Antinematodal Activity of Some Tropical Rainforest Plants against the Pinewood Nematode, *Bursaphelenchus xylophilus*

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Antinematodal Activity, *Botrytis cinerea, Bursaphelenchus xylophilus*

Sixty five methanolic extracts of Sumatran rainforest plants representing 63 species of 21 families were assayed *in vivo* for antinematodal activity against *Bursaphelenchus xylophilus* using our cotton ball-fungal mat method. Extracts of 27 plants species from 14 families exhibited antinematodal activity, while 37 species were inactive. Among them, three extracts of *Bischofia javanica*, *Knema hookeriana* and *Areca catechu* exhibited very strong activity at minimum effective dose (MED) of 0.7 mg/cotton ball (mg/bl.). Eight extracts from *Allamanda cathartica*, *Ervatamia corymbosa*, *Hoya diversifolia*, *Bischofia javanica*, *Derris malacensis*, *Melastoma melabathricum*, *Ophiorriza konsteleary* and *Brucea sumatrana* also showed strong activity (MED, 5 mg/bl.).

Introduction

The serious wilting disease in Japanese pine was found to be caused by a nematode, *Bursaphelenchus xylophilus* (Tokushige and Kiyohara, 1969; Kiyohara and Tukoshige, 1971; Mamiya and Kiyohara, 1972) and many efforts have been done for controlling such disease. Although some antinematodal chemicals such as morantel tartarate and emamectin benzoate are now commercially available, the current method for its control is still based on killing the vector, a longihorn beetle, *Monochamus alternatus* Hope, by aerial application of an insecticide (Kawazu *et al.*, 1980a). Therefore, effective controlling method against the pathogenic nematode is urgently desired. However, the chemicals that are widely used for control of plant-parasitic nematodes are far from perfect with respect to ecological safety. Several of them have been prohibited due to of concern to the environmental contamination such as ozone depletion, groundwater pollution and harmful effect on human health. On the other hand, multibillion dollar losses to the agricultural sector by lack of excellent chemical management on phytoparasitic nematodes. (Nigg and Seigler, 1992). Therefore, at present time, the development of new safe nematicides is our important objective, and many agrochemical companies and researchers are involved in searching for new antinematodal substances from natural products.

In this study, some crude extracts of tropical rainforest plants in Indonesia were examined to find potent antinematodal compounds against the pinewood nematode, *B. xylophilus*.

Materials and Methods

Plant materials

The plant materials were collected in August 1997 in the "Panti" forest region, 120 km north of Padang, the capital of West Sumatra, Indonesia. Some species were collected at other locations in the region of Padang and Bukittingi. Most of them were guided by information on ethnopharmacognostic and traditional uses. Families and species of samples were identified by Dr. Rusdi Tamin at the Department of Biology, Faculty of Mathematic and Natural Sciences, Andalas University, Padang, West Sumatra, Indonesia. The voucher specimens are kept in the Herbarium Biology Andalas (AND) and Herbarium Bogoriense (BO), Bogor Indonesia.

Preparation of plant extracts

Fresh parts of plants (50 g each) were soaked in methanol (150 ml) for one week at room temperature. After filtration, the methanol solutions were evaporated *in vacuo* to give methanolic extracts. The crude extracts were redissolved in methanol to prepare working stocks of 200 mg ml^{-1}.

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Preparation of test nematodes

The pinewood nematodes, *Bursaphelenchus xylophilus*, were collected by a Baermann funnel from wood chips of the trunk or stem of wilted pine trees in the Okayama University Experimental Forest. The fungus, *B. cinerea*, was cultured on 14 ml of Czapeck-Dox agar medium (1.3% agar) in a petri dish (9 cm in dia.) at 21 °C for ten days. The petri dishes with full grown fungus were inoculated with nematodes, *B. xylophilus*, and kept at 26 °C until fungal mycelia were completely consumed (for 5 days).

