the acid extract; E. coli was stimulated by the basic extract and the acid extract; Myco. tuberculosis was stimulated by the aqueous extract, the basic extract, and the mineral acid extract. Results are tabulated in Table 11.

12. Cynodon dactylon L. Pers. (Bermuda Grass)

Each extract was prepared from 15 grams of ground stems, roots, and leaves freshly collected, to which 15 ml of each solvent were added. Stimulation of B. subtilis was evidenced with all extracts; M. pyogenes var. aureus was stimulated by the aqueous extract and the mineral acid extract; Ps. aeruginosa was stimulated by the aqueous extract, the basic extract, and the mineral acid extract; E. coli was stimulated by all extracts excepting that of the acid extract and the mineral acid extract; Myco. tuberculosis was stimulated by the aqueous extract and the basic extract. Results are tabulated in Table 12.

13. Erythrina flabelliformis Kearney (Coralbean)

Each extract was prepared from 15 grams of ground stems and leaves collected two days previously, to which 15 ml of each solvent were added. Stimulation of B. subtilis was evidenced with the aqueous extract, the basic extract, the acid extract, and the mineral acid extract. M. pyogenes var. aureus was stimulated by the aqueous extract; Ps. aeruginosa was stimulated by the aqueous extract, the basic extract, and the acid extract; E. coli was stimulated by all the extracts except that of the ether extract; Myco. tuberculosis was stimulated by

TABLE #11

RESULTS OF THE DISC-PLATE ASSAY METHOD

Plant: Atriplex canescens (Pursch) Nutt (Saltweed)

Plant Extract	<u>B. subtilis</u>		<u>M. pyogenes</u>		<u>Ps. aeruginosa</u>		<u>E. coli</u>		<u>Myco. tuberculosis</u>	
	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr
Buffered Aqueous: pH 7.0	0	0	S	S	S	S	0	0	0	0
	0	0	S	S	S	S	0	0	S	S
Ethyl Ether	0	0	0	0	0	0	0	0	0	0
	0	0	S	S	0	0	0	0	0	0
Buffered Aqueous: pH 9.0	0	0	S	S	S	S	S	S	0	0
	S	S	S	S	S	S	S	S	S	S
Buffered Aqueous: pH 4.0	0	0	0	0	S	S	S	S	0	0
	0	0	0	0	S	S	S	S	0	0
Mineral Acid	S	S	S	S	0	0	0	0	S	S
	S	S	S	S	0	0	0	0	S	S
Controls	0	0	0	0	0	0	0	0	0	0

Results with each extract were obtained from duplicate plates.

"0" denotes characteristic growth of the organism. "S" denotes stimulation.

TABLE #12

RESULTS OF THE DISC-PLATE ASSAY METHOD

Plant: Cynodon dactylon L. Pers. (Bermuda Grass)

Plant Extract	<u>B. subtilis</u>		<u>M. pyogenes</u>		<u>Ps. aeruginosa</u>		<u>E. coli</u>		<u>Myco. tuberculosis</u>	
	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr
Buffered Aqueous: pH 7.0	S	S	S	S	S	S	S	S	S	S
	S	S	S	S	S	S	S	S	S	S
Ethyl Ether	S	S	0	0	0	0	0	0	0	0
	S	S	0	0	0	0	0	0	0	0
Buffered Aqueous: pH 9.0	S	S	0	0	S	S	0	0	S	S
	S	S	0	0	0	0	0	0	S	S
Buffered Aqueous: pH 4.0	S	S	0	0	0	0	S	S	0	0
	S	S	0	0	0	0	S	S	0	0
Mineral Acid	S	S	S	S	S	S	S	S	0	0
	S	S	S	S	0	0	S	S	0	0
Controls	0	0	0	0	0	0	0	0	0	0

Results with each extract were obtained from duplicate plates.

"0" denotes characteristic growth of the organism. : "S" denotes stimulation.

the aqueous extract, the basic extract, and the mineral acid extract. Difference in duplicate plates was encountered in the basic extract and the acid extract of Ps. aeruginosa; also, the duplicate plates of the basic extract, the acid extract, and the mineral acid extract of E. coli differed in that one set showed stimulation while the other did not. Results are tabulated in Table 13.

14. Agave schottii Engelm

Each extract was prepared from 15 grams of ground leaves and roots, collected one week prior to use, to which 30 ml of each solvent were added. B. subtilis and M. pyogenes var. aureus showed stimulation with all extracts. Ps. aeruginosa was stimulated by the aqueous extract and the ether extract, E. coli was stimulated by the ether extract, the basic extract, and the acid extract; and Myco. tuberculosis was stimulated by the mineral extract. Results are tabulated in Table 14.

15. Tribulus terrestris L. (Bull head)

Each extract was prepared from 20 grams of ground stems, leaves, and flowers, freshly collected, to which 20 ml of extract were added. This was then Seitz filtered in an effort to rid the extract of contaminating organisms which made impossible the readings of the first assay. B. subtilis and M. pyogenes var. aureus were stimulated by each extract; Ps. aeruginosa was stimulated by all the extracts with the exception of the basic extract. E. coli was stimulated by the aqueous extract, and the mineral acid extract; Myco. tuberculosis was stimulated by the aqueous extract and the ether extract. Again, a difference in duplicate plates was observed with the basic extract on M. pyogenes var. aureus and the acid extract on Ps. aeruginosa. One plate

TABLE #13

RESULTS OF THE DISC-PLATE ASSAY METHOD

Plant: Erythrina flabelliformis Kearney (Coralbean)

Plant Extract	<u>B. subtilis</u>		<u>M. pyogenes</u>		<u>Ps. aeruginosa</u>		<u>E. coli</u>		<u>Myco. tuberculosis</u>	
	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr
Buffered Aqueous: pH 7.0	S	S	S	S	S	S	S	S	S	S
	S	S	S	S	S	S	S	S	S	S
Ethyl Ether	O	O	O	O	O	O	O	O	O	O
	O	O	O	O	O	O	O	O	O	O
Buffered Aqueous: pH 9.0	S	S	O	O	O	O	S	S	S	S
	S	S	O	O	S	S	S	S	S	S
Buffered Aqueous: pH 4.0	S	S	O	O	O	O	O	S	O	O
	S	S	O	O	S	S	S	S	O	O
Mineral Acid	S	S	O	O	O	O	O	S	S	S
	S	S	O	O	O	O	S	S	S	S
Controls	O	O	O	O	O	O	O	O	O	O

Results with each extract were obtained from duplicate plates.

"O" denotes characteristic growth of the organism. : "S" denotes stimulation.

TABLE #14

RESULTS OF THE DISC-PLATE ASSAY METHOD

Plant: Agave schottii Engelm

Plant Extract	<u>B. subtilis</u>		<u>M. pyogenes</u>		<u>Ps. aeruginosa</u>		<u>E. coli</u>		<u>Myco. tuberculosis</u>	
	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr
Buffered Aqueous: pH 7.0	S	S	S	S	S	S	0	0	0	0
	S	S	S	S	S	S	0	0	0	0
Ethyl Ether	S	S	S	S	S	S	S	S	0	0
	S	S	S	S	S	S	S	S	0	0
Buffered Aqueous: pH 9.0	S	S	S	S	0	0	S	S	0	0
	S	S	S	S	0	0	S	S	0	0
Buffered Aqueous: pH 4.0	S	S	S	S	0	0	S	S	0	0
	S	S	S	S	0	0	S	S	0	0
Mineral Acid	S	S	S	S	0	0	0	0	S	S
	S	S	S	S	0	0	0	0	S	S
Controls	0	0	0	0	0	0	0	0	0	0

Results with each extract were obtained from duplicate plates.

"0" denotes characteristic growth of the organism. : "S" denotes stimulation.

showed stimulation while the other did not. B. subtilis with the aqueous extract in one set of the duplicate plates displayed a difference between the 24 hour reading and the 48 hour reading; the former showed no stimulation and the latter was indicative of stimulation. Results are tabulated in Table 15.

16. Trianthema portulacastrum L. (Rabbit weed)

Each extract was prepared from 20 grams of ground stems, leaves, and roots, freshly collected, to which 60 ml of each solvent were added. The addition of the extra solvent was necessitated by the highly viscous quality of the extracts which made transfer of them to the discs impractical. Stimulation of all organisms with all extracts was evidenced. Results are tabulated in Table 16.

Group C. Plants which showed inhibition.

17. Cirsium arizonicum (A. Gray) Petrak (Thistle)

Each extract was prepared from 15 grams of ground stems, leaves, and flowers collected two weeks prior to testing, to which 30 ml of each solvent were added. Partial inhibition was observed on one plate of B. subtilis with the acid extract, after 24 hour incubation, while the duplicate of this plate showed no inhibition; however, at the 48 hour observation period, stimulation was evident on both plates. Stimulation of B. subtilis with the aqueous extract and the mineral acid extract was noted; Myco. tuberculosis was stimulated by all the extracts with the exception of the ether extract. Results are shown in Table 17.

18. Brickellia californica (Torr and Gray) A. Gray (Pachaba)

Each extract was prepared from 20 grams of ground stems and leaves collected two days previously, to which 20 ml of each solvent

TABLE #15

RESULTS OF THE DISC-PLATE ASSAY METHOD

Plant: Tribulus terrestris L. (Bull head)

Plant Extract	<u>B. subtilis</u>		<u>M. pyogenes</u>		<u>Ps. aeruginosa</u>		<u>E. coli</u>		<u>Myco. tuberculosis</u>	
	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr
Buffered Aqueous: pH 7.0	0	S	S	S	S	S	S	S	S	S
	S	S	S	S	S	S	S	S	S	S
Ethyl Ether	S	S	S	S	S	S	0	0	S	S
	S	S	S	S	S	S	0	0	S	S
Buffered Aqueous: pH 9.0	S	S	S	S	0	0	0	0	0	0
	S	S	0	0	0	0	0	0	0	0
Buffered Aqueous: pH 4.0	S	S	S	S	S	S	0	0	0	0
	S	S	S	S	0	0	0	0	0	0
Mineral Acid	S	S	S	S	S	S	S	S	0	0
	S	S	S	S	S	S	S	S	0	0
Controls	0	0	0	0	0	0	0	0	0	0

Results with each extract were obtained from duplicate plates.
 "0" denotes characteristic growth of the organism. : "S" denotes stimulation.

