1978

Antimicrobial activity of plant extracts

Melinda J. Seid
University of the Pacific

ANTIMICROBIAL ACTIVITY OF PLANT EXTRACTS

A Thesis

Presented to

The Faculty of the

Department of Biological Sciences

University of the Pacific

In Partial Fulfillment

of the Requirements of the Degree

Master of Science

by

Melinda J. Seid

May 1978

ACKNOWLEDGEMENTS

The author wishes to express sincere appreciation to: Dr. Madhukar Chaubal for his patience, guidance, and helpful criticism; Dr. Fuad Nahhas for his constant encouragement and guidance; Dr. Kris Iyer for his assistance; Mrs. Kishori Chaubal, whose constant sense of humor was a great source of comfort at difficult moments; and to my mother for her unending patience, encouragement, and support.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

I. INTRODUCTION AND HISTORICAL REVIEW

"An antibiotic is a chemical substance, produced by microorganisms, which has the capacity to inhibit the growth (of) and even to destroy bacteria and other microorganisms, in dilute solutions" (Waksman, 1951). This definition was revised in October, 1962, by the Federal Food, Drug, and Cosmetic Act. The legal definition states "the term 'antibiotic drug' means any drug intended for use by man containing any quantity of any chemical substance which is produced by a microorganism and which has the capacity to inhibit or destroy microorganisms in dilute solution (including the chemically synthesized equivalent of any such substance)." Recently, the trend has been to expand the definition to include antimicrobial substances derived from algae, mosses, higher plants, and even animals. The search for antibiotics is based on the fact that living microorganisms may produce specific compounds which are detrimental to other organisms and hence, may be used in the prevention and cure of human diseases.

Intensive efforts have been made to discover new and clinically useful antibiotics. The search has concentrated mainly on actinomycetes, such as Streptomyces spp., and a

few fungi, since these two groups have, to date, been the most productive in yielding clinically attractive antibiotics. Unfortunately, not all antimicrobials are considered ideal. Organisms become resistant to antibiotics, and adverse reactions, such as allergy, occur in some persons. These factors make it necessary to continue the search for new sources of antibiotics that are effective against infections caused by bacteria, fungi, viruses or pathogenic protozoa.

Higher plants are potential sources of new antibiotics. In recent years increased screening of higher plants for in vitro antimicrobial activity has occurred. Several articles have been published giving the results of plant screening studies. Osborn (1943) studied the activities of 2300 species from 166 families against Staphylococcus aureus and Escherichia coli. As a result, 63 genera were shown to contain substances which inhibited one or both test organisms. Huddleston et al. (1944) found 23 genera active against E. coli, S. aureus, and Brucella abortus. Sanders et al. (1945), working with plants collected in Indiana, screened 270 species, 15 of which displayed varied degrees of bacterial inhibition against Bacillus subtilis and E. coli. Hayes (1946) tested 231 species collected in Ohio against S. aureus, E. coli, Erwinia carotovora, and Phytomonas tumefaciens. Extracts from 46 species inhibited growth of one or more of the test organisms. Atkinson (1946) screened 1200 Australian flowering plants and found 50 species to have inhibitory activity against S. aureus and four against Salmonella typhi. Carlson et al.

(1948) found 115 of 550 species with activity against S. aureus and E. coli. Sproston et al. (1948) used 73 Vermont plants and found Impatiens biflora as the most active. Bushnell et al. (1950) found inhibition of Mycobacterium pyogenes, E. coli, and Pseudomonas aeruginosa by 42 of 101 Hawaiian plants tested. The sources listed above demonstrated that antibiosis is quite common among higher plants and emphasized the need to look for useful chemotherapeutic agents among these forms of life.

Any portion of the plant may contain the active component for antimicrobial activity. The usual procedure for screening is first to test the extracts or juices from the intact plant for activity, then to test the individual parts. Heatley (1944) reported an inhibitor of certain Gram-positive and Gram-negative bacteria obtained from the buds and flower petals of Crepis taraxacifolia. Cavallito et al. (1945) and Cavallito and Bailey (1946) found antibacterial activity in the leaves of Arctium minus, and the fresh leaves and stems of Asarum canadense var. reflexum. The extract was active against Gram-positive bacteria. Crude plant juices (corn, cucumber, wild mustard, and cabbage) were reported by Little and Grubaugh (1946) to possess greater inhibitory effect against Eberthella typhosa, Salmonella paratyphi A, and E. coli (three Gram-negative bacilli) than against S. aureus (a Gram-positive organism). Spencer et al. (1957) found the highest activity against Aspergillus niger in the lower parts of the stem and the upper segments of the primary root of

Vicia faba. Chopra et al. (1957) reported the roots of Alpinia galanga and Acorus calamus to yield oils which inhibit Mycobacterium tuberculosis and certain Gram-positive bacteria. Celayeta (1960) screened the stems of 18 species of plants and found 16 active against Sphacelia segetum. Bondarenko and Zelepukha (1962) tested various parts of Fragria vesca, F. grandiflora, and F. ananassa and reported that the berries, leaves, flowers, and roots contained the active substances.

Brunfelsia hopeana (Fam. Solanaceae) is an important member of the Genus Brunfelsia. The plant is native to South America, the West Indies, and the Malayan Peninsula; where it is known by various common names such as manaca root, jerataca, jeratacaca, gertaca, geracaca, camgaba, camgamba, umburapuama, bloom of the Lent, Christmas bloom, Santa Maria, Paraquay, jasmine, vegetable mercury, white tree, and goodnight. The more frequently used name manaca, originates from an ancient tribe, which named the shrub after a lovely maiden calledManaja. Among some South American Indian tribes several species of Brunfelsia have been used as hallucinogenic drinks or as additives to other hallucinogenic drinks. All species of Brunfelsia have been used in folk medicine to reduce fevers and as antirheumatic agents. The list of varied folklore medicinal uses of B. hopeana includes; diuretic, antirheumatic, emetic, cathartic, diaphoretic, antisyphilitic, against yellow fever, snake bite, as febrifuge, purgative, arrow poison, in skin and mucous membrane infections, and to induce abortion (Iyer, 1978).