Bioassay procedure

A previously reported bioassay method (Kawazu *et al.*, 1980a) was used with some modifications. The living nematodes were washed and separated from the culture medium through double sheets of tissue paper (Type JK Wiper 150-S, Kimberly-Clark Corp.) in the Baermann funnel for 2 h. The nematodes were collected by centrifugation (650×*g*, 3 min). The suspension of nematodes of appropriate concentration was poured into a flat dish placed on a section. The nematodes were counted under a microscope (× 20). An aqueous suspension of the nematode (*ca.* 15,000 heads/ml) was prepared by appropriate dilution for use as a working stock.

The cultured fungus, *B. cinerea*, on 3 ml of the Czapeck-Dox agar medium (1.3% agar) in a petri dish (4 cm in dia.) at 21 °C for 4 days was placed a cotton ball (5 mm in dia.) containing the test concentrate at the center of the fungal mat. The nematode suspension (0.1 ml) prepared above was injected into the cotton ball, and the dish was kept at 26 °C for 96 h. The antinematodal effect (inactive or active) was estimated by observing whether the mycelia were consumed by the nematode or not, and denoted by a sign − or +, respectively. Based on author's experience, the highest dose of crude extracts was started from 20 mg/cotton ball (mg/bl.), by serial dilution method, and the assay was done to get the minimum effective dose (MED). Each test extract was evaluated in triplicate experiments. The MED values is defined as the lowest dose of the test extract to completely inhibit the nematodes by consuming the fungal mat.

Results and Discussion

Several groups of investigators have used the method for assessing antinematodal activity established by Kawazu *et al.* (1980a), and the method involves collecting both the agar medium and the nematodes and submitting them to the Baermann funnel filtration which takes at least 6 hours. In the present modification, living nematodes in the surface of medium in the petri dish were transferred and separated through double sheets of tissue paper (JK Wiper 150-S) to the Baermann funnel for 2 h. By this reduction of time, the viability of nematodes increased greatly during the subsequent assay.

Recently, much research has been conducted on chemical diversity and biological activities related in phytochemical studies of rainforest plants (Gottlieb, 1979; Kitagawa *et al.*, 1994, 1996a; Mackeen *et al.*, 1997). However, reports on antinematodal activities remain scanty. From this view, the present report describes a survey of antinematodal plants from the Indonesian rainforest.

Results of the antinematodal activity of the plant extracts are shown in Table I. Among 65 methanolic extracts of parts of the plants tested (21 families), 27 species from 14 families proved to be active. As can be seen from the Table, most of the activities was given in the range of MED, 5~20 mg/bl.. Among of 27 species, three extracts from *Bischofia javanica* (entry 29) of Euphorbiaceae, *Knema hookeriana* (entry 45) of Myristicaceae, and *Areca catechu* (entry 49) of Palmae afforded specially high activitiy, at MED of 0.7 mg/bl., respectively. In due course, we have been interested in the active components in these plants.

From the methanol extract of *K. hookeriana* in particular, two kinds of active phenolics were isolated and identified as 3-undecylphenol and 3-[(8*Z*)-tridecenyl]-phenol, with the activity (MED) of 0.0045 and 0.02 mg/bl., respectively. The study of the structural analyses will be reported in detail in a succeeding paper.

Additionally, strong activity (MED 5 mg/bl.) was shown by the extract from *Allamanda cathartica* (bark) (entry 4), *Ervatamia corrymbosa* (seeds) (entry 6), *Hoya diversifolia* (sap) (entry 14), *Bichofia javanica* (leaves) (entry 30), *Derris malaccensis* (root) (entry 36), *Melastoma melabathricum* (root) (entry 38), *Ophiorriza konstel-*

Table I. Activity of plant extracts against *Bursaphelenchus xylophilus*.