TABLE #16

RESULTS OF THE DISC-PLATE ASSAY METHOD

Plant: Trianthema portulacastrum L. (Rabbit weed)

Plant Extract	<u>B. subtilis</u>		<u>M. pyogenes</u>		<u>Ps. aeruginosa</u>		<u>E. coli</u>		<u>Myco. tuberculosis</u>	
	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr
Buffered Aqueous: pH 7.0	S	S	S	S	S	S	S	S	S	S
Ethyl Ether	S	S	S	S	S	S	S	S	S	S
Buffered Aqueous: pH 9.0	S	S	S	S	S	S	S	S	S	S
Buffered Aqueous: pH 4.0	S	S	S	S	S	S	S	S	S	S
Mineral Acid	S	S	S	S	S	S	S	S	S	S
Controls	0	0	0	0	0	0	0	0	0	0

Results with each extract were obtained from duplicate plates.

"O" denotes characteristic growth of the organism. : "S" denotes stimulation.

TABLE #17

RESULTS OF THE DISC-PLATE ASSAY METHOD

Plant: Cirsium arizonicum (A. Gray) Petrak (Thistle)

Plant Extract	<u>B. subtilis</u>		<u>M. pyogenes</u>		<u>Ps. aeruginosa</u>		<u>E. coli</u>		<u>Myco. tuberculosis</u>	
	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr
Buffered Aqueous: pH 7.0	S	S	0	0	0	0	0	0	S	S
	S	S	0	0	0	0	0	0	S	S
Ethyl Ether	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0
Buffered Aqueous: pH 9.0	0	0	0	0	0	0	0	0	S	S
	0	0	0	0	0	0	0	0	S	S
Buffered Aqueous: pH 4.0	P12	S	0	0	0	0	0	0	S	S
	0	S	0	0	0	0	0	0	S	S
Mineral Acid	S	S	0	0	0	0	0	0	S	S
	S	S	0	0	0	0	0	0	S	S
Controls	0	0	0	0	0	0	0	0	0	0

Results with each extract were obtained from duplicate plates.

"O" denotes characteristic growth of the organism.
"S" denotes stimulation.

"P" with arabic numerals denotes partial inhibition measured in mm.

were added. Partial inhibition was observed on one plate of the M. pyogenes var. aureus with the basic extract. Definite interpretation of this partial inhibition was determined by observation under the low power of the colony microscope. A lesser number of colonies was evident within the inhibited area. The duplicate plate showed no inhibition or stimulation of the normal growth of the organism. Stimulation of B. subtilis with the basic extract, the acid extract, and the mineral acid extract was noted. One set of the duplicate plates of M. pyogenes var. aureus with the aqueous extract and the mineral acid extract showed stimulation while the other set did not. Ps. aeruginosa and Myco. tuberculosis were stimulated by the aqueous extract; E. coli was stimulated by the aqueous extract, the basic extract and the acid extract. Results are shown in Table 18.

19. Encelia farinosa A. Gray, ex Torr in Emory (Brittle Bush)

Each extract was prepared from 20 grams of ground stems and leaves, collected two days prior to use, to which 40 ml of solvent were added. Partial inhibition of B. subtilis was noted with the ethyl ether extract. Stimulation of B. subtilis and E. coli with all the extracts, except that of the ether extract, was evident. M. pyogenes var. aureus was stimulated by the ether extract; Ps. aeruginosa was stimulated by the acid extract; Myco. tuberculosis was stimulated by the aqueous extract, the basic extract, and the mineral acid extract. The aqueous extract on B. subtilis and Myco. tuberculosis, as well as the ether extract on the M. pyogenes var. aureus, showed stimulation on one of the duplicate plates only. At the 24 hour reading stimulation was not observed on one of the duplicate plates of the acid extract on E. coli while it was evident at the 48 hour reading. The other of the du-

TABLE #18

RESULTS OF THE DISC-PLATE ASSAY METHOD

Plant: Brickellia californica (Torr and Gray) A. Gray (Pachaba)

Plant Extract	<u>B. subtilis</u>		<u>M. pyogenes</u>		<u>Ps. aeruginosa</u>		<u>E. coli</u>		<u>Myco. tuberculosis</u>	
	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr
Buffered Aqueous: pH 7.0	0	0	0	0	S	S	S	S	S	S
	0	0	S	S	S	S	S	S	S	S
Ethyl Ether	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0
Buffered Aqueous: pH 9.0	S	S	P23	P23	0	0	S	S	0	0
	S	S	0	0	0	0	S	S	0	0
Buffered Aqueous: pH 4.0	S	S	0	0	0	0	S	S	0	0
	S	S	0	0	0	0	S	S	0	0
Mineral Acid	S	S	0	0	0	0	0	0	0	0
	S	S	S	S	0	0	0	0	0	0
Controls	0	0	0	0	0	0	0	0	0	0

Results with each extract were obtained from duplicate plates.

"O" denotes characteristic growth of the organism

"S" denotes stimulation.

"P" with arabic numerals denotes partial inhibition measured in mm.

plicate plates of the acid extract showed stimulation at both readings. Results are shown in Table 19.

20. Xanthium saccharatum Wallr (Cockleburr)

Each extract was prepared from 20 grams of ground stems, leaves, and seeds, freshly collected, to which 40 ml of solvent were added. Partial inhibition of B. subtilis with the ether extract was observed in 24 hours. The 48 hour readings disclosed a lesser zone of partial inhibition on these same plates. Results, Table 20, show no inhibition or stimulation of the remainder of the organisms.

21. Gnaphalium wrightii A. Gray

Each extract was prepared from 20 grams of stems and leaves collected two days previously, to which were added 40 ml of the solvent. Complete inhibition on both plates of B. subtilis with the ether extract was noted. No diminution of the zones was evidenced within the 48 hour period. Partial inhibition on one plate of Ps. aeruginosa was observed with the ether extract; the other plate showed neither stimulation nor inhibition. B. subtilis was stimulated by the aqueous extract and the basic extract; M. pyogenes var. aureus, E. coli, and Myco. tuberculosis was stimulated by all the extracts with the exception of the ether extract. Results, Table 21, show no inhibitory nor stimulatory effect on the remainder of the organisms.

22. Gossypium hirsutum (Cotton)

Each extract was prepared from 20 grams of ground stems and leaves, freshly collected, to which 20 ml of solvent were added. Complete inhibition on both plates of B. subtilis with the ether extract was observed. M. pyogenes var. aureus showed the same result. No diminution of the zones was observed in the 48 hour period. Stimulation of all organisms

TABLE #19

RESULTS OF THE DISC-PLATE ASSAY METHOD

Plant: Encelia farinosa A.Gray, ex Torr in Emory (Brittle Bush)

Plant Extract	<u>B. subtilis</u>		<u>M. pyogenes</u>		<u>Ps. aeruginosa</u>		<u>E. coli</u>		<u>Myco. tuberculosis</u>	
	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr
Buffered Aqueous: pH 7.0	S	S	0	0	0	0	S	S	S	S
	0	0	0	0	0	0	S	S	0	0
Ethyl Ether	P22	P22	S	S	0	0	0	0	0	0
	P22	P22	0	0	0	0	0	0	0	0
Buffered Aqueous: pH 9.0	S	S	0	0	0	0	S	S	S	S
	S	S	0	0	0	0	S	S	S	S
Buffered Aqueous: pH 4.0	S	S	0	0	0	0	0	S	0	0
	S	S	0	0	S	S	S	S	0	0
Mineral Acid	S	S	0	0	0	0	S	S	S	S
	S	S	0	0	0	0	S	S	S	S
Controls	0	0	0	0	0	0	0	0	0	0

Results with each extract were obtained from duplicate plates.

"0" denotes characteristic growth of the organism.

"P" with arabic numerals denotes partial inhibition measured in mm.

"S" denotes stimulation.

TABLE #20

RESULTS OF THE DISC-PLATE ASSAY METHOD

Plant: Xanthium saccharatum Wallr (Cockleburr)

Plant Extract	<u>B. subtilis</u>		<u>M. pyogenes</u>		<u>Ps. aeruginosa</u>		<u>E. coli</u>		<u>Myco. tuberculosis</u>	
	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr
Buffered Aqueous: pH 7.0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0
Ethyl Ether	P18	P14	0	0	0	0	0	0	0	0
	P16	P13	0	0	0	0	0	0	0	0
Buffered Aqueous: pH 9.0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0
Buffered Aqueous: pH 4.0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0
Mineral Acid	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0
Controls	0	0	0	0	0	0	0	0	0	0

Results with each extract were obtained from duplicate plates.

"0" denotes characteristic growth of the organism.

"P" with arabic numerals denotes partial inhibition measured in mm.

TABLE #21

RESULTS OF THE DISC-PLATE ASSAY METHODPlant: Gnaphalium wrightii A. Gray

Plant Extract	<u>B. subtilis</u>		<u>M. pyogenes</u>		<u>Ps. aeruginosa</u>		<u>E. coli</u>		<u>Myco. tuberculosis</u>	
	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr
Buffered Aqueous pH 7.0	S	S	S	S	0	0	S	S	S	S
	S	S	S	S	0	0	S	S	S	S
Ethyl Ether	C23	C23	0	0	0	0	0	0	0	0
	C22	C22	P22	P22	0	0	0	0	0	0
Buffered Aqueous pH 9.0	S	S	S	S	0	0	S	S	S	S
	S	S	S	S	0	0	S	S	S	S
Buffered Aqueous pH 4.0	0	0	S	S	0	0	S	S	S	S
	0	0	S	S	0	0	S	S	S	S
Mineral Acid	0	0	S	S	0	0	S	S	S	S
	0	0	S	S	0	0	S	S	S	S
Controls	0	0	0	0	0	0	0	0	0	0

Results with each extract were obtained from duplicate plates.