Anemopsis californica (Fam. Saururaceae) is indigenous to Mexico and extends north to California, New Mexico, southern Utah, Nevada and eastern Colorado. In California, A. californica can be found in the lower Sacramento valley, San Joaquin valley, south coast ranges and Inyo county, and southern California. The common names for this plant are Yerba del Manza, Apache beads, and Va Visa. Reported uses in folklore medicine include: antispasmodic, diuretic, anti-rheumatic, antiasthamatic, antimalarial, blood purifier, local application to ulcers, cuts, and malignant sores, treatment of colds, indigestion, dysentery and venereal diseases (Tutupalli, 1975).

Heimia salicifolia (Fam. Lythraceae) is found in the tropical and subtropical regions of South America and Mexico as well as in Texas. The plant has various common names such as: "quiebra arado" (plow-breaker) in Argentina, "herva de la vida" (herb of life) in Brazil, and "sinicuiche" (twisted foot) in Mexico. "Sinicuiche" is the most common name, and refers to the user's lack of coordinated movements after drinking the plant extract. The drink is prepared by using slightly wilted leaves which are crushed and soaked in water for a day. The juice is then exposed to the sun and allowed to ferment. The resulting beverage is imbibed alone or with alcohol. The effects of ingesting this combination include giddiness, darkening of the surroundings and drowsiness or euphoria. The plant has been used for its emetic, antisyphilitic, hemostatic, febrifuge, diuretic, laxative, vulnerary, sudorific tonic and

astringent properties (Kaplan, 1965).

These results suggest that antimicrobial substances are present in one or more parts of higher plants and indicate that plants contianing these substances are widely distributed in nature.

The purpose of this study was to investigate a possible antimicrobial activity in the following plants: Brunfelsia hopeana, Anemopsis californica, and Heimia salicifolia against Staphylococcus aureus, a Gram-positive coccus, Escherichia coli, a Gram-negative fermenter, Pseudomonas aeruginosa, a Gram-negative non-fermenter, and the yeast Candida albicans.

II. MATERIALS

Test Organisms

The following organisms were used in testing the antimicrobial activity of the various plant materials.

A.	*Staphylococcus aureus*	ATCC 29523
B.	*Escherichia coli*	ATCC 25922
C.	*Pseudomonas aeruginosa*	ATCC 27853
D.	*Candida albicans*	ATCC 10231

Media[1]

Maintenance Media. Tryptic soy agar (TSA) slants were used to maintain the first three organisms. Luxurious growth of *C. albicans* required a higher dextrose content, so Sabouraud dextrose agar slants were used.

Inoculum Media. The first three organisms were transferred from TSA slants to 10 ml of tryptic soy broth (TSB) and incubated at 37°C for 24 hours. *C. albicans* was transferred from Sabouraud dextrose agar slants to 10 ml of Sabouraud dextrose broth and incubated at 25°C for 24 hours.

Test Media.

1. *Agar Diffusion Method*. TSA was used for *S. aureus* and *E. coli*. *P. aeruginosa* did not grow anaerobically unless

[1]Difco Laboratories, Inc., Detroit, MI. 48201.

nitrate was added to the media. For this reason, tryptic nitrate agar was chosen. Sabouraud dextrose agar was used for C. albicans.

2. Serial Dilution Method. TSB was used for tests with the bacterial species while Sabouraud dextrose broth was used for testing C. Albicans.

Plant Test Materials

The plant materials, under investigation for their chemical composition and pharmacological activity, were obtained from Dr. Madhukar Chaubal, of the School of Pharmacy, University of the Pacific. Various extracts from these plants as well as the pure compounds were intended to be used for microbiological testing.

Plant Extracts and/or Fractions

1. Brunfelsia hopeana[2] (See Figure 1)
   i) Extract B, F, P and Et
   ii) Fractions IV, V, VI, X, XII, XIV, XV, and Scopoletin
2. Anemopsis californica[3]
   i) Essential oil
   ii) Methyleugenol

---

[2]These extracts and compounds were prepared by Dr. R. P. Iyer, Dr. L. V. Tutupalli, and Mr. John Byrne during their phytopharmacologic investigations of the respective plants.

[3]Ibid.

3. Heimia salificolia[4] (see Figure 2)

i) Chloroform extract

ii) Fractions WC and H-11

Dilution of Plant Test Materials

Agar Diffusion Methods. Each of the plant extracts was dissolved, in most cases in methyl sufoxide (DMXO)[5] and sterile distilled water to obtain solutions of serial dilutions of 1000 ug/ml to 5000 ug/ml range as follows:

1000 ug/ml: 100 mg of extract was dissolved in 5 ml of DMSO. Three 0.25 ml aliquots were separately diluted to 5 ml each with DMSO, sterile distilled water, and 0.25% agar solution.

2000 ug/ml: 100 mg of extract was dissolved in 2 ml of DMSO. Two 0.2 ml aliquots were separately diluted to 5 ml each with DMSO and sterile distilled water.

3000 ug/ml: 100 mg of extract was dissolved in 2 ml of DMSO. Two 0.3 ml aliquots were separately diluted to 5 ml each with DMSO and sterile distilled water.

4000 ug/ml: 100 mg of extract was dissolved in 2 ml of DMSO. Two 0.4 ml aliquots were diluted separately to 5 ml each with DMSO and sterile distilled water.

---

[4] Ibid.

[5] Methyl Sulfoxide (DMSO), Spectroquality Matheson Coleman & Bell, Los Angeles, CA.

5000 ug/ml: 100 mg of extract was dissolved in 2 ml of DMSO. Two 0.5 ml aliquots were separately diluted to 5 ml each with DMSO and sterile distilled water.

Since the fractions were expected to be more concentrated than the extracts their dilution were made in the following manner to yield solutions between 1000 ug/ml to 5000 ug/ml.