Family/species	Parts	Dose (mg/bl) 20	10	5	2.5	1.3	0.7	Minimum Effective Dose (MED), mg/bl.
Apocynaceae								
1. *Alstonia scholaris*	sap	–	–	–	–	–	–	n.a.
2. *Alstonia angustivolia*	leaves	–	–	–	–	–	–	n.a.
3. *Alstonia angustiloba*	sap	+	+	–	–	–	–	10
4. *Allamanda cathartica*	bark	+	+	+	–	–	–	5
5. *Ervatamia corymbosa*	sap	–	–	–	–	–	–	n.a.
6. *Ervatamia corymbosa*	seeds	+	+	+	–	–	–	5
7. *Plumeria acumilata*	sap	+	+	–	–	–	–	10
8. *Rauwolfia corymbosa*	sap	–	–	–	–	–	–	n.a.
9. *Rauwolfia verticillata*	sap	–	–	–	–	–	–	n.a.
Araceae								
10. *Acorus calamus*	leaves	–	–	–	–	–	–	n.a.
11. *Plantago aquatica*	leaves	–	–	–	–	–	–	n.a.
12. *Typhonia javanicum*	leaves	–	–	–	–	–	–	n.a.
Asclepiadaceae								
13. *Calotropis gigantea*	bark	+	+	–	–	–	–	10
14. *Hoya diversifolia*	sap	+	+	+	–	–	–	5
15. *Hoya coronaria*	sap	–	–	–	–	–	–	n.a.
16. *Sarcostemma esculentum*	leaves	–	–	–	–	–	–	n.a.
17. *Tylophora tenuis*	leaves	–	–	–	–	–	–	n.a.
Begoniaceae								
18. *Begonia sinuata*	leaves	–	–	–	–	–	–	n.a.
19. *Begonia tuberosa*	leaves	–	–	–	–	–	–	n.a.
Caricaceae								
20. *Carica papaya*	leaves	+	+	–	–	–	–	10
Convolvulaceae								
21. *Merremia mamosa*	leaves	–	–	–	–	–	–	n.a.
22. *Merremia umbelata*	leaves	+	–	–	–	–	–	20
Compositeae								
23. *Ageratum conyzoides*	a.p.	+	–	–	–	–	–	20
Dilleniaceae								
24. *Dillenia sp.*	sap	–	–	–	–	–	–	n.a.
Euphorbiaceae								
25. *Acalypha grandis*	leaves	–	–	–	–	–	–	n.a.
26. *Acalypha wilkesiana*	sap	+	–	–	–	–	–	20
27. *Antidesma bunius*	leaves	+	–	–	–	–	–	20
28. *Antidesma montana*	sap	–	–	–	–	–	–	n.a.
29. *Bischofia javanica*	sap	+	+	+	+	+	+	0.7
30. *Bischofia javanica*	leaves	+	+	+	–	–	–	5
31. *Euphorbia hirta*	a.p.	+	–	–	–	–	–	20
32. *Jatropha curcas*	sap	+	–	–	–	–	–	20
33. *Macaranga gigantea*	leaves	–	–	–	–	–	–	n.a.
34. *Macaranga triloba*	bark	–	–	–	–	–	–	n.a.
Guttiferae								
35. *Garcinia hombroniana*	leaves	–	–	–	–	–	–	n.a.
Leguminoceae								
36. *Derris malaccensis*	root	+	+	+	–	–	–	5
37. *Parkia spiciosa*	leaves	–	–	–	–	–	–	n.a.
Melastomataceae								
38. *Melastoma melabathricum*	root	+	+	+	–	–	–	5
Moraceae								
39. *Arthocarpus blumei*	sap	–	–	–	–	–	–	n.a.
40. *Arthocarpus altissima*	sap	–	–	–	–	–	–	n.a.
41. *Ficus vistulosa*	sap	–	–	–	–	–	–	n.a.
42. *Ficus variegatta*	sap	+	+	–	–	–	–	10
43. *Ficus vulva*	sap	+	+	–	–	–	–	10

Table I (continued).