"O" denotes characteristic growth of organism.

"S" denotes stimulation.

"P" with arabic numerals denotes partial inhibition measured in mm.

"C" with arabic numerals denotes complete inhibition measured in mm.

was evidenced with all the extracts with the exception of the ether extract. Results, Table 22, show no effect either inhibitory or stimulatory on the remaining organisms.

23. Gossypium thurberi Todardo (Wild Cotton)

Each extract was prepared from 20 grams of ground stems and leaves collected two days previously, to which 20 ml of solvent were added. Complete inhibition of B. subtilis with the aqueous extract and the ether extract was noted. Also, complete inhibition of M. pyogenes var. aureus with ether extract was observed. Ps. aeruginosa and Myco. tuberculosis were stimulated by all the extracts excepting the ether extract. E. coli was stimulated by the aqueous extract, the basic extract, and the acid extract. It was observed that with the aqueous extract on E. coli and Ps. aeruginosa stimulation occurred after 24 hours. Results, Table 23, show no inhibition or stimulation on the remaining organisms.

24. Dalea (Indigobush)

Each extract was prepared from 20 grams of ground stems and leaves freshly collected, to which 20 ml of the solvent were added. Complete inhibition of B. subtilis and M. pyogenes var. aureus with the ether extract, and partial inhibition of Myco. tuberculosis with the ether extract were noted. B. subtilis was stimulated with the aqueous extract and the acid extract; M. pyogenes var. aureus and Ps. aeruginosa were stimulated with the aqueous extract, the basic extract, and the acid extract; E. coli was stimulated by all the extracts excepting the ether extract; Myco. tuberculosis was stimulated by the aqueous extract, the basic extract, and the mineral acid extract. Results, Table 24, show neither inhibitory nor stimulatory effect on the remainder of the organisms.

TABLE #22

RESULTS OF THE DISC-PLATE ASSAY METHOD

Plant: Gossypium hirsutum (Cotton)

Plant Extract	<u>B. subtilis</u>		<u>M. pyogenes</u>		<u>Ps. aeruginosa</u>		<u>E. coli</u>		<u>Myco. tuberculosis</u>	
	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr
Buffered Aqueous: pH 7.0	S	S	S	S	S	S	S	S	S	S
	S	S	S	S	S	S	S	S	S	S
Ethyl Ether	C17	C17	C12	C12	0	0	0	0	0	0
	C17	C17	C11	C11	0	0	0	0	0	0
Buffered Aqueous: pH 9.0	S	S	S	S	S	S	S	S	S	S
	S	S	S	S	S	S	S	S	S	S
Buffered Aqueous: pH 4.0	S	S	S	S	S	S	S	S	S	S
	S	S	S	S	S	S	S	S	S	S
Mineral Acid	S	S	S	S	S	S	S	S	S	S
	S	S	S	S	S	S	S	S	S	S
Controls	0	0	0	0	0	0	0	0	0	0

Results with each extract were obtained from duplicate plates.

"O" denotes characteristic growth of the organism.

"C" with arabic numerals denotes complete inhibition

"S" denotes stimulation.

tion measured in mm.

TABLE #23

RESULTS OF THE DISC-PLATE ASSAY METHOD

Plant: Gossypium thurberi Todardo (Wild Cotton)

Plant Extract	<u>B. subtilis</u>		<u>M. pyogenes</u>		<u>Ps. aeruginosa</u>		<u>E. coli</u>		<u>Myco. tuberculosis</u>	
	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr
Buffered Aqueous: pH 7.0	C21	C21	0	0	S	S	0	S	S	S
	C20	C20	0	0	S	S	0	S	S	S
Ethyl Ether	C16	C16	C19	C19	0	0	0	0	0	0
	C20	C20	C20	C20	0	0	0	0	0	0
Buffered Aqueous: pH 9.0	0	0	0	0	0	S	0	S	S	S
	0	0	0	0	S	S	S	S	S	S
Buffered Aqueous: pH 4.0	0	0	0	0	S	S	S	S	S	S
	0	0	0	0	S	S	S	S	S	S
Mineral Acid	0	0	0	0	S	S	0	0	S	S
	0	0	0	0	S	S	0	0	S	S
Controls	0	0	0	0	0	0	0	0	0	0

Results with each extract were obtained from duplicate plates.

"O" denotes characteristic growth of organism.

"C" with arabic numerals denotes complete inhibition measured in mm.

"S" denotes stimulation.

tion measured in mm.

TABLE #24

RESULTS OF THE DISC-PLATE ASSAY METHOD

Plant: Dalea (Indigobush)

Plant Extract	<u>B. subtilis</u>		<u>M. pyogenes</u>		<u>Ps. aeruginosa</u>		<u>E. coli</u>		<u>Myco. tuberculosis</u>	
	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr
Buffered Aqueous: pH 7.0	0	0	S	S	S	S	S	S	S	S
	S	S	0	0	S	S	S	S	S	S
Ethyl Ether	C26	C26	C27	C27	0	0	0	0	P17	P17
	C25	C25	C25	C25	0	0	0	0	P20	P20
Buffered Aqueous: pH 9.0	0	0	S	S	S	S	S	S	S	S
	0	0	S	S	S	S	S	S	0	0
Buffered Aqueous: pH 4.0	S	S	S	S	S	S	S	S	0	0
	S	S	S	S	S	S	S	S	0	0
Mineral Acid	0	0	0	0	0	0	0	0	S	S
	0	0	0	0	0	0	S	S	S	S
Controls	0	0	0	0	0	0	0	0	0	0

Results with each extract were obtained from duplicate plates.

"O" denotes characteristic growth of organism.

"S" denotes stimulation.

"P" with arabic numerals denotes partial inhibition measured in mm.

"C" with arabic numerals denotes complete inhibition measured in mm.

25. Cirsium wheeleri (A. Gray) Petrak (Thistle)

Each extract was prepared from 20 grams of the ground stems, leaves, and flowers, collected two weeks previously, to which 40 ml of the solvent had been added. Partial inhibition of B. subtilis with the aqueous extract, the acid extract, and the mineral acid extract were noted. About the periphery of the partial inhibition zones of the aqueous extract and the mineral acid extract of B. subtilis stimulation was observed. The partial inhibition with the acid extract on B. subtilis was evidenced only at the 24 hour reading. M. pyogenes var. aur-eus, Ps. aeruginosa, and E. coli were stimulated by the aqueous extract and the mineral acid extract; Myco. tuberculosis was stimulated by all the extracts excepting that of the ether extract. Results, Table 25, show neither inhibitory nor stimulatory effects on the remainder of the organisms.

26. Haploppapus fruticosus (Green) Blake in Benson (Rayless Goldenrod)

Each extract was prepared from 20 grams of ground stems, leaves, and flowers, freshly collected, to which 20 ml of the solvent were added. Complete inhibition of B. subtilis was noted with the aqueous extract and the mineral acid extract. Partial inhibition of B. subtilis at the periphery of the zones of complete inhibition with the aqueous extract and the ether extract were obtained. The results, Table 26, show no inhibition or stimulation with the remainder of the organisms.

27. Haploppapus laricifolius A. Gray (Turpentine bush)

Each extract was prepared from 20 grams of ground stems, leaves, and flowers, collected one day prior to use, to which 20 ml of each solvent were added. The disc plate method showed complete inhibition of B.

TABLE #25

RESULTS OF THE DISC-PLATE ASSAY METHOD

Plant: Cirsium wheeleri (A. Gray) Petrak (Thistle)

Plant Extract	<u>B. subtilis</u>		<u>M. pyogenes</u>		<u>Ps. aeruginosa</u>		<u>E. coli</u>		<u>Myco. tuberculosis</u>	
	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr
Buffered Aqueous: pH 7.0	P23s	P23s	S	S	S	S	S	S	S	S
	P21s	P21s	S	S	S	S	S	S	S	S
Ethyl Ether	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0
Buffered Aqueous: pH 9.0	0	0	0	0	0	0	0	0	S	S
	0	0	0	0	0	0	0	0	S	S
Buffered Aqueous: pH 4.0	P16	0	0	0	0	0	0	0	S	S
	P14	0	0	0	0	0	0	0	S	S
Mineral Acid	P20s	P20s	S	S	S	S	S	S	S	S
	P21s	P21s	S	S	S	S	S	S	S	S
Controls	0	0	0	0	0	0	0	0	0	0

Results with each extract were obtained from duplicate plates.

"O" denotes characteristic growth of organism.

"S" denotes stimulation.

"P" with arabic numerals denotes partial inhibition:

"s" denotes stimulation beyond inhibition zone.

measured in mm.

TABLE #26

RESULTS OF THE DISC-PLATE ASSAY METHOD

Plant: Haploppapus fruticosus (Green) Blake in Benson (Rayless Goldenrod)

Plant Extract	<u>B. subtilis</u>		<u>M. pyogenes</u>		<u>Ps. aeruginosa</u>		<u>E. coli</u>		<u>Myco. tuberculosis</u>	
	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr
Buffered Aqueous: pH 7.0	C24 P30	C24 P30	0	0	0	0	0	0	0	0
	C24 P30	C24 P30	0	0	0	0	0	0	0	0
Ethyl Ether	C26 P31	C26 P31	0	0	0	0	0	0	0	0
	C26 P31	C26 P31	0	0	0	0	0	0	0	0
Buffered Aqueous: pH 9.0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0
Buffered Aqueous: pH 4.0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0
Mineral Acid	C14	C14	0	0	0	0	0	0	0	0
	C13	C13	0	0	0	0	0	0	0	0
Controls	0	0	0	0	0	0	0	0	0	0

Results with each extract were obtained from duplicate plates.

"O" denotes characteristic growth of organism.

"S" denotes stimulation.

"P" with arabic numerals denotes partial inhibition measured in mm.

