CHAPTER I

INTRODUCTION

Plants and their products appear to have been used in the treatment of infectious diseases for a long period of time. For centuries, vegetable drugs have been used in all parts of the world as folklore remedies even before the discovery of microorganism (Abraham 1949)(Kshirsagar 1972). In the field of modern medical practice Mosse (1852)(Kshirsagar 1972) was the first to suggest the use of microorganisms for therapeutic purposes. Tyndall (1876)(Kshirsagar 1972) was the first to demonstrate the antibiotic action of a fungus. He described the antagonistic action of a species of *Penicillium* to bacterial growth. This was immediately followed by the first clear description of bacterial antagonism by Pasteur and Joubert (1877)(Kshirsagar 1972). The antibiotic research gathered momentum until it culminated in discovery of penicillin (Chain et al. 1940)(Kshirsagar 1972).

It is commonly assumed that a substance designated as an antibiotic must necessarily have been elaborated by a living organism. However antibiotics are not confined to microorganism only, but are also found in higher plants and animals. Claims have been made that certain plants have the power of healing wound, abating inflammation, and that others are effective in the treatment of diseases such as cholera, and dysentery. It might

be assumed that if these observations were true the cures claimed may have been influenced in some cases by the presence of antibiotic substances in the plants. The antibiotics of plant origin are called fytoncides -- a term that existed long before antibiotics in the restricted sense were discovered (Borgstrom 1969).

The modern term "antibiotic" is usually applied to an organic substance which will kill certain microorganism such as bacteria, fungi, or protozoa, or which will inhibit their growth.

Although modern technology has made possible the synthesis of chemicals used as important drugs, plants continue to be a major sources of medicines. Many of our present day drugs from plant sources were known and used, at least in raw form, by early man. Indeed some modern medicines have been discovered through study of their usage in primitive societies.

It is common practice to select various parts of local plants for use in herbal medicine. There are claims of the effectiveness of these plants in both prophylactic and therapeutic uses. Some are included in the pharmacopoeiae as crude drugs available for use in modern practice of medicine. The local plant flora are not exhaustively used in herbal medicines, nor are there adequate investigations into their characteristic properties. In view of these, the author decided to collect plant samples randomly and screen for the antimicrobial activities.

Historical

Over the last 3 decades, an intensive effort had been made to the systematically study of higher plants for the purpose of detecting antimicrobial substances in their tissues. However, these investigations which had been inspired largely by the desire to find new substances toxic to pathogenic microorganisms, had followed naturally from the age-old practice of using plants and their extracts as drugs for the cure of human diseases. Documents, many of which were of great antiquity, reveal that plants were used medicinally in China, Egypt and Greece, long before the beginning of the Christian era. Knowledge of the healing properties of plants increased in later times, particularly during the Middle Ages in Europe, and much information was later recorded in the herbals of the 15th to the 17th centuries. After this time knowledge became more systematized and more divorced from superstitious belief, but it was not until the early 19th century that it became realized that the medicinal properties of plants were due to active constituents present usually in minute quantities.

The discovery of microorganisms as the causative agents of many infectious diseases of man, naturally created interest in substances toxic to these organisms. Many substances, including some of vegetable origin, became recognized as antiseptics. Thus thymol, a simple phenol present in the essential oils of several plants for example *Thymus vulgaris* L. was used as an antiseptic

by Martini in 1887 (Skinner 1955). During the present century, many plants had been found to contain substances capable of inhibiting the growth of microorganisms or of killing them. Glaser and Prinz (1926)(Skinner 1955), had reported that the addition of certain oxidases from barley, grasses, malt, and horse-radish to plate cultures prevented the growth of *Escherichia coli*, *Eberthella typhosa*, *Bacillus anthracis* and certain *Streptococci*. All these oxidases had pronounced bactericidal properties which were proportional to their concentrations and which varied with the nature of the oxidase used. Jordanoff (1927)(Skinner 1955), reported that extracts of *Capsicum annuum* L. had a bactericidal effect on *Escherichia coli*, *Eberthella typhosa* and on some other Gram negative enteric bacteria. Yamagami (1927) (Skinner 1955), found that quite dilute solutions of an oil obtained from *Allium scorodoprasum* L. had an inhibiting and a lethal action toward *Vibrio cholera*. The antibacterial effect in this case was selective since a strong solution of the oil was required to inhibit the growth of the typhoid and coliform bacteria but was almost without effect on *Staphylococci*. It is now known that several species of the genus *Allium*, notably *A. cepa* L. (onion) and *A. sativum* L. (garlic) are rich in substances having an adverse effect on the growth of microorganisms (Skinner 1955).

