

STUDIES ON THE ANTIBIOTIC PLANTS IN TAIWAN

I. Screening of common local herbs for antibiotic activity against enteropathogenic *E. coli*

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Introduction

Due to the widespread use of antibiotics, the antibiotic-resistant mutant emerges, which causes trouble in clinics. Therefore, the discovery of more antimicrobial agents in nature to remedy infectious disease caused by antibiotic-resistant bacteria strains seems to be a urgent work now.

Taiwan is an island lying off the east coast of Asia; the situation in the subtropical zone provides a warm temperature and heavy rainfall. Under these physical conditions, therefore, the flora of Taiwan is very rich, containing approximately three thousand species and many of them are used as antibiotics locally. They are the important source of discovering the natural antibiotics. In previous reports Dr. Koo^(1,2) and Dr. Yen⁽³⁾ had reported the antimicrobial screening of some plants against only a few standard strains. In this paper, we have attempted the screening of 30 specimens of common local herbs in Taiwan,⁽⁴⁾ for antibiotic activity (see Table 1). A total of 49 *E. coli* strains, which were isolated from patients with diarrhea and were resistant to many commonly used antibiotics⁽⁵⁾ were used as the tested strains. These antibiotics include colistan, chloramphenicol, oxytetracyclin, streptomycin, ampicillin, neomycin, and kanamycin.

Assay for antibacterial activity was done by agar plate dilution method. An appropriate amount of test solution --- n-hexane, chloroform, alcohol and water extract of each herbs, was added into melted agar mixed thoroughly and then poured into sterilized petri dish to make a concentration of 4%, 2%, and 1% separately. A loopful of *E. coli* young culture broth was inoculated bandly on agar plate. The result was investigated after incubation at 37°C for 48 hours.

Among 30 specimens the water extract of *Sphenomeris chinensis*, *Rhus semialata* var. *roxburghiana*, *Ludwigia octovalvis*, *Centella asiatica*, and *Scutellaria virularis* (as shown in Table 2); the alcohol extracts of

Glochidion hongkongense (leaf), *Rhus semialata* var. *roxburghiana* (shown Table 3); and the n-hexane extracts of *Lygodium japonicum*, *Pteris ensiformis*, *Broussonetia papyrifera*, and *Centella asiatica* (see Table 4) showed antibacterial activity. But the chloroform extracts of all the 30 specimens showed no antibacterial activity.

In particular, it is interesting that the n-hexane extracts of *Lygodium japonicum*, *Pteris ensiformis*, and *Centella asiatica*; water extract of *Rhus semialata* var. *roxburghiana* alcohol extract of leaf of *Glochidion hongkongense* inhibited the growth of all the *E. coli* strains.

For more complete understanding of clinical applications, we will study the chemical constituent and experimental pharmacology of antibiotic herbs in future. Further investigation is now under way.

Materials and Methods

A total of 30 specimens, including some commonly used herb medicines, all wildy growing in suburban Taipei, were collected. The used part was submitted to the following extract for antibiotic assay.

Preperation of test solution:

1) Test solution of n-hexane, chloroform, and alcohol extract: A dried plant material (50 gm) extracted with solvent in the order of appropriate n-hexane, chloroform, alcohol. All heated under reflux each for 4 hrs. respectively. After filtration, each extract solution was concentrated under a reduced pressure to give a dried residue, and then dissolved or suspended in 6 ml acetone. It was used as the test solution and considered as 100%.

2) Test solution of water extract: A dried plant material (50 gm) collected in spring (1977) was extracted with appropriate distilled water under reflux for 4 hrs. After filtration, the extract was concentrated under a reduced pressure to 30 ml. It was used as the test solution. and considered as 100%.

Preperation of culture medium:

Heart infusion broth or agar (Difco), which furnishes good growth for *E. coli*, was used as the culture medium throughout the experiment.

Preparation of bacterial strains:

All the tested *E. coli* strains, which were isolated from patients with diarrhea from hospital.

Assay for antibacterial activity.:

An appropriate amount of the test solution was added into melted agar mixed thoroughly and then poured into sterilized petri dish to make a concentration of 4%, 2%, or 1% separately. A loopful of *E. coli* young culture broth was inoculated bandly on agar plate. The result was read after incubation at 37°C for 48 hrs. If there is no bacteria growth the tested solution is considered as having an antibacterial effect.

