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Research Article

THE PARTIAL PURIFICATION AND PROPERTIES OF ANTIBIOTIC SUBSTANCES FROM THE SWEET POTATO PLANT (*IPOMOEA BATATAS*) [1, 2]

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The dramatic chemical and therapeutic successes of certain antibiotics, notably the penicillins and streptomycin from *Penicillium* and *Actinomyces* molds, have spurred the search among other microorganisms and, more recently, among angiosperms for additional substances possessing antibacterial and antifungal properties.

Within the past few years it has been repeatedly shown by many investigators that some plant juices and extracts of dried whole plants and plant parts contain various kinds and intensities of antibacterial and antifungal activity (1–8).

In connection with investigations in this laboratory it has been previously reported (7) that the autoclaved expressed juice of the sweet potato plant (*Ipomoea batatas*) inhibited cultures of several plant-wilt producing *Fusaria*. The present investigations have confirmed and elaborated upon this early observation.

It is the purpose of this paper to present a procedure for obtaining crude and partially purified preparations of antibiotic substances from the sweet potato plant, to discuss some of the chemical, physical, bacteriological, and mycological properties of the various active fractions, and to present some of the speculative implications of this work in connection with the problems associated with some human, and possibly animal, diseases.

To date, no pure antibacterial or antifungal compound has been isolated. Since the crude extracts and some of the partially purified fractions were considered relatively impure and were found to contain both inhibitory and some stimulatory material, it was not deemed advisable, as yet, to set up an arbitrary unit of activity. It will thus be appreciated that the results reported here have only qualitative significance and that the interpretations of the results must be regarded as tentative.

EXPERIMENTAL

Two varieties [3] of sweet potato plants were used throughout this work. One variety was *Fusarium*-wilt-resistant (designated *Res.*), whereas the other variety was *Fusarium*-wilt-susceptible (designated *Susc.*).

Using the familiar disc and cup-plate assay method with slight modifications to be described, the test microorganisms listed in Table I were employed in this work.

Method of assay

A. Into individual, sterile, 90-mm. Petri dishes were poured base layers of 20 ml. of agar as follows:

1. Nutrient agar—for all bacteria except *Mycobacterium phlei*
2. Glycerol agar—for *M. phlei*
3. Czapek-dextrose agar (3) for the *Fol.* and *Fob.*

B. The inocula were prepared and introduced onto the plates as follows:

1. For all bacteria except *M. phlei:*

Two-tenths of a milliliter of a 24-hr. nutrient broth culture was added to 5 ml. of warm (42° C) nutrient agar. This seed layer was then evenly poured over the agar surface of a prepared Petri dish. The agar was allowed to harden, and the covered plate was then incubated for one-half hour at 37° C before "spotting" or the addition of test solution.

2. For *M. phlei:*

Five milliliters of an homogenized and filtered four-day-old culture were added to 50 ml. of warm (42° C) glycerol agar. Four milliliters of this suspension was evenly flooded over the agar surface of a Petri dish. The covered dish was then incubated for one hour at 37° C before "spotting."

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[1] Presented at the Second National Symposium on Recent Advances in Antibiotics Research held in Washington, D. C., April 11–12, 1949, under the auspices of the Antibiotics Study Section, National Institutes of Health, Public Health Service, Federal Security Agency.

[2] Report of a study made under the Research and Marketing Act of 1946.

[3] *Ipomoea batatas.* No. 153655, *Fusarium*-wilt-resistant variety, and Puerto Rico Unit 1, *Fusarium*-wilt-susceptible variety. Both varieties were grown at Beltsville, Maryland, and obtained from the Bureau of Plant Industry, Soils, and Agricultural Engineering.