"C" with arabic numerals denotes complete inhibition measured in mm.

subtilis with each extract. Stimulation of M. pyogenes var. aureus and Ps. aeruginosa was evident with all the extracts except that of the ether extract. Ps. aeruginosa produced a very strong pigmentation of blue-green color with the acid and mineral acid extracts. E. coli was stimulated by the aqueous extract, the basic extract, and the mineral acid extract. One of the duplicate plates of E. coli with the basic and mineral acid extracts showed stimulation while the other plate in each instance did not. Results, Table 27a, show no inhibition or stimulation of the remaining organisms.

For the assay of this plant with the cylinder-plate method each extract was prepared from 20 grams of ground stems, leaves, and flowers collected one week prior to use, to which 20 ml of each solvent were added. Complete inhibition of B. subtilis, M. pyogenes var. aureus, and Myco. tuberculosis was observed with each extract; partial inhibition of B. subtilis beyond the periphery of the complete inhibition zone with the mineral acid extract was noted. Results, table Table 27b, show no inhibition or stimulation of the remaining organisms.

28. Datura meteloides DC. (Jimson Weed)

Each extract was prepared from 20 grams of the ground stems and leaves, freshly collected, to which 20 ml of the solvent were added. Complete inhibition of B. subtilis was noted with the ether extract, the basic extract, and the mineral acid extract. Partial inhibition of E. coli resulted with the ether extract. The results, Table 28, show neither inhibition nor stimulation of the remainder of the organisms.

29. Euphorbia albomarginata Torr and Gray (La Golondrina)

Each extract was prepared from 20 grams of ground stems, leaves, and roots collected 18 months previously, to which 40 ml of each solvent

TABLE #27a

RESULTS OF THE DISC-PLATE ASSAY METHOD

Plant: Haplopappus laricifolius A. Gray (Turpentine bush)

Plant Extract	<u>B. subtilis</u>		<u>M. pyogenes</u>		<u>Ps. aeruginosa</u>		<u>E. coli</u>		<u>Myco. tuberculosis</u>	
	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr
Buffered Aqueous: pH 7.0	C22	C22	S	S	S	S	S	S	0	0
	C23	C23	S	S	S	S	S	S	0	0
Ethyl Ether	C22	C22	0	0	0	0	0	0	0	0
	C23	C23	0	0	0	0	0	0	0	0
Buffered Aqueous: pH 9.0	C22	C22	S	S	S	S	0	0	0	0
	C21	C21	S	S	S	S	S	S	0	0
Buffered Aqueous: pH 4.0	C20	C20	S	S	Sx	Sx	0	0	0	0
	C21	C21	S	S	Sx	Sx	0	0	0	0
Mineral Acid	C20	C20	S	S	Sx	Sx	0	0	0	0
	C22	C22	S	S	Sx	Sx	S	S	0	0
Controls	0	0	0	0	0	0	0	0	0	0

Results with each extract obtained from duplicate plates.

"O" denotes characteristic growth of organism.

"S" denotes stimulation.

"C" with arabic numerals denotes complete inhibition measured in mm.

"x" denotes pigmentation.

TABLE #27b

RESULTS OF THE CYLINDER-PLATE ASSAY METHOD

Plant: Haplopappus laricifolius A. Gray (Turpentine)

Bush

Plant Extract	<u>B. subtilis</u>		<u>M. pyogenes</u>		<u>Ps. aeruginosa</u>		<u>E. coli</u>		<u>Myco. tuberculosis</u>	
	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr
Buffered Aqueous: pH 7.0	C33	C33	C15	C15	0	0	0	0	C25	C25
	C16	C16	C17	C17	0	0	0	0	C24	C24
Ethyl Ether	C17	C17	C16	C16	0	0	0	0	C15	C15
	C22	C22	C20	C20	0	0	0	0	0	0
Buffered Aqueous: pH 9.0	C28	C28	C15	C15	0	0	0	0	C26	C26
	C24	C24	C15	C15	0	0	0	0	C20	C20
Buffered Aqueous: pH 4.0	C15	C15	C16	C16	C11	C11	0	0	C26	C26
	C16	C16	C14	C14	C12	C12	0	0	C25	C25
Mineral Acid	C14 P18:C14 P18		C17	C17	0	0	0	0	C27	C27
	C17 P20:C17 P20		C13	C13	0	0	0	0	C23	C23
Controls	0	0	0	0	0	0	0	0	0	0

Results with each extract were obtained from duplicate plates.

"O" denotes characteristic growth of organism.

"P" with arabic numerals denotes partial inhibition measured in mm.

"C" with arabic numerals denotes complete inhibition measured in mm.

TABLE #28

RESULTS OF THE DISC-PLATE ASSAY METHOD

Plant: Datura meteloides DC. (Jimson weed)

Plant Extract	<u>B. subtilis</u>		<u>M. pyogenes</u>		<u>Ps. aeruginosa</u>		<u>E. coli</u>		<u>Myco. tuberculosis</u>	
	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr
Buffered Aqueous: pH 7.0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0
Ethyl Ether	C20	C20	0	0	0	0	P32	P32	0	0
	C21	C21	0	0	0	0	P18	P18	0	0
Buffered Aqueous: pH 9.0	C15	C15	0	0	0	0	0	0	0	0
	C13	C13	0	0	0	0	0	0	0	0
Buffered Aqueous: pH 4.0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0
Mineral Acid	C18	C18	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0
Controls	0	0	0	0	0	0	0	0	0	0

Results with each extract were obtained from duplicate plates.

"O" denotes characteristic growth of organism.

"C" with arabic numerals denotes complete inhibition

"P" with arabic numerals denotes partial inhibition measured in mm.

tion measured in mm.

were added. Complete inhibition of B. subtilis, M. pyogenes var. aureus and E. coli with the basic extracts was observed, while the acid inhibited E. coli and M. pyogenes var. aureus completely. Stimulation of Myco. tuberculosis was noted with the aqueous extract, the basic extract, and the mineral acid extract. The results, Table 29, show no inhibition or stimulation of the remainder of the organisms. These results differ from the work of Atterbury on this plant in that complete inhibition of B. subtilis was obtained not with the basic extract, but with the mineral acid and ether extracts. Also, Atterbury's work did not disclose any inhibition of M. pyogenes var. aureus with the acid extract, but she did obtain complete inhibition of this organism with the mineral acid extract and the ether extract; partial inhibition of M. pyogenes var. aureus with the aqueous extract and the basic extract was also recorded. The inhibition of M. pyogenes var. aureus with the basic extract agrees with present findings. However, she obtained inhibition of E. coli with each extract whereas in this investigation this was found only with the acid and basic extracts.

30. Chilopsis linearis (Cav.) Sweet (Desert Willow)

Each extract was prepared from 20 grams of ground stems and leaves, freshly collected, to which 20 ml of each solvent were added. Complete inhibition of B. subtilis with all the extracts was evidenced. M. pyogenes var. aureus was completely inhibited by the ether, basic, and mineral acid extracts; partial inhibition was noted with the aqueous and the acid extracts. Myco. tuberculosis was completely inhibited by the aqueous, ether, and mineral acid extracts; partial inhibition was evidenced with the basic and the acid extracts. Stimulation of growth was observed about the periphery of the inhibitory zones of M. pyogenes

TABLE #29

RESULTS OF THE DISC-PLATE ASSAY METHOD

Plant: Euphorbia albomarginata Torr and Gray (La Golondrina)

Plant Extract	<u>B. subtilis</u>		<u>M. pyogenes</u>		<u>Ps. aeruginosa</u>		<u>E. coli</u>		<u>Myco. tuberculosis</u>	
	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr
Buffered Aqueous: pH 7.0	0	0	0	0	0	0	0	0	S	S
	0	0	0	0	0	0	0	0	S	S
Ethyl Ether	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0
Buffered Aqueous: pH 9.0	C20	C20	C20	C20	0	0	C20	C20	S	S
	C21	C21	C22	C22	0	0	C15	C15	S	S
Buffered Aqueous: pH 4.0	0	0	C18	C18	0	0	C14	C14	S	S
	0	0	C19	C19	0	0	0	0	S	S
Mineral Acid	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0
Controls	0	0	0	0	0	0	0	0	0	0

Results with each extract were obtained from duplicate plates.

"O" denotes characteristic growth of organism.

"C" with arabic numerals denotes complete inhibition measured in mm.

"S" denotes stimulation.

var. aureus with the basic extract, and Myco. tuberculosis with the ether extract, basic extract, and mineral extract. It is of interest to note that the gram negative organisms were not at all affected while the gram positive organisms were. Results, Table 30a, show the results mentioned above. Another set of extracts of this plant were prepared from 20 grams of ground, frosted stems to which 40 ml of each solvent were added. Complete inhibition of B. subtilis occurred with the aqueous, basic, and acid extracts. Partial inhibition of M. pyogenes var. aureus was observed with the basic and the acid extracts. Partial inhibition of Myco. tuberculosis with the aqueous extract and the acid extract was shown. Results, Table 30b, show neither inhibition nor stimulation on the remainder of the organisms. It is interesting to compare the two tests of this plant using the fresh growing stems and leaves against the frosted stems. Although inhibitory activity exists with the latter, it is much less than that shown with the growing plant.

31. Perezia thurberi A. Gray

Each extract was prepared from 20 grams of ground stems, leaves, and flowers, collected one day previously, to which 20 ml of the solvent had been added. Complete inhibition of B. subtilis with all the extracts was observed. M. pyogenes var. aureus was completely inhibited by the ether extract and partially inhibited by the basic, acid, and mineral acid extracts. Complete inhibition of Myco. tuberculosis with the ether extract was noted. Stimulation was observed on one of the duplicate plates of Ps. aeruginosa with the basic extract and also the acid extract. E. coli was stimulated by the aqueous extract, and Myco. tuberculosis was stimulated on one of duplicate plates with the basic extract and the mineral acid extract. Results, Table 31, show

TABLE #30a

RESULTS OF THE DISC-PLATE ASSAY METHOD

Plant: Chilopsis lenearis (Cav.) Sweet (Desert Willow)

Plant Extract	<u>B. subtilis</u>		<u>M. pyogenes</u>		<u>Ps. aeruginosa</u>		<u>E. coli</u>		<u>Myco. tuberculosis</u>	
	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr
Buffered Aqueous: pH 7.0	C20	C20	P11	P11	0	0	0	0	C18	C18
	C21	C21	C15	C15	0	0	0	0	P11	P11
Ethyl Ether	C25	C25	C18	C18	0	0	0	0	C10s	C10s
	C19	C19	C12	C12	0	0	0	0	C11s	C11s
Buffered Aqueous: pH 9.0	C20	C20	C12s	C12s	0	0	0	0	P10s	P10s
	C18	C18	C14s	C14s	0	0	0	0	0	0
Buffered Aqueous: pH 4.0	C18	C18	P13	P13	0	0	0	0	P11s	P11s
	C22	C22	P15	P15	0	0	0	0	P10s	P10s
Mineral Acid	C18	C18	C10	C10	0	0	0	0	C12	C12
	C20	C20	C10	C10	0	0	0	0	C10	C10
Controls	0	0	0	0	0	0	0	0	0	0

Results with each extract were obtained from duplicate plates.