Results

The distribution of antibacterial activities of water, alcohol, n-hexane, and chloroform extracts of the 30 tested specimens was quite different. The water extracts of *Sphenomeris chinensis*, *Rhus semialata* var. *roxburghiana*, *Ludwigia octovalvis*, *Centella asiatica*, and *Scutellaria virularis*, showed an inhibitory effect on the growth of *E. coli* (Table 2). The extract of *Rhus semialata* var. *roxburghiana* inhibited the growth of all the tested 49 *E. coli* strains. The growth of 44 strains out of 49 tested strains was inhibited by *Scutellaria virularis*; 35 out of 49 strains by *Sphenomeris chinensis*, 4 out of 49 by *Centella asiatica*, 2 out of 49 by *Ludwigia octovalvis*. The water extract of *Rhus semialata* var. *roxburghiana*, was the most effective one among the water extracts of the 30 tested specimens.

The alcohol extracts of only two specimens, *Glochidion hong-*

The alcohol extracts of only two specimens, *Glochidion hongkongense* (leaf) and *Rhus semialata* var. *roxburghiana* showed antibacterial effect (Table 3). Only the leaf, not the stem, of *Glochidion hongkongense* was effective against the growth of all the 49 strains, while *Rhus semialata* var. *roxburghiana* was effective against only 14 strains.

The n-hexane extracts of 4 plants showed inhibiting effect on *E. coli* growth (Table 4). Three of them, *Lygodium japonicum*, *Pteris ensiformis* and *Centella asiatica*, inhibited the growth of 49 strains, while *Broussonetia papyrifera* inhibited only 4 strains.

The chloroform extracts of all the tested 30 specimens were ineffective.

The majority of the effective extract showed an inhibiting effect at concentration of 4% (Table 5). Although a few still showed inhibiting effect at the concentration of 2% or even 1%, the number of strains inhibited was much less than that at the concentration of 4%, with the exception of n-hexane extract of *Centella asiatica*, which at the concentration of 2% still could inhibit the growth of all the 49 strains. But at the concentration of 1% only the growth of 6 strains was inhibited.

Discussion

The antibacterial activities against the antibiotic-resistant enteropathogenic *E. coli* of the water, alcohol and n-hexane extracts of the various plants were quite different. The results indicated that, usually, only one of the extract of a given species showed antibacterial effect, with the exception of *Rhus semialata* var. *roxburghiana* and *Centella asiatica*. Both the water and alcohol extracts of *Rhus semialata* var. *roxburghiana* showed inhibiting effect on enteropathogenic *E. coli* growth, but the number of strains inhibited were different. The water extract inhibited 49 strains, while the alcohol extract inhibited only 14 strains growth. This may be due to the fact that the responsible substance is more soluble in polar solvent. The

n-hexane extract of *Centella asiatica*, was effective against the growth of all the enteropathogenic *E. coli* isolated from 49 patients, while its water extract inhibited the growth of only 4 strains.

Although among the 30 specimens the antibacterial activities of *Pteris ensiformis*, *Centella asiatica*, *Lydwigia octovalis*, *Polygonum perfoliatum*, and *Brussonetia payrifera* and been reported (1,4,5,6,7) the results of our experiment are not the same as the previous reports. For example, in the previous papers reported (6,7) that *Polygonum perfoliatum* showed inhibitory effect on the growth of *E. coli* NIHJ, *E. coli* 0-127, but in this experiment the results showed that it was not effective against the enteropathogenic which were isolated from clinical specimen and were resistant to many commonly used antibiotics; *Pteris ensiformis* and *Centella asiatica* also failed to show any antibacterial effect on *E. coli* but in this experiment the n-hexane extracts of these two plants are effective against all the enteropathogenic isolated from 49 patients suffering from diarrhea. The n-hexane extract of *Centella asiatica* is the most effective one. These differences may be caused by the different susceptibility of the tested strains of different origin, the ways of preparation of test solution, or the place, time for collection of the plants.

As for the potency of water, alcohol, and n-hexane extracts of the various plants, n-hexane extracts, particularly that of *Centella asiatica* which at concentration of 2% still inhibited the growth of 49 enteropathogenic *E. coli*, is the most effective.