1000 ug/ml: 10 mg of the fraction was dissolved in 2 ml of DMSO; 1.0 ml aliquot was diluted to ml with sterile distilled water.

2000 ug/ml: 10 mg of the fraction was dissolved in 0.2 ml of DMSO; 0.2 ml aliquot was diluted to 5 ml with sterile distilled water.

3000 ug/ml: 10 mg of the fraction was dissolved in 2 ml of DMSO; 0.3 ml aliquot was diluted to 5 ml with sterile distilled water.

4000 ug/ml: 10 mg of the fraction was dissolved in 2 ml of DMSO; 0.4 ml aliquot was diluted to 5 ml with sterile distilled water.

5000 ug/ml: 10 mg of the fraction was dissolved in 0.2 ml of DMSO and diluted to 2 ml with sterile distilled water.

Serial Dilution Method. In order to obtain uniform distribution of the plant extract in the aqueous test media they were suspended in 0.25% agar solution to obtain serial dilutions of range 1000 ug/ml to 5000 ug/ml as follows:

1000 ug/ml: 100 mg of extract was dissolved in 5 ml of a

0.25% agar solution; 0.1 ml aliquots were added to 0.5 ml of the medium.

2000 ug/ml: 100 mg of extract was dissolved in 5 ml of a 0.25% agar solution; 0.2 ml aliquots were added to 0.5 ml of the medium.

3000 ug/ml: 100 mg of extract was dissolved in 5 ml of a 0.25% agar solution; 0.3 ml aliquots were added to 0.5 ml of the medium.

4000 ug/ml: 100 mg of extract wad dissolved in 5 ml of a 0.25% agar solution; 0.4 ml aliquots were added to 0.5 ml of the solution.

50000 ug/ml: 100 mg of extract was dissolved in 5 ml of a 0.25% agar solution; 0.5 ml aliquots were added to 0.5 ml of the medium.

III. METHODS

Preparation of Stock Cultures for Testing

Stock test cultures of the test organisms E. coli, S. aureus, and Ps. aeruginosa were prepared by growth in 10 ml TSB while those of C. albicans were grown in 10 ml Sabouraud dextrose broth. The organisms were incubated at their respective temperatures for 24 hours or until a moderate turbidity prevailed when the test tube was held in front of a card containing a black line.

Agar Diffusion

The agars were prepared and dispensed in 10 ml aliquots into test tubes (125 x 16 mm) and autoclaved at 121°C and 15 psi for 15 minutes. After cooling to 45°C - 50°C, 0.5 ml of stock cultures of the respective organisms were pipetted into each test tube; mixed thoroughly, then poured into petri dishes (100 x 15 mm). The seeded petri dishes were allowed to set for an hour at room temperature and then placed in the refrigerator (2-8°C) for an hour to hasten hardening. Five 4 mm holes were made in the seeded agar and filled with the plant extracts (2-3 drops). The prepared plates were incubated for 20 hours at 37°C, with the exception of C. albicans (25°C) and inhibition zones, when present, were measured and then averaged. Positive

and negative controls were as follows: a) plates seeded with organsims and their respective agar mediums; b) plates seeded with each organism and holes filled with each solvent; and c) an unseeded plate of each agar medium.

Serial Dilution

A comparison investigation was used simultaneoulsy to evaluate whether diffusion was an inhibitory factor in the agar diffusion method. The antimicrobial activity of all the extracts was verified by the dilution method of Catalfomo and Schultz (1966).

The study was carried out in small, sterile, disposable tubes (75 x 12 mm) containing 0.5 ml of TSB for S. aureus, E. coli, and Ps. aeruginosa, and 0.5 ml Sabouraud dextrose broth for C. albicans. The tubes were incubated by adding 0.05 ml of the respective culture. However, before inoculation with the microorganisms the extract was added to the tube and allowed to be uniformly distributed in the medium. Controls consisted of tubes containing microorganisms and medium, and blanks consisted of uninoculated medium. All tubes (tests, controls, and blanks) were examined for turbidity (bacterial growth) after 24 hours incubation at 37°C (C. albicans at 25°C). Afterwards, a sample of each tube was swabbed onto a petri dish containing an agar medium and incubated 24 hours as a confirmation measure. The results were recorded by comparison with the growth on the control plates (inoculated and uninoculated agar medium).

It was found that at higher concentrations (50,000 ug/ml) the extracts were water insoluble which made recording the results difficult since a precipitate formed throughout the liquid medium. Therefore, the modified procedure of Catalfomo and Schultz (1966) was used to dissolve the extracts.

IV. RESULTS

The antibiotic activity of the plant extracts and fractions comparing the agar diffusion method and the serial dilution method are shown in Tables 1 through 6. Tables 1 through 4 are the results for Brunfelsia hopeana. Extracts B, P, and Et were ineffective against the growth of all organisms. Extract F was effective against S. aureus from a range of 5000 ug/ml to 3000 ug/ml. The fraction F-XV was the only active fraction from extract F and inhibited S. aureus at a range of 5000 ug/ml to 2000 ug/ml. The serial dilution method was not used because not enough plant fractions were available. It is assumed that there would have been activity against S. aureus due to the results of the agar dilution method. Scopoletin (a pure compound) was only active at 5000 ug/ml against C. albicans with the agar diffusion method. There was no activity with the serial dilution method.

The inhibitory effect of Anemopsis californica is summarized in Table 5. The essential oil and methyleugenol (a pure compound and main constituent of the oil) displayed no inhibition against any of the organisms when the agar diffusion method was used. However, with the serial dilution method the essential oil displayed activity against S. aureus, E. coli, and C. albicans at concentrations of 5000 ug/ml and 4000 ug/ml.

Methyleugenol was active at 5000 ug/ml against C. albicans.