"O" denotes characteristic growth of organism.

"P" with arabic numerals denotes partial inhibition measured in mm.

"C" with arabic numerals denotes complete inhibition measured in mm.

TABLE #30b

RESULTS OF THE CYLINDER-PLATE ASSAY METHOD

Plant: Chilopsis lenearis (Cav.) Sweet (Desert Willow)

Plant Extract	<u>B. subtilis</u>		<u>M. pyogenes</u>		<u>Ps. aeruginosa</u>		<u>E. coli</u>		<u>Myco. tuberculosis</u>	
	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr
Buffered Aqueous: pH 7.0	C17	C17	0	0	0	0	0	0	P15	P15
	C17	C17	0	0	0	0	0	0	P13	P13
Ethyl Ether	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0
Buffered Aqueous: pH 9.0	C17	C17	P15	P15	0	0	0	0	0	0
	C15	C15	P12	P12	0	0	0	0	0	0
Buffered Aqueous: pH 4.0	C16	C16	P14	P14	0	0	0	0	P12	P12
	C12	C12	P11	P11	0	0	0	0	P11	P11
Mineral Acid	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0
Controls	0		0	0	0	0	0	0	0	0

Results with each extract were obtained from duplicate plates.

"O" denotes characteristic growth of organism.

"C" with arabic numerals denotes complete in-

"P" with arabic numerals denotes partial inhibition measured in mm.

hibition measured in mm.

TABLE #31

RESULTS OF THE DISC-PLATE ASSAY METHOD

Plant: Perezia thurberi A. Gray

Plant Extract	<u>B. subtilis</u>		<u>M. pyogenes</u>		<u>Ps. aeruginosa</u>		<u>E. coli</u>		<u>Myco. tuberculosis</u>	
	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr
Buffered Aqueous: pH 7.0	C20	P30	0	0	0	0	S	S	0	0
	P22	P22	0	0	0	0	S	S	0	0
Ethyl Ether	C28	C28	C17	C17	0	0	0	0	C18	C18
	C22	C22	C15	C15	0	0	0	0	C17	C17
Buffered Aqueous: pH 9.0	C30	C30	P20	P20	0	0	0	0	0	0
	C30	C30	P20	P20	S	S	0	0	S	S
Buffered Aqueous: pH 4.0	C30	C30	P22	P22	0	0	0	0	0	0
	C30	C30	P20	P20	S	S	0	0	0	0
Mineral Acid	C22	C22	P23	P23	0	0	0	0	S	S
	C24	C24	P24	P24	0	0	0	0	0	0
Controls	0	0	0	0	0	0	0	0	0	0

Results with each extract were obtained from duplicate plates.

"O" denotes characteristic growth of organism.

"S" denotes stimulation.

"P" with arabic numerals denotes partial inhibition measured in mm.

"C" with arabic numerals denotes complete inhibition measured in mm.

neither inhibition nor stimulation of the remaining organisms. Further work was not done with this plant because of failure to locate more of the specimen with which to work.

32. Baccharus sarothroides A. Gray (Desert Broom)

Each extract was prepared from 20 grams of the ground stems, leaves, and flowers, freshly collected, to which 20 ml of the solvent were added. B. subtilis was inhibited completely with the ether extract. Complete inhibition on one of duplicate plates and partial inhibition on the other of the duplicate plates with the basic extract and the mineral acid extracts was evident, and partial inhibition with the acid extract on both plates was noted. M. pyogenes var. aureus was completely inhibited with the ether extract and partially inhibited with the basic and the mineral acid extracts. Complete inhibition of Myco. tuberculosis was evidenced on both plates with the ether extract and on one of the duplicate plates with the mineral acid extract; partial inhibition of this organism occurred on both plates with the basic extract and on one of the duplicate plates with the acid and mineral acid extracts. The results, Table 32, show neither inhibition nor stimulation of the other organisms.

33. Oenothera hookeri Torr and Gray (Primrose)

Each extract was prepared from 20 grams of ground stems, leaves, and flowers, collected three weeks prior to use, to which 40 ml of solvent were added. B. subtilis was partially inhibited by the basic and the mineral acid extracts; however, it was completely inhibited with the basic extract at the 24 hour reading and partially inhibited with this extract in the 48 hour period. M. pyogenes var. aureus, Ps. aeruginosa, and E. coli, were completely inhibited by all of the extracts except

TABLE #32

RESULTS OF THE DISC-PLATE ASSAY METHODPlant: Baccharus sarothroides A. Gray (Desert Broom)

Plant Extract	<u>B. subtilis</u>		<u>M. pyogenes</u>		<u>Ps. aeruginosa</u>		<u>E. coli</u>		<u>Myco. tuberculosis</u>	
	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr
Buffered Aqueous: pH 7.0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0
Ethyl Ether	C33	C33	C17	C17	0	0	0	0	C18	C18
	C28	C28	C11	C11	0	0	0	0	C15	C15
Buffered Aqueous: pH 9.0	C16	C16	0	0	0	0	0	0	P14	P14
	P20	P20	P19	P19	0	0	0	0	P10	P10
Buffered Aqueous: pH 4.0	P14	P14	0	0	0	0	0	0	P14	P14
	P12	P12	0	0	0	0	0	0	0	0
Mineral Acid	P24	P24	P20	P20	0	0	0	0	C19	C19
	C20	C20	P14	P14	0	0	0	0	P12	P12
Controls	0	0	0	0	0	0	0	0	0	0

Results with each extract were obtained from duplicate plates.

"0" denotes characteristic growth of organism.

"P" with arabic numerals denotes partial inhibition measured in mm.

"C" with arabic numerals denotes complete inhibition measured in mm.

the ether extract. Complete inhibition of Myco. tuberculosis was evidenced with the aqueous, basic, and mineral acid extracts. Stimulation was noted about the periphery of the inhibitory zones with the aqueous and the mineral acid extract on Myco. tuberculosis, and with the aqueous and mineral acid extracts on M. pyogenes var. aureus. Stimulation of B. subtilis with the aqueous extract and of Myco. tuberculosis with the acid extract was observed. Intense pigmentation by the Ps. aeruginosa occurred with all extracts except that of ether. It is of interest to observe that the ether extract exhibited no effect on any of the organisms. Results of the above are given on Table 33.

34. Humulus americanus Nutt (Wild hop)

Each extract was prepared from 20 grams of the ground stems, leaves, and flowers, freshly collected, to which 20 ml of each solvent were added. Complete inhibition of B. subtilis was noted with all the extracts except the ether extract. Of the five extracts only the basic extract failed to completely inhibit M. pyogenes var. aureus. Complete inhibition of Myco. tuberculosis was noted with the ether extract. Stimulation of B. subtilis with the aqueous extract and of Myco. tuberculosis with all the extracts except the ether extract was evidenced. The results, Table 34a, show no inhibition or stimulation on the remaining organisms.

In a second assay of this plant the cylinder-plate method was used. Each extract was prepared from 20 grams of the ground stems, leaves, and flowers, freshly collected, to which 20 ml of each solvent were added. Complete inhibition of B. subtilis, M. pyogenes var. aureus, and Myco. tuberculosis was noted with all the extracts. Stimulation of Ps. aeruginosa was evidenced with all the extracts except

TABLE #33

RESULTS OF THE DISC-PLATE ASSAY METHOD

Plant: Oenothera hookeri Torr and Gray (Primrose)

Plant Extract	<u>B. subtilis</u>		<u>M. pyogenes</u>		<u>Ps. aeruginosa</u>		<u>E. coli</u>		<u>Myco. tuberculosis</u>	
	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr
Buffered Aqueous pH 7.0	S	S	C20	C20s	xC20	xC20	C22	C22	C30s	C30s
	S	S	C21	C21s	xC21	xC21	C20	C20	C27s	C27s
Ethyl Ether	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0
Buffered Aqueous pH 9.0	P20	P15	C23	C23	xC22	xC22	C24	C20	C30s	C30s
	P24	P24	C20	C20	xC21	xC21	C20	C20	C27s	C27s
Buffered Aqueous pH 4.0	C24	P13	C22	C22	xC18	xC18	C30	C27	S	S
	C18	P14	C18	C18	xC19	xC19	C24	C22	S	S
Mineral Acid	P15	P15	C22s	C22s	xC20	xC20	C20	C20	C28	C28
	P19	P19	C23s	C23s	xC18	xC18	C23	C23	C26	C26
Controls	0	0	0	0	0	0	0	0	0	0

Results with each extract were obtained from duplicate plates.

"0" denotes characteristic growth of organism.

"P" with arabic numerals denotes partial inhibition measured in mm.

"s" denotes stimulation beyond inhibition zone.

: "S" denotes stimulation.

: "C" with arabic numerals denotes complete inhibition measured in mm.

: "x" denotes pigmentation.

the ether extract. Results are given in Table 34b. It is of interest to compare the results of the disc-plate method with those of the cylinder-plate method. This latter method shows considerable increase in activity against the Myco. tuberculosis, and an advent of stimulation of the Ps. aeruginosa that was not evident in the disc-plate method.