All the enteropathogenic *E. coli* strains isolated from patients suffering from diarrhea were multi-drug resistant, all of them are resistant to antibiotics ranging from 2 to 7 kinds, but they are sensitive to the plants extracts. The results suggested that there is no cross-resistant existing between these antibacterial substances in the extracts and the antibiotics mentioned above. It seems they may be a promising antibacterial agent. Whether they can act as a chemotherapeutic agent for infectious disease, especially, those caused by antibiotic-resistant strain, needs further study.

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Summary

A total of 30 specimens of plant, all wildy growing in suburban Taiepi, were collected. The water, alcohol, n-hexane, and chloroform extracts were screened for antibacterial effect on *B. coli* which were isolated from patients with diarrhea and were resistant to antibiotics ranging from 2 to 7 kinds.

Among the 30 tested specimens the water extracts of *Sphenomeris chinensis*, *Rhus semialata* var. *roxburghiana*, *Luwsigia octovalvis*, *Centella asiatica*, and *Scutellaria virularis*; the alcohol extracts of *Glochidion hongkongense* (leaf) and *Rhus semialata* var. *roxburghiana*; n-hexane extracts of *Lygodium japonicum*, *Pteris ensiformis*, *Centella asiatica* showed an inhibitory effect on the growth of all the 49 strains. The results suggested that there is no cross-resistant existing between these antibacterial compounds of plant origin and the commonly used antibiotics. Whether they can act as an antibacterial agent from treatment of infectious disease caused by antibiotic-resistant strain needs further study.

Literature Cited

- (1) Chen, J.Y., T.I. Yang, Y.C. Liu and C.S. Yang, 1973. Search for biologically active substances in Taiwan medical plants, 3. Further screening for anti-tumor and anti-microbial substance. *Chinese J. Microbiol.* 7: 104-106.
- (2) Kan, W.S., 1965. Manual of vegetable drugs in Taiwan, part 1, 2, 3.
- (3) Kee, W.Y., K.Y. Fong, 1957. Antibiotic activity of *Forsythia suspensa* Vahl., *Lonicera japonica* Thunb, and *Arctimu Lappa* L.J. *Taiwan Pharm. Assoc.*, 9: 15-21.
- (4) Koo, W.Y., 1962. A study of the antibiotic activity of 50 species of Taiwan plants. *J. Taiwan Pharm. Assoc.* 14: 66-72.
- (5) Lin, Y.C., T.I. Yang, J.Y. Chen and C.S. Yang, 1972. Search for biologically active substances in Taiwan medical plants. 1. Screening for anti-tumor and anti-microbial substances. *Chinese J. Microbiol.*, 5: 76-81.
- (6) Lin, Y.C., T.I. Yans, J.Y. Chen and C. S. Yang, 1973. Search for biologically active substances in Taiwan medical plants, 2. Further screening for anti-tumor and anti-microbial substances. *Chinese J. Microbiol.*, 6: 97-99.
- (7) Yon, K.Y., W.H. Lee, 1966. Studies on the pharmaceutical effective components of local plants in Taiwan, 1. The antibiotic activity of 150 local plants. *J. Taiwan Pharm. Assoc.* 18: 49-55.

臺灣產抗生藥材之研究

第一報：民間常用抗生藥材活性之檢索

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採自臺北郊外臺灣民間常用抗痢疾之藥用植物之三十種，其水、醇、氯仿及正己烷四種抽取液，作對 *E.coli* 的四十九株菌株的抗生作用，*E.coli* 係從腹瀉病患者分離出來的，均具抗藥性，有的對二種，有的高達八種的抗生素。

在此預試驗的植物中具抗生作用：烏蕨、埔鹽、大本水丁香、老公根、半枝蓮之水抽取液；大本饅頭果（葉）及埔鹽之醇抽取液；海金沙、鳳尾草、構樹、老公根之正己烷抽取液；而氯仿抽取液皆無抗生作用。且值得一提之事，埔鹽水抽取液、大本饅頭（葉）之醇抽取液和海金沙、鳳尾草、老公根之正己烷抽取液均能完全抑制四十九株 *E.coli* 菌株的生長。

由實驗結果，可知這些植物抽取液與一般常用的抗生素之間似乎無交差抗藥性的情形存在。

因此這些具抗生作用的藥用植物是否可治療對抗生素具抗藥性的腹瀉疾病，實在值得我們更進一步的研究。