The antiseptic and preservative substances present in hops formed the subject of investigations by several workers in the late 19th century. This work was reviewed by Pyman *et al.* (1922)(Skinner 1955), and the subject was further investigated by

teams of workers connected with the brewing industry from this date onwards. At least two antibiotic substances, lupulon and humulon, had been isolated from hops.

As a result of the surveys and the numerous more detailed research works on individual plant species, it is clear that antibiotic principles are distributed widely among the higher plants, particularly among the Angiosperms (Table 1 pp. 15-17). Substances inhibiting or toxic to one or more microorganisms had been detected in some members of all the families listed below.

1. Pteridophytes.

Equisetaceae, Gleicheniaceae, Lycopodiaceae, Polypodiaceae, Psilotaceae, Selaginellaceae.

2. Gymnosperms.

Cupressaceae, Ginkgoaceae, Gnetaceae, Pinaceae, Taxaceae.

3. Angiosperms:-

a) Monocotyledon.

Alismataceae, Amaryllidaceae, Araceae, Bromeliaceae, Commelinaceae, Cyperaceae, Dioscoreaceae, Gramineae, Iridaceae, Juncaceae, Liliaceae, Musaceae, Palmaceae, Pontederiaceae, Stemonaceae, Typhaceae, Zingiberaceae.

b) Dicotyledons.

Acanthaceae, Aceraceae, Aizoaceae, Amaranthaceae, Anacardiaceae, Annonaceae, Apocynaceae, Aquifoliaceae, Araliaceae, Aristolochiaceae, Asclepiadaceae, Balsaminaceae, Berberidaceae,

Betulaceae, Bignoniaceae, Bixaceae, Boraginaceae, Burseraceae, Cactaceae, Canellaceae, Capparidaceae, Caprifoliaceae, Caricaceae, Caryophyllaceae, Casuarinaceae, Celastraceae, Chenopodiaceae, Cistaceae, Combretaceae, Compositae, Convolvulaceae, Cornaceae, Crassulaceae, Cruciferae, Cucurbitaceae, Datiscaceae, Dilleniaceae, Dipsacaceae, Droseraceae, Ebenaceae, Elaeocarpaceae, Empetraceae, Ericaceae, Euphorbiaceae, Fagaceae, Flacourtiaceae, Fumariaceae, Gentianaceae, Geraniaceae, Guttiferae, Hamamelidaceae, Hippocrateaceae, Hydrophyllaceae, Hypericaceae, Juglandaceae, Koeberliniaceae, Labiatae, Lauraceae, Leguminosae, Linaceae, Loganiaceae, Lythraceae, Magnoliaceae, Malvaceae, Martyniaceae, Melastomataceae, Meliaceae, Melianthaceae, Menispermaceae, Monotropaceae, Moraceae, Moringaceae, Myricaceae, Myrsinaceae, Myrtaceae, Nyctaginaceae, Nymphaeaceae, Nyssaceae, Oleaceae, Onagraceae, Oxalidaceae, Papaveraceae, Passifloraceae, Phytolaccaceae, Piperaceae, Pittosporaceae, Plantaginaceae, Plumbaginaceae, Polemoniaceae, Polygalaceae, Polygonaceae, Portulacaceae, Proteaceae, Punicaceae, Ranunculaceae, Resedaceae, Rhamnaceae, Rosaceae, Rubiaceae, Rutaceae, Salicaceae, Sapindaceae, Sapotaceae, Saururaceae, Sarraceniaceae, Saxifragaceae, Scrophulariaceae, Simarubaceae, Solanaceae, Sterculiaceae, Theaceae, Tremandraceae, Tropoeolaceae, Umbelliferae, Urticaceae, Valerianaceae, Verbenaceae, Violaceae, Vitidaceae, Zygophyllaceae.