The agar-streak method was also used as a third means for the assay of this plant. To 235 grams of ground stems, leaves, and flowers, collected two weeks prior to use, 300 ml of ethyl ether were added and allowed to stand 6 hours. The extract was decanted through cheesecloth, treated with Norite A to remove chlorophyll from the solution, and filtered. The latter step was repeated twice. The extract was then evaporated to dryness in air and weighed. To 1.52 grams of crude extract 3.06 ml of ethyl alcohol (95%) were added. This solution was Seitz filtered. Serial increments of this filtrate were added to agar tubed in 10 ml amounts, and poured into petri plates; these contained 8,000 mcg/ml, 6,000 mcg/ml, 4,000 mcg/ml, 2,000 mcg/ml, 1000 mcg/ml, 900 mcg/ml, 700 mcg/ml, 500 mcg/ml, 300 mcg/ml, and 100 mcg/ml of the crude extract. The test organisms used were E. coli, M. pyogenes var. aureus, M. smegmatis, and B. subtilis. Duplicates were run of each plate. No inhibition of the growth of E. coli on any dilution was noted; M. pyogenes var. aureus and Myco. smegmatis attained normal growth only with the highest dilution used, 100 mcg/ml in a 72 hour reading. However, B. subtilis was totally inhibited even with the highest dilution used. These results are shown in Table 34c. A test of four species of mycobacteria only was made using the above method and employing the same dilutions and preparation. Myco. avium was totally inhibited by all the dilutions except that of 100 mcg/ml where normal growth was attained.

TABLE #34a

RESULTS OF THE DISC-PLATE ASSAY METHOD

Plant: Humulus americanus Nutt (Wild hop)

Plant Extract	<u>B. subtilis</u>		<u>M. pyogenes</u>		<u>Ps. aeruginosa</u>		<u>E. coli</u>		<u>Myco. tuberculosis</u>	
	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr
Buffered Aqueous: pH 7.0	S	S	C21	C21	0	0	0	0	S	S
	S	S	C21	C21	0	0	0	0	S	S
Ethyl Ether	C31	C31	C26	C26	0	0	0	0	0	0
	C28	C28	C30	C30	0	0	0	0	C12	C12
Buffered Aqueous: pH 9.0	C22	C22	0	0	0	0	0	0	S	S
	C21	C21	0	0	0	0	0	0	S	S
Buffered Aqueous: pH 4.0	C24	C24	C22	C22	0	0	0	0	S	S
	C24	C24	C24	C24	0	0	0	0	S	S
Mineral Acid	C20	C20	C21	C21	0	0	0	0	S	S
	C20	C20	C21	C 21	0	0	0	0	S	S
Controls	0	0	0	0	0	0	0	0	0	0

Results with each extract were obtained from duplicate plates.

"0" denotes characteristic growth of organism.

"S" denotes stimulation.

"C" with arabic numerals denotes complete inhibition measured in mm.

TABLE #34b

RESULTS OF THE CYLINDER-PLATE ASSAY METHOD

Plant: Humulus americanus Nutt (Wild Hop)

Plant Extract	<u>B. subtilis</u>		<u>M. pyogenes</u>		<u>Ps. aeruginosa</u>		<u>E. coli</u>		<u>Myco. tuberculosis</u>	
	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr
Buffered Aqueous: pH 7.0	C18	C18	C16	C16	S	S	0	0	C17	C17
	C17	C17	C16	C16	S	S	0	0	C16	C16
Ethyl Ether	C18	C18	C11	C11	0	0	0	0	C22	C22
	C18	C18	C9	C9	0	0	0	0	C20	C20
Buffered Aqueous: pH 9.0	C19	C19	C16	C16	S	S	0	0	C26	C26
	C18	C18	C16	C16	S	S	0	0	C26	C26
Buffered Aqueous: pH 4.0	C15	C15	C14	C14	S	S	0	0	C18	C18
	C16	C16	C11	C11	S	S	0	0	C16	C16
Mineral Acid	C18	C18	C16	C16	S	S	0	0	C20	C20
	C17	C17	C12	C12	S	S	0	0	C23	C23
Controls	0	0	0	0	0	0	0	0	0	0

Results with each extract were obtained from duplicate plates.

"O" denotes characteristic growth of organism.

"S" denotes stimulation.

"C" with arabic numerals denotes complete inhibition measured in mm.

TABLE #34C

RESULTS OF THE AGAR-STREAK METHODPlant: Humulus americanus Nutt (Wild Hop)

Extract in mcg/ml media	<u>E. coli</u>			<u>M. pyogenes</u> var. <u>aureus</u>			<u>Myco. Smegmatis</u>			<u>B. subtilis</u>		
	24	48	72	24	48	72	24	48	72	24	48	72
8000	+	+	+	-	-	-	-	-	-	-	-	-
	+	+	+	-	-	-	-	-	-	-	-	-
4000	+	+	+	-	-	-	-	-	-	-	-	-
	+	+	+	-	-	-	-	-	-	-	-	-
1000	+	+	+	-	-	-	-	-	-	-	-	-
	+	+	+	-	-	-	-	-	-	-	-	-
900	+	+	+	-	-	-	-	-	-	-	-	-
	+	+	+	-	-	-	-	-	-	-	-	-
700	+	+	+	-	-	-	-	-	-	-	-	-
	+	+	+	-	-	-	-	-	-	-	-	-
500	+	+	+	-	-	-	-	-	-	-	-	-
	+	+	+	-	-	-	-	-	-	-	-	-
300	+	+	+	-	-	-	-	-	-	-	-	-
	+	+	+	-	-	-	-	-	-	-	-	-
100	+	+	+	-	-	-	-	±	+	-	-	-
	+	+	+	-	-	-	-	±	+	-	-	-
Control	+	+	+	+	+	+	-	+	+	+	+	+

Results with each organism were obtained from duplicate plates.

"- " denotes no growth of the organism.

"±" denotes slight growth of the organism.

"++" denotes normal growth."

"±" denotes slightly less than normal growth.

TABLE #34d

RESULTS OF THE AGAR-STREAK METHOD

Plant: Humulus americanus Nutt (Wild Hop)

Extract in mcg/ml media	<u>Myco. avium</u>			<u>Myco. phlei</u>			<u>Myco. Ranae</u>			<u>Myco. tuberculosis</u>		
	24	48	72	24	48	72	24	48	72	24	48	72
8000	-	-	-	-	-	-	-	-	-	-	-	-
4000	-	-	-	-	-	-	-	-	-	-	-	-
1000	-	-	-	-	-	-	-	-	-	-	-	-
900	-	-	-	-	-	-	-	-	-	-	-	-
700	-	-	-	-	-	-	-	-	-	-	-	-
500	-	-	-	-	-	-	-	-	-	-	-	-
300	-	-	-	-	-	-	-	+	+	-	-	-
100	-	+	+	-	-	-	-	+	+	-	-	-
Control	-	+	+	-	+	+	-	+	+	-	+	+

Results of each extract were obtained from duplicate plates.

"-" denotes no growth of the organism.

"+" denotes normal growth.

Myco. phlei and Myco. tuberculosis were totally inhibited by all the dilutions used; Myco. ranae attained normal growth with the 300 mcg/ml dilution. These results are shown in Table 34d.

35. Artemisia dracunculoides Pursch

Each extract was prepared from 20 grams of ground leaves, collected one week previously, to which 20 ml of each solvent were added. B. subtilis was completely inhibited in both plates with the ether extract, and on one of the duplicate plates with the basic extract. M. pyogenes var. aureus was partially inhibited on one of the duplicate plates with the ether extract; Myco. tuberculosis was completely inhibited with the ether extract. The complete inhibition of Myco. tuberculosis was so pronounced with the basic extract, the acid extract, and the mineral acid extract that no zoning could be determined. The results, Table 35a, show no inhibition or stimulation of the remaining organisms.

In the second assay of this plant the cylinder-plate method was used. Each extract was prepared from 20 grams of ground leaves, collected three weeks previously, to which 20 ml of each solvent were added. Complete inhibition of B. subtilis and M. pyogenes var. aureus with the ether and acid extracts was evidenced. Myco. tuberculosis was completely inhibited by the ether and acid extracts in both plates, and with the mineral acid and basic extracts on one of the duplicate plates. Stimulation of Myco. tuberculosis with the aqueous extract was observed. Results, Table 35b, show neither inhibitory nor stimulatory effects with the remaining extracts.

The agar-streak technic was also used as a third means for the assay of this plant. An ether extract of this plant was prepared from 285 grams of ground leaves to which 300 ml of ether were added. This

TABLE #35a

RESULTS OF THE DISC-PLATE ASSAY METHOD

Plant: Artemisia dracunculoides Porsch

Plant Extract	<u>B. subtilis</u>		<u>M. pyogenes</u>		<u>Ps. aeruginosa</u>		<u>E. coli</u>		<u>Myco. tuberculosis</u>	
	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr
Buffered Aqueous: pH 7.0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0
Ethyl Ether	C17	C17	P19	P19	0	0	0	0	C25	C25
	C18	C18	0	0	0	0	0	0	C27	C27
Buffered Aqueous: pH 9.0	0	0	0	0	0	0	0	0	*	*
	C18	C18	0	0	0	0	0	0	*	*
Buffered Aqueous: pH 4.0	0	0	0	0	0	0	0	0	*	*
	0	0	0	0	0	0	0	0	*	*
Mineral Acid	0	0	0	0	0	0	0	0	*	*
	0	0	0	0	0	0	0	0	*	*
Controls	0	0	0	0	0	0	0	0	0	0

Results with each extract were obtained from duplicate plates.

"O" denotes characteristic growth of organism.

"P" with arabic numerals denotes partial inhibition measured in mm.

"*" denotes complete inhibition of undetermined diameter

"C" with arabic numerals denotes complete inhibition measured in mm.

