CHAPTER IV

DISCUSSION

The evaluations in a screening program designed to uncover new antimicrobial substances potentially useful against bacterial and fungal infections are usually carried out by means of standard *in vitro* methods, because they provide a rapid methods for detecting antimicrobial substances.

The method selected in this investigation has advantage of being simple, rapid and does not necessitate of large quantity of extracts in order to give some ideas of the distribution of the antimicrobial substances within the plant tissues.

Many antibiotics are highly active even in a very low concentration and display a marked specificity in their action toward different microorganisms (Skinner 1955). Thus, the amount of sample used in this study should be enough in making decision of plants being interested.

In this study, both fresh and dried plant materials are also available depending on the facilities of collections, although many plant antimicrobial substances disappear from the tissue as the plants dry out (Osborn 1943), but another antimicrobial substances may also develop in the tissue on drying (Winter and Willecke 1952)(Skinner 1955).

It is preferable to use cold method for making extracts in order not to destroy any thermo-labile antimicrobial substances in the plant tissues. Some antimicrobial substances exist in the plant as inactive precursors from which the active principles are liberated by enzymic action on rupture of the cells. In such cases, heating will tend to destroy the enzymes and lead to the production of only inactive extracts, for example allicin in garlic (Cavallito, Bailey, and Buck 1945). However, hot extracts may be more toxic to microorganisms than cold extracts (Fleck and Richards 1924)(Skinner 1955).

Storage of the absorbed discs before testing, in order to get rid of solvents, may have the problem of losing volatile substances in the extracts. For example, the crushed garlic tissues were found to be most active when fresh, it became inactive in a few days owing, presumably, to the disappearance of a volatile component (Bocher 1938)(Skinner 1955).

In many cases, it may happen that plants which have been reported to have antimicrobial activities by one worker, have given negative results when tested by another. Such disparity in results is undoubtedly often due to the followings:-

1. The distribution of antimicrobial principles within individual plant and within group of plants.

Antimicrobial substances vary greatly as to their potency and distribution within plants (Cavallito, Bailey, and Kirchner 1945)

(Lucas and Lewis 1944) (Huddenson et al. 1944) (Osborn 1943) (Hayes 1946). Thus, it is preferred to test as many separate parts of the plants as possible. For example, in this study, it was found that extracts of the flowers of *Datura metel* L. inhibit the growth of test microorganisms but the green leaves and fruits showed negative test.

Some extracts had no effect on microorganisms, and on the other hand, stimulated growth of them. When testing plant extracts, stimulation of the growth of the test organisms is often observed (Fig. 29-32 p. 129). In diffusion tests, rings of stimulated growth were often surrounded the inhibition zones (Fig. 29-32 p.129). Boas (1943) called attention to the fact that stimulatory substances may be present in plant tissues together with antimicrobial substances. Simultaneous action might explain these phenomena on plates particularly if the stimulant diffuses faster than the antibiotic or vice versa. It is possible too, that some inhibitors, if present in very low concentration, may have a stimulatory effect on the growth of some test organisms (Skinner 1955).

Many other factors affected the antimicrobial activities of plant extracts are antagonistic and synergistic actions of the effective chemical constituents of plant itself. Molecular size of the antimicrobial substances from plant extract will effect the rate of diffusion of those substances through agar medium, resulting in many bands of inhibition zone (Fig. 29-32 p. 129).

Many medicinal plants studied are found to contain various

different kinds of effective chemical constituents that are active against microorganisms. Most of the constituents are quinones for examples anthraquinones in genus *Cassia* (Caesalpiniaceae), *Rheum* (Polygonaceae); naphthoquinones in *Tectona grandis* L. (Verbenaceae), *Lawsonia inermis* L. var. *alba* Hassk. (Lythraceae) and *Impatiens balsamina* L. (Balsaminaceae), the last two species contain the same specific naphthoquinones called "lawsone". Besides these the plumbagin substance in the family Plumbaginaceae is also the quinones in nature. All quinones mentioned are found to be more or less active against many kinds of microorganisms (Thomson 1971).

A possible reason for the divergence of some results between difference workers may be the seasonal variation in antimicrobial substances content of certain plants (Hayes 1946). Such seasonal variation may be due in some cases to a difference in concentration with the season, and in others, to difference in the nature of the antimicrobial substances, and these may be influenced by the variations of soil (Shellard 1974).

2. The effects of the technique used in testing on the results obtained.

The detection of the presence of the antimicrobial substances in plant extracts will be influenced by a number of factors such as the ability of the active principles to withstand various treatments during preparation and testing of the extracts, the solubility of the principle in the solvent or solvents used and

the ability of the principle to respond to the particular test employed. Many methods for detecting antimicrobial 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. However, diffusion method is not quite as sensitive as the dilution method and the antimicrobial substances that will not diffuse through agar, will give a negative result, and size of inhibition depend also on the rate of diffusion of the antimicrobial through the agar (Tobie and Ayres 1944).

The correlation of the observed activities by the agar diffusion method helped in detecting any level of activity present in the extracts. This was considered essential as we could not exactly quantify the active constituents from each of the extracts. Such observations served as the first essential step in the screening program of these randomly collected plants. In this study the procedure of extraction, the fixed quantity of plant sample and volume of solvents could approximately indicate the amount of active constituents present.

3. Kinds of microorganisms.

The results will also be profoundly affected by kinds of microorganisms. Since the microorganisms used in this investigation are confined to the representative of each of three groups of bacteria and two groups of fungi,therefore the lower-estimated of antimicrobial spectrum may obtain. Some plant extracts showing

negative activity against a tested representative microorganism may have positive activity against another microorganism(s) of the same group of represented one.

In comparison to previous reports among local publications of antimicrobial activities of medicinal plants (Disyaboot et al. 1975 and Chumsri et al. 1976-1977), some of the results obtained are correlated, for examples, some plant samples showed relative degree of positive activities against certain bacteria such as *Plumbago rosea* L. and *Piper betel* L.

Some others, such as *Curcuma longa* L. and *Clerodendron enerme* Gaertn. showed no activities against the same microorganism - *Escherichia coli* by the two previous mentioned workers, Disyaboot et al. and Chumsri et al. 1976-1977, including this present paper, eventhough different test methods were applied.

Among the positive ones, the results obtained were found in various different degree of activities, for examples, *Acorus calamus* L. and *Cassia tora* L. However, some were in contrast, one reported positive whereas the other(s) reported negative or vice versa, for examples, Chumsri et al. reported the positive result of *Acacia auriculaeformis* A.Cunn. against *Staphylococcus aureus* but the negative result was obtained by this present paper. In *Acorus calamus* L. this present paper showed positive result against *Staphylococcus aureus* when the negative result was obtained by Chumsri et al.

In the same manner the positive results of *Phyllanthus emblica* L. and *Momordica cochinchinensis* Spreng. could get by Disyaboot et al. but this present paper showed negative results.

These different results can be fallen into two categories as follows:

1. The conditions of test plants.

The fresh or dried condition of plant used will effect the results as mentioned before.

2. The methods of extraction.

The test method is very much concerned. If the amount of active constituents in plant are very low, a positive result could hardly get by simple method, unless it is highly active antibiotic, otherwise the exhaustive extraction by fractionation could do. However, the simple method of extraction for screening activities of medicinal plants will be worthwhile of getting relatively good and rapid results in very large number of samples.