In the course of this quest for new substances active against microorganisms pathogenic for man, many workers had taken

plants at random though others had tested groups of plants already known or suspected to possess useful medicinal properties (Table 2 pp. 18-20). It would seem that some of the material recorded in the older herbals might be of value to modern investigators by indicating to them those which might well repay detailed study. Infact, very few workers had availed themselves of ancient literatures, in the year 1953 Winter and Willecke attempted to use information given in the herbal of Matthiolus (1611)(Skinner 1955). The plants mentioned by Matthiolus frequently could not be identified with any degree of certainty but they could usually be assigned to families recognized by modern botanists. Accordingly, Winter and Willecke (1953)(Skinner 1955) tested extracts prepared from many plants belonging to these families. Two groups of families were considered: those families containing plants which Matthiolus had supposed to be of value in the treatment of the urinary system and those families containing species reputed to be of value for the treatment of wounds. Of the first group of 21 families, 18 (86 %) contained plants which gave extracts active against one or more of the test bacteria (*Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*). Of the second groups of 31 families, 27 (87 %) were similarly active. These percentages of active families were rather higher than would reasonably be expected had the plants been taken at random. Nevertheless, it seems generally agreed that reference to very old literature is of limited value today, partly because of the often inadequate descriptions of plants given, and partly

because a plant regarded as being of value against a particular disease might act more directly on the human subject rather than on the organism causing that disease.

The survey of Spencer et al. (1947) (Skinner 1955) deserved special mention, since it was an attempt to discover new naturally occuring substances active against malarial parasites. Tests were made on extracts of parts of about 600 different species representing 123 families of Phanerogams and 3 families of Cryptogams. Though many plants gave extracts which were active *in vivo* against at least one of the species of the parasites used, *Plasmodium gallinaceum* in chicks; *Plasmodium cathemerium* and *Plasmodium lophurae* in ducklings, none contained active principles which appeared promissing for use against malarial infections in man.

It might , in fact, be fairly stated that the results of all the numerous investigations conducted for the purpose of finding in plants new antibiotics active against microorganisms pathogenic for man, had been disappointing. Many potentially useful antibiotics had been isolated, but most of them had proved to be too toxic to human and animal subjects to be of value (Skinner 1955). As yet, no antibiotic medicine at all comparable with that held by certain drugs of microbial origin such as penicillin.

In food technology, they found that many cruciferous plants contained mustard oils, which killed aerobic organisms,

especially molds and other fungi. Allyl- and ethyl-isothiocyanate (mustard oil) had been found to suppress the growth of mold even dilutions of 1 to 10 ppm. In Turkey and Finland, for example, mustard oil was used in the preservation of fruit juices. The presence of 10 ppm in apple and grape juices caused a marked reduction in the thermal resistance of some molds and yeasts (Kosker et al. 1951)(Borgstrom 1969). The therapeutic properties of garlic and onion were due to sulfur components (Binet 1962) (Borgstrom 1969). A key substance and precursor to a series of different chemicals is alliin. Water extracts of cabbages and turnips showed bactericidal activity against *Escherichia coli*, *Aerobactor aerogenes*, *Pseudomonas campestris* and the natural mixed flora from the exterior of these plants. The substance in question was destroyed by heat (Borgstrom 1969). Tomatin, an antibiotic isolated from tomato, was present throughout the plant and fruit. It was found to inhibit Gram positive and Gram negative bacteria ans certain pathogenic plants and animal fungi (Irving et al. 1945). Many antifungal compounds, such as benzoic acid were found in carrots (Borgstrom 1969). In India, fishes were preserved efficiently over long periods by pickling them in brine containing Malabar tamarind (dried freshy fruits of *Garcinia cambogia* Desrouss). This preservative contains citric and tartaric acids. Several anthocyanins are antibacterial and might play a role in the bacterial stability of wine and fruit juices (Jensen 1954)(Borgstrom 1969).

Orange oil was reported as an effective preservative in fish sausages. Its active ingredient, α-limonene was later traced by Indian scientists (Krishnaswamy and Rudra Seitz 1966)(Borgstrom 1969). Many other food ingredients, such as laurel leaves, spices such as cloves and cinnamon, algae and Capparis fruits contained compounds with antimicrobial properties (Anand and Johur 1959, Bundeally and Shah 1962)(Borgstrom 1969).

Literatures Survey

The biological screening of plant extracts are mostly in connection with the determination of antimicrobial activity. These evaluations are usually carried out by means of standard methods *in vitro* (disc, cup, cylinder and diffusion methods) utilizing a broad selection of pathogenic as well as non-pathogenic microbes. In most cases, a minimum of one Gram positive and one Gram negative organism, usually *Staphylococcus aureus* and *Escherichia coli* are included for initial screening. However, filamentous fungi, yeasts and acid-fast organisms are often included (Farnsworth 1966).