TABLE #35b

RESULTS OF THE CYLINDER-PLATE ASSAY METHOD

Plant: Artemisia dracunculoides Pursch

Plant Extract	<u>B. subtilis</u>		<u>M. pyogenes</u>		<u>Ps. aeruginosa</u>		<u>E. coli</u>		<u>Myco. tuberculosis</u>	
	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr
Buffered Aqueous: pH 7.0	0	0	0	0	0	0	0	0	S	S
	0	0	0	0	0	0	0	0	S	S
Ethyl Ether	C20	C20	C15	C15	0	0	0	0	C48	C48
	C19	C19	C13	C13	0	0	0	0	C50	C50
Buffered Aqueous: pH 9.0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	C15	C15
Buffered Aqueous: pH 4.0	C14	C14	C12	C12	0	0	0	0	C20	C20
	C20	C20	C11	C11	0	0	0	0	C26	C26
Mineral Acid	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	C14	C14
Controls	0	0	0	0	0	0	0	0	0	0

Results with each extract were obtained from duplicate plates.

"O" denotes characteristic growth of the organism. : "S" denotes stimulation.

"C" with arabic numerals denotes complete inhibition measured in mm. :

extract was treated in a similar manner to that described above for Humulus americanus. Dilutions of 500, 300, 100, 90, 70, 50, 30, and 10 mcg/ml were prepared. This method was applied to mycobacterium cultures only. Myco. tuberculosis and Myco. smegmatis attained normal growth with the 60 mcg/ml dilution; Myco. avium showed normal growth with the 100 mcg/ml dilution, and Myco. phlei evidenced normal growth with the 30 mcg/ml dilution. Results are shown in Table 35c. A second assay using the same method of preparation, but using dilutions from 600, mcg/ml to 10 mcg/ml was made. In these results Myco. ranae, Myco. tuberculosis, and Myco. phlei attained normal growth at 60 mcg/ml, and Myco. avium attained normal growth at 40 mcg/ml. These results are tabulated in Table 35d. The duplicate assay showed similar results with Myco. ranae and Myco. tuberculosis, while Myco. avium attained normal growth at 50 mcg/ml and Myco. phlei attained normal growth at 40 mcg/ml. Two more assays were made using the same method and preparation with the exception that tap water was used with one assay. The results with the distilled water prepared assay show normal growth of Myco. tuberculosis and Myco. avium at 20 mcg/ml, of Myco. smegmatis, Myco. phlei, and Myco. ranae at 40 mcg/ml. The results are shown in Table 35e. The control duplicate set of plates showed practically the same end-points. With the tap-water prepared assay normal growth of all the mycobacteria tested was attained at 100 mcg/ml. Very similar end-points were shown in the duplicate plates. These results are shown in Table 35f. As the author was primarily interested in the results of this plant on the acid-fast bacteria, no other organisms were used in this method.

TABLE #35c

RESULTS OF THE AGAR-STREAK METHOD

Plant: Artemisia dracunculoides Pursch

Extract in mcg/ml media	<u>Myco. tuberculosis</u> :			<u>Myco. avium</u> :			<u>Myco. smegmatis</u> :			<u>Myco. phlei</u>		
	24	48	72	24	48	72	24	48	72	24	48	72
500	-	-	-	-	-	-	-	-	-	-	-	-
300	-	-	-	-	±	±	-	-	-	-	-	-
100	-	±	±	-	±	±	-	±	±	-	-	-
90	-	±	±	-	±	±	-	±	±	-	-	-
70	-	+	+	-	+	+	-	±	±	-	±	±
50	-	+	+	-	+	+	-	+	+	-	±	±
30	-	+	+	-	+	+	-	+	+	-	+	+
10	-	+	+	-	+	+	-	+	+	-	+	+
Control	-	+	+	-	+	+	-	+	+	-	+	+

Results with each extract were obtained from duplicate plates.

"-" denotes no growth of the organism.

:"+" denotes normal growth.

"±" denotes slight growth of the organism.

:"±" denotes slightly less than normal growth.

TABLE #35d

RESULTS OF THE AGAR-STREAK METHODPlant: Artemisia dracunculoides Pursch

Extract in mcg/ml media	<u>Myco. Ranae</u>			<u>Myco. tuberculosis</u>			<u>Myco. avium</u>			<u>Myco. phlei</u>		
	24	48	72	24	48	72	24	48	72	24	48	72
600	-	-	-	-	-	-	-	-	-	-	-	-
400	-	-	-	-	-	-	-	-	-	-	-	-
200	-	-	-	-	-	-	-	-	-	-	-	-
100	-	+	+	-	-	-	-	+	+	-	+	+
80	-	+	+	-	+	+	-	+	+	-	+	+
60	-	+	+	-	+	+	-	+	+	-	+	+
40	-	+	+	-	+	+	-	+	+	-	+	+
20	+	+	+	+	+	+	-	+	+	-	+	+
10	-	+	+	-	+	+	-	+	+	-	+	+
Control	-	+	+	-	+	+	-	+	+	-	+	+

Results with each organism were obtained from duplicate plates.

TABLE #35e

RESULTS OF THE AGAR-STREAK METHOD

Plant: Artemisia dracunculoides Pursch

Extract in mcg/ml media	<u>Myco. tuberculosis</u>			<u>Myco. smegmatis</u>			<u>Myco. ranae</u>			<u>Myco. phlei</u>			<u>Myco. avium</u>		
	24	48	72	24	48	72	24	48	72	24	48	72	24	48	72
400	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
300	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
200	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
100	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
80	-	-	-	-	±	±	-	-	-	-	-	-	-	-	-
60	-	-	-	-	±	±	-	±	±	-	±	±	-	±	±
40	-	±	±	-	+	+	-	+	+	-	±	±	-	+	+
20	-	+	+	-	+	+	-	+	+	-	+	+	-	+	+
Control	-	+	+	-	+	+	-	+	+	-	+	+	-	+	+

Results with each extract were obtained from duplicate plates.

"-" denotes no growth of the organism.

"+" denotes normal growth.

"±" denotes slight growth of the organism.

"±" denotes slightly less than normal growth.

TABLE #35f

RESULTS OF THE AGAR-STREAK METHOD

Plant: Artemisia dracunculoides Pursch

Extract in mcg/ml media	<u>Myco. tuberculosis</u>			<u>Myco. smegmatis</u>			<u>Myco. ranae</u>			<u>Myco. phlei</u>			<u>Myco. avium</u>		
	24	48	72	24	48	72	24	48	72	24	48	72	24	48	72
400	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
300	-	±	±	-	-	-	-	-	-	-	-	-	-	±	±
200	-	±	±	-	±	±	-	±	±	-	-	-	-	±	±
100	-	+	+	-	+	+	-	±	±	-	±	±	-	+	+
80	-	+	+	-	+	+	-	+	+	-	+	+	-	+	+
60	-	+	+	-	+	+	-	+	+	-	+	+	-	+	+
40	-	+	+	-	+	+	-	+	+	-	+	+	-	+	+
20	-	+	+	-	+	+	-	+	+	-	+	+	-	+	+
Control	-	+	+	-	+	+	-	+	+	-	+	+	-	+	+

Results with each extract were obtained from duplicate plates.

"- " denotes no growth of the organism.

"+ " denotes normal growth.

"± " denotes slight growth of the organism.

"± " denotes slightly less than normal growth

DISCUSSION

As defined by Waksman (1948) "An antibiotic or an antibiotic substance is a substance produced by microorganisms, which has the capacity of inhibiting the growth and even of destroying other microorganisms." This definition states that an antibiotic must be a product exclusively of microorganisms, from which it may be inferred that antibiotic-like substances produced by macroorganisms are not to be considered antibiotics but must be given other designations. However, in a personal communication to Irving and Herrick (1949), Waksman modified this statement as follows: "An antibiotic is a chemical substance produced by microorganisms and other living systems which has the capacity to inhibit the growth of bacteria and other microorganisms and even of destroying them." This sufficiently broadens the definition to include the higher plants. It seems appropriate to include the definition of an antibiotic as given by Benedict and Langlyke (1947): "An antibiotic is a chemical compound derived from or produced by living organisms, which is capable, in small concentration, of inhibiting the life processes of microorganisms." It will be noted in this definition that a relative qualification to the concentration is added. The latter definition is the one used in this thesis.

The first observation of antibiotic action can be traced to the year 1876 when Tyndall, as stated by Florey et al (1949), described the antagonistic action of a species of penicillium upon a bacterium. Apparently, he failed to recognize the significance of this phenomenon. The antagonistic effects of one organism upon another was first scientifically demonstrated by Pasteur and Joubert in 1877 while experimenting with the anthrax bacillus. Within the next thirty years considerable literature accumulated in regard to the use of several antibacterial substances as

possible therapeutic agents, such as pyocyanase which was obtained from cultures of Ps. aeruginosa. For the following twenty-five years contributions, according to Florey et al (1949), were made to the field of antibiotics with the development of assay methods; but it was not until the re-discovery of penicillin during the last decade and the demonstration of its effectiveness as a chemotherapeutic agent that antibiotics attained the prominent place they now hold.

In recent years several surveys have been made of potential antibiotic-producing plants; among the more extensive investigations are those of Osborn in England (1943); Sanders, Weatherwax, and McClung in Indiana (1945); Atkinson and Rainsford in Australia (1946); Carlson et al in southern Oregon (1946); and Hayes in Ohio (1947.) Some of the noncrystalline antibiotics isolated from higher plants include Puchiin, a milky liquid obtained from crushed Eleocharis tuberosa by Chin et al (1945), which is active against M. pyogenes var. aureus, E. coli, and A. aerogenes in the cylinder-plate method; Rao et al (1946) extracted pteryospermin from the root of the Indian plant, Moringa pterygosperma.

Several antibiotics from higher plants have been crystallized and, in some instances, identified. Crepin, a crystalline extract from Crepis taraxacifolia, was reported by Heatly (1944) to be most active against gram positive bacteria but it was too toxic for in vivo experiments. Cavallito et al (1945) isolated two crystalline antibiotics from another of the Compositae, namely Arctium minus. The antibacterial principle of Allium sativum, Cavallito et al (1944), has been found to be equally active toward gram-positive and gram negative bacteria.