The chloroform extract of Heimia salicifolia (Table 6) was active against S. aureus from range 5000 ug/ml to 2000 ug/ml using both methods. E. coli was inhibited by the extract in the agar diffusion method at concentrations 5000 ug/ml and 4000 ug/ml. In the serial dilution method H. salicifolia inhibited E. coli from 5000 ug/ml to 1000 ug/ml. Due to the small quantity of the test materials, the fractions WC and H-II were tested only at 1000 ug/ml against S. aureus and E. coli. Fraction H-III inhibited S. aureus and E. coli; whereas, fraction WC was not active at this concentration.

B. hopeana contained antibacterial agents effective against only the Gram-positive organism. A. californica appeared to have antibacterial and antifungal activity. H. salicifolia was effective against both the Gram-positive and Gram-negative organisms.

V. DISCUSSION

In view of the numerous interesting folklore medicinal uses of Brunfelsia hopeana, Anemopsis californica, and Heimia salicifolia, an antimicrobial screening was undertaken. However, any attempt to screen plant samples involves inherent problems that must be taken into consideration when results are interpreted.

The preliminary study has shown that these plants contain antimicrobial agents. However, the extracts were prepared from various plant parts (roots, aerial parts, and overground parts) and it is quite possible that different plant tissues could have different biological activity when compared with the effects of the whole plant since the active principles can vary greatly as to their distribution, concentration, and potency within a plant.

Isolation and identification of the active principles are often difficult and tedious. It may involve a system of many physiochemical procedures (Tutupalli, 1975; Iyer, 1978) which are beyond the scope of this study. Such extraction procedures (Figures 1 and 2) may require the use of more than one solvent since it is possible that the 'active' substances that are present can be extracted only by several methods. Therefore, the screening of plants for the presence

of antibiotic substances would require the testing of extracts prepared by the use of more than one solvent (Carlson and Douglas, 1947; Carlson et al., 1948).

The test concentration used is important. Mitscher et al. (1972) used concentrations of 1000 ug/ml and 100 ug/ml in their antimicrobial screen. However, very weak antimicrobial substances or those produced in very small quantity would not be detected according to their procedure. On this basis, tests on plant extracts in this study were carried out at a range of concentrations from 5000 ug/ml to 1000 ug/ml. It was hoped that the screen would be sensitive enough to detect active principles that may be present in crude extracts at low concentrations and the pure compounds showing greater activity at the same concentrations. Results for B. hopeana (Tables 1-4) and H. salicifolia (Table 6) suggest this trend as shown by zones of inhibition.

False-negative results may be obtained in this antimicrobial screen. Active compounds could be isolated from plant extracts even though the extracts themselves were shown to be devoid of activity. A logical explanation would seem to be that the active compound(s) was (were) present in insufficient quantity in the crude extract to manifest any activity at the concentration used. If they were present in sufficient concentration then there must be an alternative explanation, such as the possibility of antagonistic effects due to the heterogeneous character of phytoconstituents in the extracts evluated.

The nature of the solvents used in preparation of the test samples can also influence the interpretation of results. Mitscher et al. (1972) dissolved the plant extracts in water, alcohol, acetone or dimethylsulfoxide (DMSO) because preliminary studies showed no inhibition of the cultures with these solvents. However, irregular inhibition occurred with DMSO in this study. To rule out this activity, the extracts and fractions were also dissolved in sterile distilled water and both sets of tests were run simultaneously. The results in Tables 1-6 reflect the use of sterile distilled water as the solvent in preparation of the test samples for the agar diffusion method and a 0.25% agar solution for the serial dilution method.

Another point to consider in the interpretation of data is the methods used in antimicrobial screening. In the agar diffusion technique numerous factors are responsible for variations in results obtained. The most important probably are composition of the media, bacterial enzymes, inoculum density, quantity of extract used, size of agar wells, diffusibility of the constituents of the extract, and stability under the conditions of the test. In addition, diffusion tests are influenced by the growth rate of the organisms and by the type, depth and concentration of the agar used (Wick et al., 1974).

To discover whether diffusibility of the extract was a significant factor, a broth serial dilution technique was used for comparison. However, some of the factors that contribute to variations in the agar diffusion technique are also operative

in dilution methods (Wick et al., 1974). Therefore, the small tube modification of Catalfomo and Schultz (1966) was used. Since a no-growth endpoint is used and determined by visual inspection of small tubes this eliminated the task of measuring zones of inhibition. The effectiveness of most diffusion methods is dependent on those factors which govern the ability of the agent to diffuse both horizontally and vertically throughout the medium. Through the use of small tubes and thin layers of media, the extent of horizontal and vertical diffusion has been limited, thus rendering diffusion factors negligible. This is shown in Table 1, where the extract Et of B. hopeana displayed activity against S. aureus and Ps. aeruginosa with the serial dilution method; whereas, the agar diffusion method indicated no activity. In Table 5 A. californica displayed activity only with the serial dilution method. The chloroform extract of H. salicifolia (Table 6) did not display activity in the lower concentration (3000 ug/ml to 1000 ug/ml) with E. coli, but the corresponding serial dilution indicates activity. These examples illustrate that diffusibility of the extract can be an important factor in interpreting data.

In the small tube dilution methed the extracts formed a precipitate at higher concentrations (50,000 ug/ml) when sterile distilled water was used as a solvent for the preparation of test samples. Since DMSO possesses antimicrobial activity, a modified procedure was used whereby the extracts and fractions were suspended in a 0.25% agar solution. This greatly reduced the problem of deciding whether or not turbidity was

due to bacterial growth or precipitate. In cases where a slight precipitate prevailed upon addition of the suspension to the test media, a swab sample is taken after incubation and inoculated onto a sterile agar plate, then incubated for 24 hours. The results would reveal whether or not turbidity was due to bacterial growth.