In 1934 Tesumoto (Skinner 1955) published an account of one of the first surveys of plant materials for the purpose of detecting antibacterial activity, he tested 16 fruit juices and 19 Japanese condiments of vegetable origin for activity against *Eberthella typhosa* and *Vibrio cholera*. Some of these materials, particularly the condiments, were reported to be very effective. Boas and Steude (1935) and Keding (1939) (Skinner 1955) noted the bactericidal effect of anemonin, a substance contained in *Ranunculus acris*. Anemonin has since been isolated from several other members of the Ranunculaceae. Sherman and Hodge (1936) (Skinner 1955) discovered that raw juices obtained from the heads of cabbages and the roots of turnips were active against *Escherichia coli*, *Bacterium aerogenes*, and *Xanthomonas campestris*.

Valette and Liber (1938) (Skinner 1955) observed that the bactericidal action of sodium taurocholate against the pneumococcus was greatly enhanced by the presence of convolvulin (a glycoside from *Ipomoea purga* Hyne.) or of jalapin (a glycoside present also in this plant and in the roots of other members of the Convolvulaceae). Foter and Golick (1938) and Foter (1940) demonstrated the bactericidal action of crushed horse-radish (*Cochlearia armoracea* L.) root vapours an of the allyl-isothiocyanate and other mustard oils present in this plant. Many studies on the fungicidal activity of certain vegetable oils were made by Clayton and Foter (1939) (Skinner 1955).

The realization of the importance of penicillin in therapeutic medicine in about 1940 gave a tremendous stimulus to the search for microorganisms capable of yielding new antibiotics. This search was also extended to cover the higher plants. The beginning of this new period of intensive research was marked by the work of Osborn (1943), who made an enormous survey of higher plants in order to detect the presence of substances antibiotic toward *Staphylococcus aureus* and *Escherichia coli*. In this survey, extracts were made from approximately 2,300 species of plants collected in England.

During 1943-1953 many such surveys had been made, references to their authors and to salient features of the investigations are given in Table 1 pp. 15-17.

The studies of Lucas, Gottshall, Frisby et al.(1949-1955)

(Farnsworth 1966) are typical surveys of plants for antimicrobial activity. These investigators screened hundreds of extracts for inhibitory activity against *Staphylococcus aureus*, *Salmonella typhimurium* and *Mycobacterium tuberculosis*, and subsequent studies on the most promising plants by this group led to the isolation of many active principles (Weller, Boll and Dull 1953-1957) (Farnsworth 1966). Antifungal substances from higher plants similarly were reviewed by Sehgal (1961) (Farnsworth 1966).

Of particular interest is a study by Winter (1955) (Farnsworth 1966) in which he compared the antimicrobial properties of 2 groups of plants. One group included randomly collected native plants mentioned in a 300-year-old herbal, which suggested that they were useful for the treatment of infections. Only 29.5 % of plants from the randomly selected group exhibited antimicrobial activities, while 65 % of plants selected because of their mention in the herbal were found to be active.

Karel and Roach (1951) and Baron (1950) (Farnsworth 1966) had compiled lists of antibiotic substances isolated from higher plants as well as from microbes and other sources.

Interest in higher plant extracts exhibiting antimicrobial activity had been on the increase by years and several reports on this subject had been published. Those reports include those of Malcolm and Sofowora (1969) on the antimicrobial activity of some Nigerian plants; the antimicrobial activity of aquatic plants from Minnesota (Su, Abul-Hajj and Staba 1973); the

evaluation of the antimicrobial activity of pure plant products and plant extracts (Mitscher et al. 1972) (Boakye-Yiadom and Konning 1975) and the screening of Chilean plants for antimicrobial activity (Bhakuni et al. 1974).

Vajanakup (1951) had conducted an experiment on antibacterial activity of 20 species of Thai medicinal plants against *Staphylococcus aureus*. The results showed the water extract of eight species of plants could inhibit the growth of such organism.

Antibacterial properties of 63 plant species belonging to 35 families against eight representative microorganisms causing diseases (dysentery, diarrhoea, typhoid fever, dental caries and infectious skin diseases) were reported. Sixty two out of 63 test species were found to be effective (Disyaboot et al. 1975).

A similar study was also obtained by Chumsri et al. (1976-1977).

Those papers, therefore are evidence to encourage a continued interest and lead us to perform the study of antimicrobial activities of the higher plants.

Table 1*

Surveys of Higher Plants for Antibiotic Activity.