TABLE I

Microorganisms used in assays of sweet potato extracts

Microorganism and source*	Gram-stain
Fusarium oxysporum f. *batatas* Wr. (Snyder and Hansen) (D F7357e) (designated *Fob.*)	
F. oxysporum f. *lycopersici* (Snyder and Hansen) (W No. R-5-6) (designated *Fol.*)	
Escherichia coli (Migula) Castellani and Chambers (NRRL No. B210)	−
Staphylococcus aureus Rosenbach (NRRL No. B313)	+
Serratia marcescens Bizio (ATCC no. 60)	−
Xanthomonas translucens f. sp. *Hordei-avenae* Hagborg (ATCC No. 9000)	−
Mycobacterium phlei Lehmann and Neumann (ATCC No. 355)	+ (Acid-fast)
Sarcina lutea Schroeter (ATCC No. 272)	+
Bacillus cereus Fr. and Fr.(C No. 401)	+
Rhodococcus roseus Winslow and Rogers (ATCC No. 177)	g. var.
Bacillus subtilis Cohn (NRRL No. 558)	+

* ATCC, American Type Culture Collection;
NRRL, Northern Regional Research Laboratory;
C, H. R. Curran, Bureau of Dairy Industry, Washington, D. C;
D, S. P. Doolittle, Bureau of Plant Industry, Soils, and Agricultural Engineering, Beltsville, Maryland;
W, F. L. Wellman, formerly Bureau of Plant Industry, Soils, and Agricultural Engineering, Beltsville, Maryland.

3. For *Fol.* and *Fob.*:

Six milliliters of sterile distilled water were used to wash down a four-day-old potato-dextrose slant of the organism. This aqueous suspension of spores was filtered through a thin, sterile, cotton plug to remove bits of the mycelium which would tend to give rise to irregular growth, if plated. One and three-tenths ml. of this filtrate were added to 100 ml. of liquid Czapek-agar (42° C), and 4 ml. of the resulting suspension were used to flood the prepared plate evenly. After hardening of the agar, the covered plate was incubated for 24 hours at 28° C before the addition of the test solutions.

The technique of "spotting" consisted of saturating, by imbibition, a standard filter-paper assay disc (S & S 740-E) with the test solution and immediately placing the disc on the inoculated surface of the prepared Petri dish. If the antibiotic was dissolved solely in an organic solvent, the filter-paper assay disc was saturated and placed in a vacuum desiccator until the solvent had evaporated. The dried disc was then saturated with water and immediately placed on the inoculated surface. The covered dish was then inverted and incubated under the same optimum conditions for the growth of the organism. Biological activity was determined by the presence or absence, and the relative sizes, of zones of inhibition or stimulation.

Preliminary survey of sweet potato plants

Direct hot and cold aqueous extractions of the dried and ground whole Res. sweet potato plant resulted in solutions which generally stimulated the growth of the characteristic test microorganisms, *Fol.*, *Fob.*, *Escherichia coli*, and, to a slighter degree, *Staphylococcus aureus*. No effect was noticed with *M. phlei*. Similar results were noted for aqueous extracts of Res. tubers, leaves, stems, and Susc. tubers and stems. The water extracts of the Susc. leaves and of the roots, as well as the water-insoluble extraction residues of all plant parts except Susc. tubers, very slightly inhibited *Fob.* Direct aqueous extractions were abandoned as being unsatisfactory.

When portions of dried and ground Res. and Susc. sweet potato plant parts were individually extracted with methanol, the extract reduced to dryness by removal of the methanol, and the resulting residue taken up in water, the aqueous solutions thus produced, in marked contrast with the direct aqueous extracts, inhibited cultures of microorganisms previously unaffected or largely stimulated. Only the extracts of Susc. stems inhibited *M. phlei*. In almost all cases stimulation of some microorganisms was apparent, concurrently with concentric zones of inhibition.

Of the various plant parts extracted and assayed, the leaves and stems of both the plant-wilt-resistant and the susceptible varieties seemed to contain at least as much, if not more, inhibitory material than did the roots and tubers. Extracts of Susc. stems appeared to be the most active of all.