Many antimicrobial studies have been undertaken on species of family Solanaceae of which B. hopeana is a member (Hayes, 1947; Carlson et al., 1948; Sporston et al., 1948; Gottshall et al., 1949; Bushnell et al., 1950; Pates and Madsen, 1955; Farnsworth et al., 1966; Pitts et al., 1968; Beaman-Mbaya and Muhammed, 1976; Saber, 1976). These studies indicate that the family Solanaceae is a good source for potential antimicrobial agents. In this study scopoletin, a pure compound isolated from B. hopeana, was screened. Previous studies indicate that this compound has antimicrobial activity. Duquenois (1955) found that scopoletin inhibits Brucella at 1:2000 - 1:2500. Vasyukova et al. (1970) discovered that this compound displayed fungal toxicity and completely inhibited the growth of Fusarium solani spores. Yet the results of this study as shown in Table 1 show only partial inhibition against C. albicans at 5000 ug/ml. The serial dilution method revealed no activity. Perhaps the microorganisms used in this study are resistant to scopoletin.

The major constituent of the essential oil of A. californica is methyleugenol (Tutupalli, 1975). This compound is similar in structure to thymol since they both have phenol as a base.

Thymol is a disinfectant with fungicidal properties (Osol et al., 1955). Therefore one could assume that methyleugenol would also possess antifungal activity. Graczal and Zarandi (1964) reported that methyleugenol inhibits Gram-positive bacterial at a 1:500 dilution. The results in Table 5 show that methyleugenol is only active at 5000 ug/ml against C. albicans. Since the essential oil is active against S. aureus, E. coli, and C. albicans at 500 ug/ml and 4000 ug/ml, this suggests the possibility that more than one component may be responsible for the activity.

Jiu (1966) screened H. salicifolia against E. coli, Bacillus subtilis, C. albicans, and Trichophyton mentagrophytes and found no activity. In comparison Table 6 shows high activity against E. coli and S. aureus. The discrepancy in results could be due to the use of different solvents (20% aqueous ethanol mixture compared with chloroform) for plant extraction.

VI. SUMMARY

Extracts and fractions of *Brunfelsia hopeana* (Fam. Solanaceae), *Anemopsis californica* (Fam. Saururaceae), and *Heimia salicifolia* (Fam. Lythraceae), obtained from the research laboratories of the School of Pharmacy at the University of the Pacific, were evaluated *in vitro* for antimicrobial activity against *Staphyloccus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Candida albicans*. It was shown that *B. hopeana* possessed antibacterial activity against *S. aureus*. Scopoletin, a pure compound isolated from *B. hopeana*, showed slight inhibition against *C. albicans*. *A. californica* displayed antibacterial and antifungal activity. Methyleugenol, the main constituent of *A. californica* essential oil, inhibited *C. albicans*. *H. salicifolia* was effective against both the Gram-positive and Gram-negative organisms.

TABLE 1

Brunfelsia hopeana: Results of Inhibition Tests Using Agar Diffusion and Broth Dilution Methods at 5000 ug/ml

Extract or Fraction	Concentration (ug/ml)	Agar Diffusion Method				Serial Dilution Method			
		Staphylococcus aureus	Escherichia coli	Pseudomonas aeruginosa	Candida albicans	Staphylococcus aureus	Escherichia coli	Pseudomonas aeruginosa	Candida albicans
B	5000	-	-	-	-	-	-	-	-
P	5000	-	-	-	-	-	-	-	-
Et	5000	-	-	-	-	-	-	-	-
F	5000	C; 7.0mm	-	-	-	+	-	-	-
F - IV	5000	-	-	-	-	NT	NT	NT	NT
F - V	5000	-	-	-	-	NT	NT	NT	NT
F - VI	5000	-	-	-	-	NT	NT	NT	NT
F - X	5000	-	-	-	-	NT	NT	NT	NT
F - XI	5000	-	-	-	-	NT	NT	NT	NT
F - XII	5000	-	-	-	-	NT	NT	NT	NT
F - XIII	5000	-	-	-	-	NT	NT	NT	NT
F - XIV	5000	-	-	-	-	NT	NT	NT	NT
F - XV	5000	C; 9.2mm	-	-	-	NT	NT	NT	NT
Scopoletin	5000	-	-	-	P; 10.0mm	-	-	-	-

Legend: -, no inhibition; +, inhibition; C, complete inhibition; P, partial inhibition; NT, extract or fraction not tested; mm, average zone of inhibition.

TABLE 2

Brunfelsia hopeana: Results of Inhibition Tests Using Agar Diffusion and Broth Dilution Methods at 4000 ug/ml

Extract or Fraction	Concentration (ug/ml)	Agar Diffusion Method				Serial Dilution Method			
		Staphylococcus aureus	Escherichia coli	Pseudomonas aeruginosa	Candida albicans	Staphylococcus aureus	Escherichia coli	Pseudomonas aeruginosa	Candida albicans
B	4000	-	-	-	-	-	-	-	-
P	4000	-	-	-	-	-	-	-	-
Et	4000	-	-	-	-	-	-	-	-
F	4000	C; 7.0mm	-	-	-	+	-	-	-
F - IV	4000	-	-	-	-	NT	NT	NT	NT
F - V	4000	-	-	-	-	NT	NT	NT	NT
F - VI	4000	-	-	-	-	NT	NT	NT	NT
F - X	4000	-	-	-	-	NT	NT	NT	NT
F - XI	4000	-	-	-	-	NT	NT	NT	NT
F - XII	4000	-	-	-	-	NT	NT	NT	NT
F - XIII	4000	-	-	-	-	NT	NT	NT	NT
F - XIV	4000	-	-	-	-	NT	NT	NT	NT
F - XV	4000	C; 8.2mm	-	-	-	NT	NT	NT	NT
Scopoletin	4000	-	-	-	-	-	-	-	-

Legend: -, no inhibition; +, inhibition; C, complete inhibition; P, partial inhibition; NT, extract or fraction not tested; mm, average zone of inhibition.