Author	No. plants tested	No. showing activity against one or more test organisms.	Test organisms	Remarks
Osborn, 1943	c. 2300 spp. (of 166 families)	Plants belonging to 63 genera (28 families)	*E. coli, Staph. aureus*	
Huddleson et al., 1944	Plants in 23 genera (of 15 families)	6 spp., and many varieties of onion	*Brucella abortus, Staph. aureus*	
Lucas and Lewis, 1944	Not stated	7 active genera named	*E. coli, Phytomonas campestris, P. phaseoli and Staph. aureus*	
Panisset and Louis-Marie, 1945	26 spp.	At least 5 spp.	*E. coli, Staph. aureus*	Survey of some Canadian green plants
Sanders, Weatherwax, and McClung, 1945	c. 120 spp.	24 spp.	*Bacillus subtilis, E. coli*	Survey of plants of Indiana, USA.
Atkinson, 1946	c. 1100 spp.	c. 50 spp.	*Bact. typhosum and Staph. aureus*	See also, Atkinson and Rainsford, 1946
Carlson, Bissell and Mueller, 1946	> 200 spp.	5 active spp. described in detail	Several test organism used	Survey of plants of semi-arid region of S. E. Oregon, USA.
Guerra et al., 1946	11 spp.	3 spp. showed marked activity		Tested plants which were believed by the ancient Aztecs to have medicinal value
Hayes, 1946	231 spp.	46 spp.	*E. coli, Erwinia carotovora, Phytomonas tumefaciens, Staph. aureus*	
Little and Grubaugh, 1946	20 plants	13 plants	Several human and plant pathogenic bacteria and several fungi	Twenty varieties of common garden plants tested
Alamanni et al., 1947	30 spp.	A number of active extracts	Several test organism used	Survey of some Sardinian plants
Spencer et al., 1947	Extracts from c. 600 spp.	Many extracts active	*Plasmodium gallinaceum* (in chicks), *P. cathemerium* and *P. lophurae* (in ducklings)	Survey of plants (from 123 families of Phanerogams and 3 families of Cryptogams) for antimalarial activity. Tests made in vivo.

*From F.A. Skinner : Antibiotic, Modern Methods of Plant Analysis, 1955.

Table 1 (cont.)

Surveys of Higher Plants for Antibiotic Activity.

Author	No. Plants tested	No. showing activity against one or more test organisms.	Test organisms	Remarks
CARDOSO and SANTOS, 1948	c. 105 spp.	5 spp.	*E. coli*, *Proteus X-19* and *Staph. aureus*	
CARLSON and DOUGLAS, 1948a	13 spp. treated in detail	12 spp.	*E. coli*, *Staph. aureus*	Extracts of parts of plants prepared with five different solvents
CARLSON, DOUGLAS, and ROBERTSON, 1948	2115 extracts from 550 spp.	At least 114 spp. active	*E. coli*, *Staph. aureus*	Tested extracts of plants from Ohio and Oregon, USA
SPROSTON, LITTLE and FOOTE, 1948	73 extracts from 11 spp. (11 families)	20 extracts active	Several micro-organisms including *E. coli* and *Staph. aureus*	Tested plants from Vermont, USA
COLLIER and VAN DE PIJL, 1949	Leaves of 290 plants	42 plants	*E. coli*, *Pasteurella pestis*, *Staph. aureus*	Survey of Indonesian plants
GAW and WANG, 1949	45 spp.	17 spp.	*E. coli*, *Staph. aureus*	Tested concentrated aqueous extracts of Chinese drugs prepared from various parts of the 45 spp.
GEORGE and PANDALAI, 1949	100 plants	Many active extracts	Several gram-positive and gram-negative bacteria	Preliminary survey of 100 important Indian medicinal plants
GOTTSHALL et al., 1949	c. 160 spp.	c. 40 spp.	*E. coli*, *Mycobacterium tuberculosis* and *Staph. aureus*	
MITRA, CHANDRAN and RAO, 1949	57 spp. (from 32 families)	11 spp.	Nine micro-organisms	
SARTORY, QUEVAUVILLIER and RICHARD, 1949	c. 300 plants	Extracts of 44 spp. (18 families) active	Eight spp. of bacteria including *E. coli* and *Staph. aureus*	
SCHNELL and THAYER, 1949	c. 350 spp.	c. 118 spp.	*E. coli*, *Staph. aureus* and spores of *Neurospora crassa*	
BUSHNELL et al., 1950	101 spp.	13 spp.	*E. coli*, *Pseudomonas aeruginosa* and *Staph. aureus*	Survey of plants, many of which are mentioned in Hawaiian materia medica
BISHOP and MACDONALD, 1951	940 extracts from 209 spp. (65 families)	146 extracts active against Staph. aureus; 44 against E. coli	*E. coli* and *Staph. aureus*	Survey of Nova Scotian plants

Table 1 (cont.)