Many workers have given their attention to plants of reputed medicinal value. Zangin et al (1945) undertook an investigation of 47 ancient

drugs used by the Chinese for the past 4,000 years; Lucas and Lewis (1944) assayed certain plants primarily because of their mention as folk remedies; Guerra et al (1946) carried out a survey of 11 plants described in Aztec manuscripts and herbals. This latter work revealed an extract of Loesalia coccinea known as cuauchile or huitzitziloxochitl that was very active against a strain of Str. haemolyticus but not against M. pyogenes var. aureus. Additional research reported in the literature will be mentioned further only if it is relevant to this investigation.

As a result of the more intensive studies of plants it seems possible that a postulate could be formed that a similarity exists between genera of certain members of the families in the possession of active principles. Osborn (1943) noted that members of the families of Lilaceae and Ranunculaceae possessed similar antibacterial activity. The principal work on the latter has been done with the genera, Anemone and Ranunculus (buttercup), whose antibiotic principles appear to be identical. Carlson and Douglas (1947) worked with the buttercup obtained from a semi-arid region in Southeastern Oregon; later work by these investigators indicated that saline extracts inhibited both gram positive and gram negative bacteria, fungi and certain non-pathogenic protozoa. The vapors of this plant were also found to be bacteriostatic and bactericidal in vitro, toxic but not lethal in vivo. The Compositae has proved to be another fertile source of antibiotic principles; crepin, mentioned above, and the two antibiotics from Arctium minus serve to illustrate this association. A correlation between closely related genera was noted in three instances within this survey. The activity of Gossipium thurberi and Gossipium hirtus showed very close correlation; Cirsium wheeleri and Cirsium arizonicum were similar as were Haplopappus fruticosus and Haplopappus lari-

cifolius.

It is a well established fact that soil and soil factors effect the basic chemical constituents of the plant. For instance the use of Sudan grass in the Southwest for fodder is considered a safe practice; however, in the middle west the use of this plant is very dangerous because of the presence of a prussic acid component highly poisonous to stock. The marihuana plant grown in the valley and lowlands of the Border States exhibits strong narcotic properties while the same species found growing at elevations over 6,000 feet has lost this property.* The common Amaranthus palmeri becomes highly toxic to ruminants when its nitrate content exceeds 2 per cent, yet within a twenty mile radius of Tucson, Arizona, one can find this plant varying in nitrate content from zero to 20 per cent depending upon the soil and soil factors.** There is another factor, seldom considered, which influences plant components, namely, the geological period to which the surface soil owes its origin. Trelease and Beath (1949) in a comprehensive treatise concerning the cause and origin of selenium poisoning brings out the interesting correlation between the geological period of the top stratum and the selenium content in plants. With the above considerations in mind it is possible that the type of soil, soil factors, and soil origin of the semi-arid region of the Southwest might influence the presence or absence of antibacterial substances in the indigenous plants.

In research on antibiotics,, the majority of workers have employed methods identical or similar to that of the cylinder-plate technic used

*Dr. H. L. Shantz, personal communication.

**Dr. Bartley Cardon, personal communication.

in these studies. However, in an effort to examine as many plants as possible in a limited time, it was found that the disc-plate technic accelerated the screening procedures. This method has been thoroughly examined by Vincent and Vincent (1944), Loo et al (1945), Sherwood and de Beer (1945), and others who found that in many ways the use of the disc-plate was not only quicker and less tedious, but in many cases superior to the cylinder plate assay. As long as the extracts were soluble and more or less free of contaminating organisms, the disc-plate method is capable of a high degree of accuracy; furthermore, this technic will permit of higher concentrations of organic solvents in the aqueous solutions to be tested than are possible with other methods. In order to eliminate the possibility of contaminating bacteria or molds which might result in false observations, the cylinder-plate procedure was employed as a second step in three of these assays. The unique advantage of the latter method over that of the disc-plate is that the liquid to be tested need not be sterile, because most bacteria present will be confined to the inside of the cylinders.

Waksman and Reilly (1947) preferred an agar-streak method for obtaining the potency of an antibiotic because they felt that the results could be read with greater accuracy. For the research reported herein, it proved to be superior to the dilution method commonly used by many investigators. Huddleson et al (1944) studied the juices of plants against M. pyogenes var. aureus, Br. abortus, and E. coli; Osborn (1943) employed aqueous extracts against M. pyogenes var. aureus, and E. coli. The work of Atterbury (1949) who used aqueous, ether, acid, basic, and mineral acid extracts against B. subtilis, M. pyogenes var. aureus, E. coli, Ps. aeruginosa, N. catarrhalis, and Str. faecalis Lancefield D.

represents an attempt to include a broader bacterial spectrum and a variety of extracted chemical components. In this thesis, while the choice of organisms and extracts followed basically the procedure as followed by Atterbury (1949), slight modifications were made such as the inclusion of Myco. tuberculosis as a test organism, and the omission of N. catarrhales and Str. faecalis. The Myco. tuberculosis was added because of its peculiar properties as an acid fast organism and the present necessity of adequate treatment of tuberculosis. Contrary to the procedure of Atterbury who treated the macerated plant with distilled water before the addition of the solvent, it was thought that sufficient breakdown of the plant cells could be accomplished by the solvents alone without risking the inactivation of an active substance or substances by the distilled water.

Over 50 per cent of the plants tested in this research were chosen because of their medicinal history; among these Malva parviflora and Rumex hymenosepalus are mentioned by Curtin (1947) as plants that are highly valued by many of the native peoples of the Southwest. Others were selected for various reasons; i.e., Atriplex canescens, for its allergenic qualities, Datura meteloides because of its intoxicant value, and Humulus americanus because of its close generic relationship to Humulus lupulus, the source of lupelone.

The results of this investigation, for the most part, confirm those of Atterbury (1949). The differences that occurred with Solanum elaeagnifolium, Prosopis juliflora, and Euphorbia albomarginata could probably be attributed to the fact that different parts of the plant were tested as well as seasonal difference in the collection of the plants. That different organs of the same plant may have different chemical properties

is demonstrated by the work of Ivanovics and Horvath (1947) who extracted a substance from the seeds of the radish which was lacking in the roots and leaves, and of Osborn (1943) who found inhibitory substances against certain bacteria in the seeds of Brassica oleracea and in the bark of Magnolia acuminata but not in other parts of the same plants.

Several observations recorded by other investigators in their studies on antibiotics have been observed in the present assay of plants. Haploppapus laricifolius exhibited partial inhibition beyond the complete inhibition zone. Pratt and Dufrenoy (1949) have explained this as being possibly the result of the following: first, the number of organisms present in the seeded plate were of such magnitude that the antibiotic failed to completely inhibit them in its more dilute concentrations; second, susceptibilities of the strain of organism used varied; and third, weaker concentration of the diffused extract failed to kill the bacteria. Another common occurrence was observed in the assay of Oenothera hookeri in that stimulation was noted on the periphery of the inhibition zone. Possibly the concentration of the antibiotic substance at the periphery of the inhibitory zone was such that it speeded up the metabolic processes without irreversibly shifting them out of balance. Intense and characteristic pigmentation of Ps. aeruginosa was evident with each inhibiting extract of this same plant. It is regrettable that further work on this plant was prohibited because of lack of specimens available at the time of the assay.

In the review of literature, Florey et al (1949), Irving and Herrick (1949), there are fewer antibacterial substances active against the gram negative than against the gram positive organisms. Thus the wide spectrum of Oenothera hookeri, which shows activity against not only the gram

negative and gram positive organisms but against the mycobacterium as well, warrants further investigation of this species. Another plant which showed marked activity against the gram negative bacteria was Euphorbia albomarginata. The stability of the principle, or principles, of the latter plant was also of interest because although the specimen had been held in a desiccated state for over eighteen months the results show a remarkably close correlation with those of Atterbury (1949) with this same plant. The remarkable activity of the crude ether extract of Humulus americanus is interesting in view of Salle et al (1949)'s work with a related species and should be further investigated. This crude ether extract showed inhibition of Myco. tuberculosis with less than 100 mcg/ml amounts. It must be stressed that at present there is no way of knowing just how much of the soluble crude extract is active; a purified extract from Humulus lupulus isolated by Salle et al (1949) was active against the mycobacteria in 25 mcg/ml amounts.

According to Atkinson and Rainsford (1946), in view of the widespread incidence of tuberculosis in the world today and the need for effective weapons against it, any plant extract which shows inhibitory action against the mycobacteria is worthy of further investigation regardless of its total spectrum. The work with Artemisia dracunculoides was carried out with this in mind. The initial magnitude of the inhibitory zones on Myco. tuberculosis stimulated further study of this plant. Final results from the agar-streak method, using a crude ether extract, indicated a potency of approximately 30 mcg/ml of the medium which inhibited the growth of five species of mycobacteria, namely, Myco. ranae, Myco. phlei, Myco. smegmatis, and Myco. tuberculosis.

In view of the fact that of 35 plants investigated, 19 showed in-

hibitory action against the bacteria used, there appears to be justification for a continuation of the search in the Southwest for the presence of antibiotics from the higher plants.

SUMMARY

1. This survey involved an investigation of thirty-five plants in Southern Arizona for antibiotic substances.
2. Three procedures were employed: The disc-plate method for rapid survey; the cylinder-plate method to verify the former if appreciable activity was evident; and the agar-streak method for an estimation of potency.
3. Five extracts were prepared from each plant: An aqueous extract buffered at pH 7.0; an aqueous extract buffered at pH 4.0; an aqueous extract buffered at pH 9.0; an ethyl ether extract; and a weak solution of a strong mineral acid buffered at pH 7.0.
4. Routinely the constituents present in each extract were tested for antibiotic activity against five organisms: B. subtilis, #102; M. pyogenes var. aureus, #6538; E. coli, #6790; Ps. aeruginosa, #97; and Myco. tuberculosis, #607.

In the agar-streak method, the crude extract was tested for potency of activity against five organisms: Myco. tuberculosis, #607; Myco. ranae#110; Myco. phlei, #355; Myco. smegmatis, #278; and Myco. avium, #4269.
5. Approximately 54 per cent of the plants assayed showed some antibiotic activity.

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