TABLE 3

Brunfelsia hopeana: Results of Inhibition Tests Using Agar Diffusion and Broth Dilution Methods at 3000 ug/ml

Extract or Fraction	Concentration (ug/ml)	Agar Diffusion Method				Serial Dilution Method			
		Staphylococcus aureus	Escherichia coli	Pseudomonas aeruginosa	Candida albicans	Staphylococcus aureus	Escherichia coli	Pseudomonas aeruginosa	Candida albicans
B	3000	-	-	-	-	-	-	-	-
P	3000	-	-	-	-	-	-	-	-
Et	3000	-	-	-	-	-	-	-	-
F	3000	C; 9.2mm	-	-	-	+	-	-	-
F - IV	3000	-	-	-	-	NT	NT	NT	NT
F - V	3000	-	-	-	-	NT	NT	NT	NT
F - VI	3000	-	-	-	-	NT	NT	NT	NT
F - X	3000	-	-	-	-	NT	NT	NT	NT
F - XI	3000	-	-	-	-	NT	NT	NT	NT
F - XIII	3000	-	-	-	-	NT	NT	NT	NT
F - XIV	3000	-	-	-	-	NT	NT	NT	NT
F - XV	3000	C; 5.2mm	-	-	-	NT	NT	NT	NT
Scopoletin	3000	-	-	-	-	-	-	-	-

Legend: -, no inhibition; +, inhibition; C, complete inhibition; P, partial inhibition; NT, extract or fraction not tested; mm, average zone of inhibition.

TABLE 4

Brunfelsia hopeana: Results of Inhibition Tests Using Agar Diffusion and Broth Dilution Methods at 2000 ug/ml

Extract or Fraction	Concentration (ug/ml)	Agar Diffusion Method				Serial Dilution Method			
		Staphylococcu aureus	Escherichia coli	Pseudomonas aeruginosa	Candida albicans	Staphylococcus aureus	Escherichia coli	Pseudomonas aeruginosa	Candida albicans
B	2000	-	-	-	-	-	-	-	-
P	2000	-	-	-	-	-	-	-	-
Et	2000	-	-	-	-	-	-	-	-
F	2000	-	-	-	-	-	-	-	-
F - IV	2000	-	-	-	-	NT	NT	NT	NT
F - V	2000	-	-	-	-	NT	NT	NT	NT
F - VI	2000	-	-	-	-	NT	NT	NT	NT
F - XI	2000	-	-	-	-	NT	NT	NT	NT
F - XII	2000	-	-	-	-	NT	NT	NT	NT
F - XIII	2000	-	-	-	-	NT	NT	NT	NT
F - XIV	2000	-	-	-	-	NT	NT	NT	NT
F - XV	2000	C; 8.0mm	-	-	-	NT	NT	NT	NT
Scopoletin	2000	-	-	-	-	-	-	-	-

Legend: -, no inhibition; +, inhibition; C, complete inhibition, P, partial inhibition; NT, extract or fraction not tested; mm, average zone inhibition.

TABLE 5

Anemopsis californica: Results of Inhibition Tests Using Agar Diffusion and Broth Dilution Methods From 5000 ug/ml to 1000 ug/ml

Extract or Fraction	Concentration (ug/ml)	Agar Diffusion Method				Serial Dilution Method			
		Staphylococcus aureus	Escherichia coli	Pseudomonas aeruginosa	Candida albicans	Staphylococcus aureus	Escherichia coli	Pseudomonas aeruginosa	Candida albicans
E. oil	5000	-	-	-	-	+	+	-	+
Methyl-eugenol	5000	-	-	-	-	-	-	-	+
E. oil	4000	-	-	-	-	+	+	-	+
Methyl-eugenol	4000	-	-	-	-	-	-	-	-
E. oil	3000	-	-	-	-	-	-	-	-
Methyl-eugenol	3000	-	-	-	-	-	-	-	-

Legend: -, no inhibition; +, inhibition.

TABLE 6

Heimia salificolia: Results of Inhibition Tests Using
Agar Diffusion and Broth Dilution Methods From 5000 ug/ml to 3000 ug/ml

Extract or Fraction	Concentration (ug/ml)	Agar Diffusion Method				Serial Dilution Method			
		Staphylococcus aureus	Escherichia coli	Pseudomonas aeruginosa	Candida albicans	Staphylococcus aureus	Escherichia coli	Pseudomonas aeruginosa	Candida albicans
Chloroform Extract	5000	C; 8.0 mm	C; 8.0 mm	-	-	+	+	-	-
WC Fraction	5000	NT	NT	NT	NT	NT	NT	NT	NT
H-II Fraction	5000	NT	NT	NT	NT	NT	NT	NT	NT
Chloroform Extract	4000	P; 8.0 mm	P; 7.0 mm	-	-	+	+	-	-
WC Fraction	4000	NT	NT	NT	NT	NT	NT	NT	NT
H-II Fraction	4000	NT	NT	NT	NT	NT	NT	NT	NT
Chloroform Extract	3000	P; 8.0 mm	-	-	-	+	+	-	-
WC Fraction	3000	NT	NT	NT	NT	NT	NT	NT	NT
H-II Fraction	3000	NT	NT	NT	NT	NT	NT	NT	NT
Chloroform Extract	2000	P; 8.0 mm	-	-	-	+	+	-	-
WC Fraction	2000	NT	NT	NT	NT	NT	NT	NT	NT
H-II Fraction	2000	NT	NT	NT	NT	NT	NT	NT	NT
Chloroform Extract	1000	-	-	-	-	-	+	-	-
WC Fraction	1000	NT	NT	NT	NT	-	-	NT	NT
H-II Fraction	1000	NT	NT	NT	NT	+	+	NT	NT

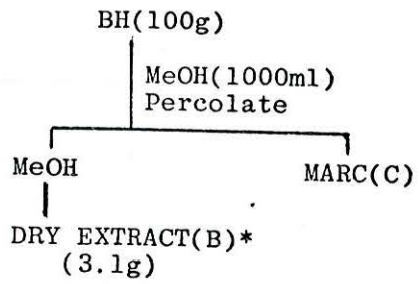
Legend: -, no inhibition; +, inhibition; C, complete inhibition; P, partial inhibition; NT, extract or fraction not tested; mm, average zone of inhibition.