Surveys of Higher Plants for Antibiotic Activity.

Author	No. Plants tested	No. showing activity against one or more test organisms.	Test organisms	Remarks
Freerksen and Bönicke, 1951	550 spp.	330 spp.		
Hughes, 1952	545 spp. (295 genera of 73 families)	151 spp. (102 genera of 42 families)	*E. coli* and *Staph. aureus*	Tested crude juices from wild plants of Southern California, USA.
Joshi and Magar, 1952	63 spp.	58 spp.	*E. coli* and *Staph. aureus*	Tested extracts of Indian medicinal plants
Madson and Pates, 1952	>1500 extracts from 126 plant parts (102 spp.)	58 spp. gave active extracts	*Candida albicans*, *Pseudomonas aeruginosa* and *Staph. aureus*	Survey of plants of Florida, USA.
Winter and Willecke, 1952a	100 spp.	37 spp.	*Bacillus subtilis*, *E. coli and Staph. aureus*	Tested extracts of green and withered leaves of the 100 spp.
Winter and Willecke, 1952b	51 spp. of grasses	At least 16 spp.	*Bacillus subtilis*. *E. coli* and *Staph. aureus*	Tested extracts of green and withered leaves of the grasses
MacDonald and Bishop, 1953	177 spp.	59 spp.	*E. coli*, *Staph. aureus*	Survey of Nova Scotian plants
Winter and Willecke, 1953	1283 spp.	378 spp.	*Bacillus subtilis*, *E. coli* and *Staph. aureus*	Survey of plants likely to be of medicinal value according to information given in the herbal of Matthiolus (1611)

Table 2*

Named Antibiotic Substances or Preparations and Their Sources

Name of Antibiotic	Category**	Source of Antibiotic
Allicin	A	*Allium sativum*
Anacardic acid	A	*Anacardium occidentale*
Anacardol	A	*Anacardium occidentale*
Anemonin	A	*Members of Ranunculaceae*
Asiaticoside	B	*Centella asiatica*
Berberine	A	*Members of Berberidaceae*
Cardol	A	*Anacardium occidentale*
Cassic acid	A	*Cassia reticulata*
Catechol	A	*Allium cepa*
Cepheranthine	B?	*Stephania cepherantha*
Chaksine	B	*Cassia absus*
Cheirolin	A	*Cheiranthus cheiri*
Chlerythrine	A	*Chelidonium majus*
Chelidonine	A	
Chelidoxanthine	A?	
Chlorophorin	B	*Chorophora excelsa*
Conessine	A	*Holarrhena antidysenterica*
Convolvulin	A	*Members of Convolvulaceae*
Creptin	B	*Crepis taraxacifolia*
Curcumin	A	*Curcuma spp.*

* From F.A. Skinner : Antibiotic, Modern Methods of Plant Analysis, 1955.

** A = Compound of known chemical structure;

B = Isolated active substance, the structure of which is incompletely known or unknown;

C = Imperfectly characterized preparation.

Table 2 (cont.)

Named Antibiotic Substances or Preparations and Their Sources

Name of Antibiotic	Category	Source of Antibiotic
Datiscetin	A	*Datisca cannabina*
Dicoumarol	A	*Melilotus spp.*
Febrifugine	B	*Dichroa febrifuga*
Iso-febrifugine	B	*Dichroa febrifuga*
Fulvoplumericin	B	*Plumeria acutifolia*
Gindricine	B?	*Stephania grabra*
Humulon	A	*Humulus lupulus*
Jalapin	B	*Ipomoea purga*
Juglone	A	*Juglans spp.*
Kawain	A	*Piper methysticum*
Lupulon	A	*Humulus lupulus*
Lycoperisicin	C	*Lycopersicum spp.*
2-Methoxy-1,4-naphtha-quinone	A	*Impatiens balsamina*
4-O-Methylresorcylic-aldehyde	A	*Decalepis hamiltonii*
Morellins	B	*Garcinia morella*
Nimbidin	C	*Melia azadirachta*
Nordihydroguiaretic acid	A	*Larrea divaricata*
Oxyasiaticoside	B	*Sorbus aucuparia*
Parasorbic acid	A	*Pyrus malus*
Phoretin	A	*Pinus sylvestris*
Pinosylvine	A	
Pinosylvine monoethyl ether	A	*lumbago europaea*
Plumbagin	A	*Plumeria multiflora*
Plumericin	B	*Podophyllum peltatum*

Table 2 (cont.)