Procedure for obtaining crude active extracts

Weighed samples of dried and ground Susc. sweet potato stems were extracted with methanol for eight hours in a Soxhlet extraction apparatus. The dark green methanolic extracts were then concentrated to dryness *in vacuo*, and the residues were thoroughly extracted, portionwise, with sufficient warm distilled water to make the final volumes of clear, reddish-brown aqueous extract

TABLE II

Assay of crude active extracts obtained from Susc. sweet potato stems*

Test organism	Aq.-Conc.	MeOH-post-Aq.-Conc.†
Fol.	=++	+++
Fob.	≡++	++
Escherichia coli	+−	0
Staphylococcus aureus	+++=	−+
Serratia marcescens	++=	0
Mycobacterium phlei	+++	0
Baccillus subtilis	+++=	0
Xanthomonas translucens	++−	0
Sarcina lutea	+++	()
Bacillus cereus	+++=	()
Rhodococcus roseus	++−	()

* +++, complete inhibition; ++, partial inhibition; +, slight indication of inhibition; ≡, complete stimulation; =, partial stimulation; −, slight indication of stimulation; 0, no effect; (), no assay

All the organisms listed were not used against all 13 preparations of each concentrate.

† Dilutions were made such that no more than three drops of organic solvent were added to each 10 ml. of agar on any assay plate.

represent 1 gram of original stem material per ml. The aqueous solutions thus prepared were considered "concentrates" and designated "Aq.-Conc." They were found to contain most of the antibacterial activity. The non-water-soluble residues were taken up in minimal amounts of methanol and designated "MeOH-post-Aq.Conc." The latter methanolic solutions, so prepared, contained most of the antifungal activity.

The approximate average assay results of 13 separate preparations of Aq.-Conc. and MeOH-post-Aq.Conc. are summarized in Table II.

Partial purification of active concentrates

Measured weights and volumes of the clear, reddish-brown aqueous concentrate, Aq.-Conc., were stirred batchwise for 15 min. at 25° C with from 2.0% to 2.5% (by weight) of activated carbon (Darco G-60) [4] while the pH of the solution was controlled in the range of 4.0 to 4.5 by the dropwise addition of 1 *N* hydrochloric acid solution. The carbon was filtered and washed with several small portions of distilled water, and the washings were added to the deep orange-brown filtrate (effluent). The carbon cake was then transferred to a beaker and to it was added a volume of distilled water—equal to that of the original sample of Aq.-Conc. used. The mixture was again stirred for 15 min. while the pH of the solution was adjusted and controlled at from 7.0 to 7.5 by the dropwise addition of 1 *N* ammonium hydroxide solution.

The carbon was then filtered from the solution, washed with several small portions of distilled water, and discarded. The washings were added to the sparkling, clear, pale-yellow eluate.

RESULTS

The average assay results obtained from three separate batchwise adsorption-elution runs are summarized in Table III.

These results seem to indicate that the water-soluble material that stimulates fungi may be separated from the materials that inhibit bacteria. The material inhibitory toward Gram-positive bacteria appeared to be concentrated in the neutral to slightly basic eluate.

Columnar adsorption using an intimate mixture of equal parts of activated carbon (Darco G-60) and Kieselguhr (Hyflo Super-Cel) resulted in irreversible adsorption of most of the active material in the original concentrates. No significant quantity of active material could be eluted, at reasonable pH values, with water, methanol, or various water-methanol mixtures. Percolation of active concentrates through Kieselguhr (Hyflo Super-Cel) filter beds containing no carbon, likewise resulted in irreversible adsorption and consequent loss of activity.

TABLE III

*Assay of partially purified aqueous concentrates obtained from Susc. sweet potato stems**

Organism	Aq.-Conc.	Effluent†	Eluate‡
Fol.	=	=	0
Fob.	=	=	0
Escherichia coli	+−	+−	0
Staphylococcus aureus	+++−	++−	+++
Xanthomonas translucens	++−	++=	++−
Sarcina lutea	+++	+	+++
Mycobacterium phlei	+++	0	+++
Bacillus cereus	+++	0	+++

* The symbols are the same as those used in Table II.
† Average pH value adjusted to 6.8.
‡ Average pH value adjusted to 6.6.

The use of chromatographic column containing activated carbon (Darco G-60) with no siliceous filler produced indeterminate results not inconsistent with those in Table III. However, these attempts presented such mechanical difficulties that the method was abandoned. A search is now being conducted for a physically and chemically suitable and non-adsorbent filler for columnar use with finely divided carbon.