Figure 1

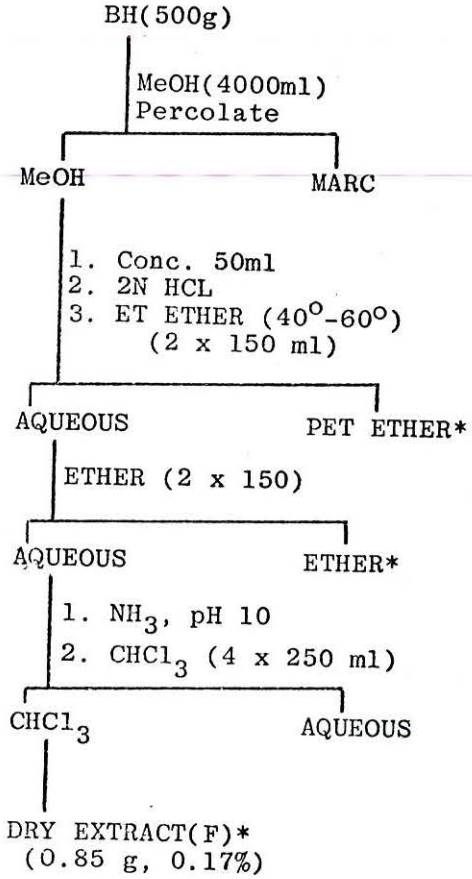
Schema for Extraction of

Brunfelsia hopeana

SCHEME 1



SCHEME 2



SCHEME 3

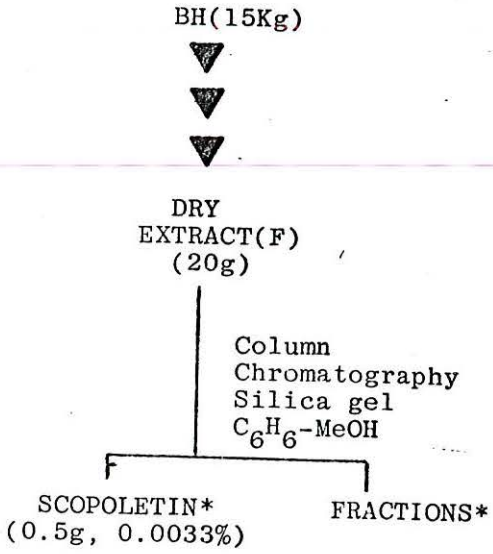
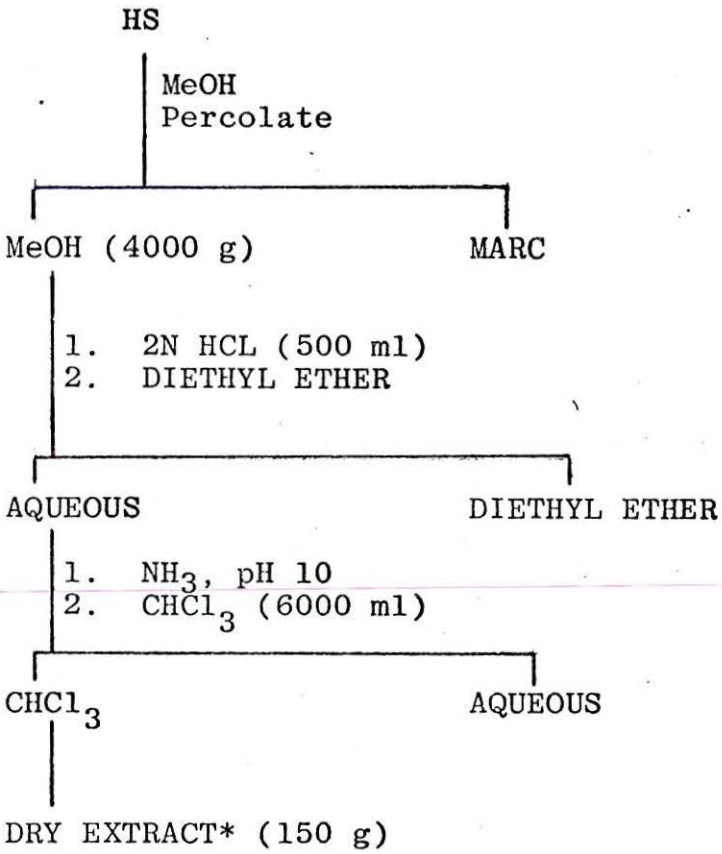


Figure 2

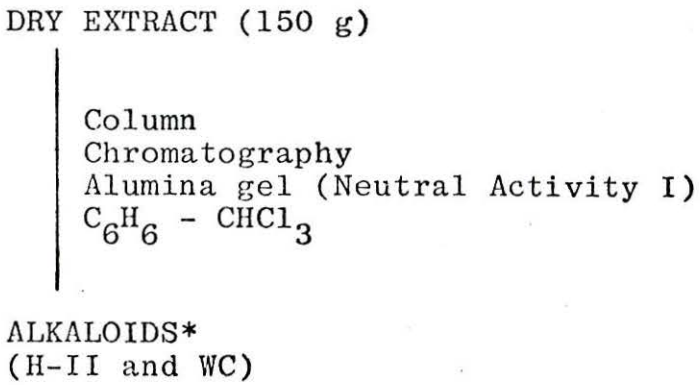
Schema for Extraction of

Heimia salicifolia

SCHEME 1



SCHEME 2



IX. LITERATURE CITED

Atkinson, N. 1946. Antibacterial Activity in Members of the Native Australian Flora. Nature(London) 158:865-877.

Beaman-Mbaya, V. and S. I. Muhammed. 1976. Antibiotic Action of Solanum incanum Linnaeus. Antimicrob. Ag. Chemother. 9:920-924.

Bondarenko, A.S. and S. I. Zelepukha. 1962. Antibacterial Properties of Strawberries. Mikrobiol. zh., Akad. Nauk Ukr. RSR 24(1):41-45.

Bushnell, O. A., M. Fukada and T. Makinodan. 1950. The Antibacterial Properties of Some Plants Found in Hawaii. Pacific Sci. 4(3):167-183.