Named Antibiotic Substances or Preparations and Their Sources

Name of Antibiotic	Category	Source of Antibiotic
Podophyllin	C	*Podophyllum peltatum*
Pristimerin	B	*Pristimera indica*
Protoanemonin	A	Members of *Ranunculaceae*
Protocatechuic acid	A	*Allium cepa*
Pterigospermin	C	*Moringa pterigosperma*
Puchiin	C	*Eleocharis tuberosa*
Purothionin	B	*Triticum spp.*
Quercitin	A	*Quercus spp.*
Quinine	A	*Cinchona spp.*
Raphanin	B	*Raphanus sativus*
Rhein	A	Synonymous with Cassic acid
Simarubidin	B	*Simaruba amara*
Solanine	A	Members of *Solanaceae*
Thujapicins	A	*Thuja plicata*
Thujic acid	A	*Thuja plicata*
Tomatin	C	Synonymous with lycopersicin
Tomatidine	B	*Lycopersicum spp.*
Trilobin	A	*Cocculus trilobus*
Umbellatine	B	Members of *Berberidaceae*
Vinalin	B?	*Prosopis rucifolia*

Purpose and Scope of Investigation

For various reasons, the search for new antibiotics has been most intensive among the lower plants, with special emphasis on various *Streptomycetes* and a few fungi. Certain disease entities, however, remain serious problems and some of the major antibiotics have considerable drawbacks in terms of limited antimicrobial spectrum or serious side effects. These factors impel a continuing search for new agents. Especially needed are safe antibiotics effective against clinical infections caused by Gram negative organisms, fungi, viruses and mycobacteria.

It is reasonable to suppose that clinically and commercially significant new antibiotics with activities supplemental to and structures widely different from those in current use might be found in sources which have not as yet been as thoroughly explored as the traditional microorganisms. Reports are appearing in the antibiotic literature with increasing frequency describing new antibiotics from such microbial sources as the *Micromonospora Nocardia, Microbispora* certain fungi, etc. This represents one potentially fruitful newer avenue of approach to this problem. Another potentially useful area for exploration is the higher plants.

The purpose and scope of this study is to investigate the antibacterial and antifungal activities of various higher plants, not only the plants which are commonly found in each preparation of folklore remedies, or were reported by earlier investigations to possess antimicrobial activity, but also the plants which are

conveniently available among the Chulalongkorn campus and/or are presented in the medicinal herb shops.

Antibacterial and antifungal studies were carried out by *in vitro* filter paper disc method against one Gram positive, *Staphylococcus aureus* Rosenbach; one Gram negative, *Escherichia coli* (Migula) Castellani et Chalmers; one spore-forming bacilli, *Bacillus subtilis* (Ehrenberg) Cohn; and two representative fungi, *Aspergillus niger* van Tieghem and *Penicillium chrysogenum* Thom.

Methodology

Antimicrobial substances in plants are detected by observing the growth response of various microorganisms to those plant tissues or extracts which are placed in contact with them. Many methods for detecting such substances are available but since they are not all equally sensitive or even based upon the same principle, the results obtained will be influenced by the method selected. Results will also be profoundly affected by the micro-organisms used to make the tests.

I. The Preparation of Plant Extracts

1. Collection of Plant Samples: Extracts may be prepared from fresh or dried material. In general, fresh material is to be preferred, since many plant antimicrobial substances disappear from the tissues as the plants dry out (Osborn 1943). It must be remembered, however that antimicrobial substances may also develop in the tissues on drying. Thus, Winter and Willecke (1952) (Skinner 1955) found that though the antibiotic power of extracts of the leaves decreased, the antibiotic power of similar extracts of other species increased. In some cases, on withering, a loss of the inhibitor contained in the fresh leaves coincided with the build-up of new substances with quite different biological spectra (i.e. active against different sets of microorganisms). The choice of material will obviously depend upon the purpose of investigation.

2. Extracts Prepared with Solvents other than Water: Antimicrobial substances have been demonstrated in the aqueous extracts of a great number of plants. Nevertheless, not all antimicrobial substances in plant tissues are soluble in, or directly extractable with water. In fact, there is now abundant evidence to show that the use of water alone does not provide an adequate test for the efficient screening of plants, extracts prepared with several different solvents should be tested.