Preliminary work with synthetic ion-exchange resins, such as the "Amberlites," has been started. As yet, insufficient data have been accumulated to warrant drawing any general conclusions other than that they offer some promise for purification and concentration of the active materials.

Some properties of active preparations

The control of pH to within rather narrow limits appeared to be a critical factor for adsorption and elution of antibacterial substances. Except at the optimum pH values, an overall distribution of non-selective inhibitory and stimulatory material occurred. This was accompanied by some loss of total activity. All the active material was found to be soluble in polar, and insoluble in non-polar, solvents. The antifungal material appeared to be very soluble in methanol but not appreciably water-soluble, whereas all the antibacterial and all the stimulatory material appeared to be very soluble in water.

Samples of active preparations seemed to retain their activity after being heated for as long

[4] The mention of this and other commercial products does not imply that they are endorsed or recommended by the Department of Agriculture over others of a similar nature not mentioned.

as eight hours at 100° C. Samples refrigerated for one week at 4° C showed no perceptible diminution of activity. The results of several investigations of serial dilutions on agar slants, made with different preparations of Aq.-Conc. against *M. phlei,* demonstrated that dilutions as low as 0.05 ml. Aq.-Conc. per ml. of total solution completely inhibited the organism. This inhibition was entirely retained after 10 days. The inhibitory material was considered bactericidal to *M. phlei,* in the aforementioned concentration, since it was found impossible to initiate growth of the organism upon transfer of the attenuated culture to a fresh slant.

DISCUSSION AND CONCLUSIONS

Crude extracts of sweet potato plant parts contained a conglomerate mixture of non-selective inhibitory and stimulatory activity. Since it has been found possible to separate, from crude extracts, individual aqueous and methanolic fractions showing, respectively, the predominate bacterial activity and the major portion of antifungal activity (Table II), and since it has been further found possible to obtain fractions of the aqueous concentrate, Aq.-Conc., which exhibit selective inhibition to Gram-positive microorganisms (Table III), it has been postulated that at least two discrete inhibitory materials and one stimulatory material may be present in the total crude concentrates.

Although no sweeping conclusions can be drawn at this time, the method successfully employed for the partial purification of the aqueous concentrates indicated that the antibiotic factor or factors responsible for the activity against Gram-positive microorganisms may be polar in nature. If the inhibitory effect against Gram-positive organisms resulted from the action of an individual substance, then, as was indicated by the results of serial dilution investigations with *M. phlei,* this substance may be a bactericide in relatively low concentrations.

It is fully appreciated by the authors that the present investigations were largely exploratory in nature and that the results reported represent work done on crude extracts and partially purified fractions. However, the partial isolation of active material selectively inhibitory, *in vitro,* to Gram-positive microorganisms, particularly *M. phlei,* encourages speculation concerning the possible therapeutic applicability of this material in the treatment of some human, and possibly animal, mycobacterial infections. It is further realized that before any thought of possible therapeutic use of this active material can be seriously entertained, investigations in addition to those now in progress must demonstrate satisfactory and desirable kinds and degrees of activity and potency *in vivo,* stability under various conditions, and, among other properties, either no toxicity or sufficiently low toxicity to render feasible any administration.

SUMMARY

1. Aqueous and methanolic extracts of dried whole sweet potato plants and dried and ground plant parts were found, by the familiar disc and cup plate assay method, to contain both antibiotic and stimulatory activity when tested against certain Gram-negative and Gram-positive bacteria (including one acid-fast form) and two forms of plant-wilt fungi.

2. Of the plant parts from wilt-resistant and wilt-susceptible varieties that were extracted and assayed, the stems of the wilt-susceptible variety appeared to be the most desirable source of antibiotic substances.

3. By use of batchwise and columnar adsorption and elution techniques, it was possible to separate partially the antifungal from the antibacterial activity and to prepare partially purified concentrates exhibiting selective inhibitory, and possibly bactericidal, activity against Gram-positive microorganisms.

4. Preliminary evidence is cited for postulating the existence of at last three discrete biologically active materials in the sweet potato plant.

5. Some of the chemical, physical, bacteriological, and mycological properties of the active solutions are discussed.

6. Preliminary antibacterial and antifungal spectra of the various active extracts and fractions are presented.

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