Carlson, H. J., H. G. Douglas. 1947. Screening Methods for Determining Antibiotic Activity of Higher Plants. J. Bacteriol. 55:235-240.

Carlson, H. S., H. G. Douglas and J. Robertson. 1948. Antibacterial Substances Separated from Plants. J. Bacteriol. 55:241-248.

Catalfomo, P. and H. W. Schultz. 1966. Small Tube Method for the Evaluation of Antifungal and Antibacterial Activity. J. Pharm. Sci. 55(1):117-119.

Cavallito, C. J., J. H. Bailey and F. K. Kirchner. 1945. Antibacterial Principle of Arctium minus. I. Isolation, Physical Properties and Antibacterial Action. J. Am. Chem. Sco. 67:948-950.

Cavallito, C. F., J. H. Bailey. 1946. Antibacterial Substances From Asarum canadense. I. Isolation, Physical Properties, and Antibacterial Action. J. Am. Chem. Soc. 68:489-492.

Celayeta, F. D. 1960. Action of the Tissues of Various Plants on the Growth of Sphacelia segetum. Farmacognosia. 20:91-101.

Chopra, I. C., B. N. Khajuria and C. L. Chopra. 1957. Antibacterial Properties of Volatile Principles From Alpinia galanga and Acorus calamus.

Duquenois, P. 1955. Recent Data on the Bacteriostatic Activity Exhibited by Several Unsaturated Lactones. Bull. Soc. Pharm. Bordeaux 94:156-160.
Chem. Abst., 1957, 51, 2115c.

Farnsworth, N. R., L. K. Henry, G. H. Svoboda, R. N. Blomster, M. F. Yates and K. L. Euler. 1966. Biological and Phytochemical Evaluation of Plants. I. Biological Test Procedures and Results From Two Hundred Accessions. Lloydia 29(2):101-122.

Gottshall, R. Y., E. H. Lucas, A. Lickfeldt and J. M. Roberts. 1949. The Occurrence of Antibacterial Substances Active Against Mycobacterium tuberculosis in Seed Plants. J. Am. Clin. Invest. 28:920-923.

Graczal, L. and A. Zarandi. 1964. Uber die wirkstoffe von Asarum europaeum L. Pharmazie 19(11):722-723.

Hayes, L. E. 1946. Survey of Higher Plants for Presence of Antibacterial Substances. Bot. Gaz. 108(3):408-414.

Heatley, N. G. 1944. An Antibiotic From Crepis taraxacifolia (Thuill). Brit. J. Exp. Pathol. 25:209-211.

Huddleston, I. F., J. Dufrain, K. C. Barrows and M. Giefel. 1944. Antibacterial Substances in Plants. J. Am. Vet. Assoc. 105:394-397.

Iyer, R. P., 1978, "Brunfelsia hopeana - Pharmacologic Screening: Isolation and Characterization of Hopeanine," Ph.D. dissertation, University of the Pacific, Stockton, California.

Jiu, J. 1966. A Survey of Some Medicinal Plants of Mexico for Selected Biological Activities. Lloydia 29(3): 250-259.

Kaplan, H. R.,1965, "Pharmacodynamic Studies on the Alkaloids of Heimia salicifolia Link and Otto," M.S. thesis, University of the Pacific, Stockton, California.

Little, J. E. and K. K. Grubaugh. 1946. Antibiotic Activity of Some Crude Plant Juices. J. Bacteriol. 52:587-591.

Mitscher, L. A., R. P. Leu, M. S. Bathala, W. N. Wu and J. L. Beal. 1972. Antimicrobial Agents From Higher Plants. I. Introduction, Rationale, and Methodology. Lloydia 35(2):157-167.

Osborn, E. M. 1942. On the Occurrence of Antibacterial Substances in Green Plants. Brit. J. Exp. Pathol. 24(6): 227-231.

Osol, A., Farrar, G. E., and Pratt, R., 1955, "The Dispensatory of the United States of America," Lippincott, Philadelphia.

Pates, A. L. and G. C. Madsen. 1955. Occurrence of Antimicrobial Substances in Chlorophyllose Plants Growing in Florida. Bot. Gaz. 166(3):250-261.

Pitts, O. M., H. S. Thompson and J. H. Hoch. 1969. Antibacterial Activity of Solanum carolinense L. J. Pharm. Sci. 58(3):379-380.

Saber, M. S. 1976. Antimicrobial Substances in Certain Members of Solanaceae. I. Preliminary Investigations. Zentrabl. Bakeriol. 131(1):40-45.

Sanders, D. W., P. W. Weatherwax and L. S. McClung. 1945. Antibacterial Substances From Plants Collected in Indiana. J. Bacteriol. 49(6):611-615.

Spencer, D. M., J. H. Topps and R. L. Wain (Univ. London). 1957. An Antifungal Substance From the Tissues of Vicia faba. Nature 179:651-652.

Sproston, T., J. E. Little and M. W. Foote. 1948. Antibacterial and Antifungal Substances From Vermont Plants. Vermont Agr. Exp. Sta. Bull. 543:3-7.

Tutupalli, L. V., 1975, "Comparative Phytochemistry of Saururaceae Essential Oils," Ph.D. dissertation, University of the Pacific, Stockton, California.

U. S. Federal Food, Drug, and Cosmetic Act, as Amended (1962) sec. 507.
"The United States Dispensatory," 1973, 27th ed., J. B. Lippincott Co., Philadelphia. p. 110.

Vasyukova, N. I., O. L. Ozeretovskaya and L. V. Metlitskii. 1970. Phytoalexins of Potatoes. Prikl. Biokhim. Mikrobiol. 6(4):431-436.

Waksman, S. A. 1951. Antibiotics-A New Field of Science of Life-Savings Drugs. Antibiot. Chemother. 1(1):1.

Wick, W. E., Preston, D. A., and Hawley, L. C., 1974, "Laboratory Testing of Bacterial Susceptibility to Antibiotics: Theory and Practice," Lily Research Laboratories, Indianapolis.