3. The pH value of Aqueous Extracts: A test micro-organism may not be able to grow in medium which has been rendered too acid or too alkaline by the presence of an extract irrespective of whether that extract contains any specific antibiotic or not. However, in this screening, it does not matter whether the anti-microbial activities were from any principle, or they may be the organic acid that is present in those plant tissues. Bushnell et al. (1950) (Skinner 1955) concluded that pH effects were not generally responsible for inhibition of the test organisms. This also seems to be the experience of other workers (MacDonald and Bishop, 1953).

4. Storage of Extracts: It is probably best to test the extracts as soon as they are prepared but they may be stored at low temperatures. No precise rules about temperature and duration of storage can be laid down as the stability of any anti-microbial substance in the extracts cannot be foretold.

II. Testing of Plant Extracts

In order to detect an antimicrobial activity, three conditions must be fulfilled. Firstly, the preparation (e.g. a plant extract) must be brought into contact with the microorganism which has been selected for the tests. Secondly, conditions must be so adjusted that the microorganism is able to grow provided no specific antimicrobial substance is present. Thirdly, there must be some means of judging the amount of growth, if any, made by the test organism during that period of time chosen for the test.

1. Methods: The available methods fall mainly into two groups -- dilution methods and diffusion methods. Dilution methods are very useful for assay purpose but are not suitable for the qualitative examination of large numbers of plant extracts. Diffusion methods are easily adapted for qualitative work, and have been very widely used by many investigators. The principle of the method is by placing a sample suspected of containing an antimicrobial substance onto a solid nutrient agar medium which has previously been seeded with a suitable test microorganism. During incubation, the antimicrobial substance, if present, will diffuse into the medium and affect the growth of the test organism. The distance to which a completely inhibitory concentration has extended is indicated by absence of growth of the test organism. Thus, the presence of an antimicrobial substance is shown by a clear zone of inhibition of growth around the sample being tested.

The filter Paper Disc Method is by using small discs of

filter paper as containers for the antimicrobial solutions to be tested. Uniform discs of filter paper or filter fabric are laid on the surface of a seeded plate, then two or three drops or loopfuls of the solution to be tested, are placed on the disc. Vincent and Vincent (1944) and Epstien et al. (1944) dipped the sterile disc into the solution to be tested before placing it on the plate. This last seems to be the best method since very uniform amounts of fluid were taken up by successive discs.

2. Type of Medium: Any agar medium may be used provided that it permits rapid growth of the test organism. For most bacteria, a nutrient medium of the conventional beef extract --- peptone type is suitable. Other media will be required for certain fastidious bacteria and for the cultivation of yeast and mould fungi.

3. Seeding the Agar: The medium may be seeded throughout while still moltened or on the surface only when set. For qualitative work, the method of seeding needs be dictated only by convenience; but for accurate assay, surface-seeding of the hardened plates is, in general, to be preferred, since such plates tend to give sharper zones of inhibition than bulk seeded plates. Surface-seeding by spreading a small amount of inoculum over the surface of a hardened agar plate with a wire loop or a curved sterile glass rod is adequate for qualitative work but not for assays, since the potency of some antimicrobial substances depends on the number of bacteria or fungi present. In such cases, the even

distribution of a standardized amount of inoculum is essential.

When a great number of tests have to be made it is convenient to seed all the medium in bulk while it is moltened and then distributed it into petri-dishes.

4. Incubation Temperature and Times: Human and animal pathogenic bacteria are usually incubated at $37^{\circ}C$ but rather lower temperatures are commonly used for saprophytic and plant-pathogenic bacteria, e.g. $18^{\circ}C$-$28^{\circ}C$. These lower temperatures are also generally used for fungi. At $37^{\circ}C$ the plates are usually incubated for 16 to 24 hours though longer periods are usual at the lower temperatures.

III. Test Organisms

When testing plant extracts for antimicrobial properties, it is advisable to use more than one test microorganism since by this means, the chance of detecting antimicrobial principles in the materials tested will be increased. Use of more than one test organism may, of course, necessitate the use of more than one culture medium.

The test organisms usually employed in the methods of testing which have been used are aerobic saprophytes, particulary bacteria and filamentous fungi. Strains of *Staphylococcus aureus* and *Escherichia coli* have been used frequently and extensively. The choice of test organism will obviously depend greatly on the purpose of the investigation. If one is interested in the possible

role of antimicrobial substances in the natural resistance of many plants to disease, it is clear to the point to employ the organisms causing these diseases. However, if the investigation is of the general character, the test organisms selected should be as diverse as posible and preferably representatives of important groups. Anaerobic bacteria have been rarely used and then only when some special reason for doing so has existed.