Family/species	Parts	Dose (mg/bl) 20	10	5	2.5	1.3	0.7	Minimum Effective Dose (MED), mg/bl.
Myristicaceae								
44. *Horsfieldia glabra*	sap	–	–	–	–	–	–	n.a.
45. *Knema hookeriana.*	sap	+	+	+	+	+	+	0.7
46. *Myristica faragrans*	leaves	–	–	–	–	–	–	n.a.
Nephentaceae								
47. *Nephentes ampularia*	flower	–	–	–	–	–	–	n.a.
Oxalidaceae								
48. *Averhoa bilimbi*	leaves	–	–	–	–	–	–	n.a.
Palmae								
49. *Areca catechu*	seeds	+	+	+	+	+	+	0.7
Pterocarpaceae								
50. *Pterocarpus indicus.*	sap	+	+	+	–	–	–	10
51. *Pterocarpus macrocarpus.*	bark	–	–	–	–	–	–	n.a.
Rubiacea								
52. *Gardenia annisophylla*	bark	–	–	–	–	–	–	n.a.
53. *Morinda citriflora*	leaves	+	+	+	–	–	–	10
54. *Hedyotis auricularia*	leaves	–	–	–	–	–	–	n.a.
55. *Hedyotis rigida*	leaves	–	–	–	–	–	–	n.a.
56. *Ixora chinensis*	leaves	–	–	–	–	–	–	n.a.
57. *Ophiorriza konsteleary*	a.p.	+	+	+	–	–	–	5
58. *Ophiorriza marginata*	a.p.	+	–	–	–	–	–	20
59. *Psychotria obovata*	leaves	–	–	–	–	–	–	n.a.
60. *Psychotria sarmentosa*	leaves	–	–	–	–	–	–	n.a.
Simaroubaceae								
61. *Brucea sumatrana*	seeds	+	+	+	–	–	–	5
62. *Eurycoma longivolia*	bark	+	+	–	–	–	–	10
63. *Quassia indica*	bark	+	+	–	–	–	–	10
Verbenaceae								
64. *Tectona grandis*	bark	–	–	–	–	–	–	n.a.
65. *Vitex trifolia*	bark	–	–	–	–	–	–	n.a.

a.p. = aerial part; n.a. = no activity
The activity profiles were classified based on the following criteria: very strong (MED 1.3 ~ 0.7 mg/bl.), strong (MED 5 ~ 2.5 mg/bl.), moderate (MED 10 mg/bl.), and weak (MED 20 mg/bl.). Each test extract plant was evaluated in triplicate experiments.

eary (a.p.) (entry 57) and *Brucea sumatrana* (seeds) (entry 61). We reported previously the antinematodal activity of methanol extracts from some plants species in Caprifoliaceae, Compositeae, and Euphorbiaceae families harvested in Japan (Kawazu *et al.*, 1980b). In this previous study it was of particular interest that 4 species *Aleurites cordata*, *Aleurites fordii*, *Sapium japonicum* and *Triadica sebifera* among 9 species of the Euphorbiacea family, grown in Japan, were shown to have activity at a concentration of 20 mg/bl. In the case of the Euphorbiaceae family in Indonesia, 5 among 9 species were found to be active (entries 26, 27, 29, 30, 31 and 32). So, the Euphorbiaceae family from Indonesia and Japan showed very similar results although the kind of the active species were entirely different. These results indicate that plants from Euphorbiaceae family include a number of species which contain antinematodal components in general. This feature may be related to the fact that plants of the Euphorbiaceae family contain high levels of general antinematodal phorbol ester and related compounds.

Moderate activity (MED 10 mg/bl) was observed in 10 species and weak activity (MED 20 mg/bl) was observed in 7 species.

From Simaroubaceous plants, more than 250 kinds of quassinoids and their glycosides have been isolated from this family (Okano *et al.*, 1981;

Cabral *et al.*, 1993; Imamura *et al.*, 1993; Kitagawa *et al.*, 1996b and Alen *et al.*, 1996), and many of them have been reported to show promising biological functions such as anticancer, antileukemic, antimalarial and amoebicidal activities. Therefore, the antinematodal activity observed in the present study for three species in Simaroubaceous plants in Indonesia (entries 61, 62 and 63) might be due to chemical constituents similar to those responsible for the above biological activities. This has to be investigated by chemical and nematicidal studies in future.

Although a strict comparison or evaluation of the activity may not be easy because of the nature of the biological assay system, the salient activity observed in the present study *K. hookeriana* appears to be a promising candidate as an antinematodal agent for practical application to prevent growth of *B. xylophilus*. The study also suggests that the bark of *K. hookeriana* may be used directly as an antinematodal agent, in the form of green manure in fields with safety and economical benefit. Such material is readily available in developing tropical countries. Hopefully, the nematicidal activity also will be assessed against several phytonematode species having different parasitic habits.

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