

Screening of Tropical Plants for the Presence of Bioactive Compounds

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ABSTRAK

Penggunaan racun serangga dan rumpai sintetik untuk mengawal pembiakan serangga dan rumpai telah menimbulkan berbagai masalah kerana pencemaran persekitaran, resistan serangga dan ketoksikan mamalia yang tinggi. Dibalikinya, racun serangga yang diterbitkan daripada tumbuhan adalah lebih mudah mengalami degradasi biologi, kurang mencemarkan persekitaran dan kurang toksik kepada organisme bukan tumpuan. Oleh kerana itu banyak penyelidikan telah ditumpukan bagi mendapatkan punca baru bagi sebatian-sebatian tersebut daripada berbagai tumbuhan. Satu kaedah penyaringan dengan menggunakan udang pepai sebagai biocerakinan umum untuk menentukan kehadiran sebatian bioaktif di dalam tumbuhan telah dilakukan dan beberapa spesies tumbuhan telah dikenali untuk kajian yang lebih mendalam. Ekstrak mentah dan sampel-sampel yang dituliskan selanjutnya diuji dengan menggunakan kaedah biocerakinan yang lebih spesifik.

ABSTRACT

The use of synthetic pesticides and herbicides to control insect and weed pests has posed substantial problems due to their persistence in the environment, insect resistance and high mammalian toxicity. On the other hand, plant-derived insecticides are more readily biodegraded, less likely to contaminate the environment and less toxic to nontarget organisms. Hence, the search for new sources of such compounds from plants has gained popularity amongst researchers. A screening procedure for the presence of bioactive compounds from plant sources by using brine shrimp as the general bioassay has been carried out and a number of plant species has been identified for further detailed studies. The crude extracts and purified samples were further tested using other more specific bioassay methods.

INTRODUCTION

Secondary metabolites from plant sources such as rotenone, pyrethrum, nicotine and sabadilla have been used widely as insect repellents and toxicants for a very long time. With the discovery of synthetic insecticides such as DDT, chlorinated hydrocarbon etc., the use of these plant compounds was greatly reduced. However, these synthetic compounds proved to be either toxic, ecologically unfriendly in the form of bioaccumulation or induced insect resistance. Thus their use has been restricted or banned totally. The shift of interest to botanical compounds is quite understandable

and there has been a big surge of interest in this area recently, especially in compounds from tropical flora.

Plants produce enormous varieties of chemicals which are believed to be important in mediating the interaction between plants and their environment. Due to the diversity of tropical flora, there is a need to carry out mass-screening of plant extracts for the presence of bioactive compounds which could be used to control insect populations. Previously two fish species have been used to detect the presence of active components (Rahmani *et al.* 1989 and Sukari *et al.* 1992). This

paper reports a simple bioassay system using brine shrimp (*Artemia salina*), a tiny crustacean, as a general tool to detect the presence of these active compounds from various plant species (Jasper 1977). By using this system it is also possible to carry out bioassay-directed fractionation during chromatography whereby the active fraction(s) could be identified easily. More specific bioassay systems are needed to evaluate their effectiveness against various plant pathogens.

RESULTS AND DISCUSSION

McLaughlin has developed a general bioassay method for this purpose using the larvae of brine shrimp (*A. salina*) (Meyer *et al.* 1982; Alkofahi *et al.* 1989). This method is very good because it is cheap, convenient, reliable, the work is easily carried out by competent phytochemists and requires very little space. The shrimp eggs can be obtained readily from aquarium shops which use them to feed tropical fish. The eggs will remain in good condition for many years if kept dry and refrigerated. Once the eggs are placed in brine solution they will hatch into tiny larvae (nauplii) within 48 hours and swim towards a light source.

Crude ethanol extracts at various concentrations were tested against these larvae. Initial concentrations of 1000, 500, 100, 50 and 10 ppm were chosen and tested against ten larvae in 5 ml brine solution placed in 10 ml vials. The experiment was carried out in five replicates and the number of survivors was monitored after 24 hours and the percentage mortality at each concentration was calculated and recorded. In cases where

the effective dosage is low, new sets of experiments were carried out using samples with lower series of concentrations. From these data, the LC_{50} value of each sample was determined and used as an indicator for the presence of bioactive compounds in the extracts.

Table 1 gives the results of this bioassay work. As expected, the ethanol extracts of *Derris elliptica* (pokok tuba) and *Piper nigrum* (lada hitam) gave very strong test results with LC_{50} of 0.078 ppm and 1.65 ppm respectively. Further isolation was not

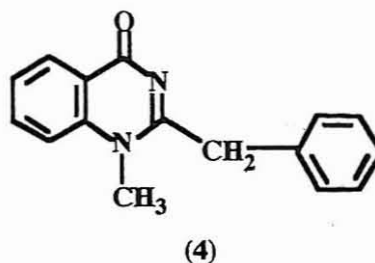
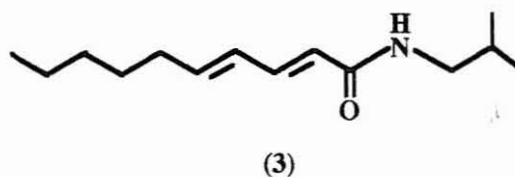
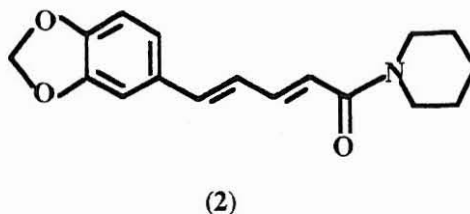
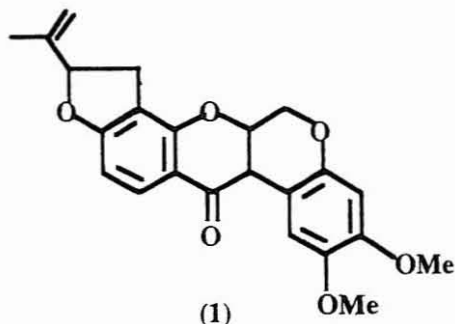


TABLE I
Bioactivity of different plant extracts using
the brine shrimp method.

Plant material	Family	LC_{50} (ppm)
1. <i>Abrus precatorius</i>	Euphorbiaceae	60
2. <i>Antidesma tomentosum</i>	Euphorbiaceae	360
3. <i>Azadirachta indica</i>	Meliaceae	23
4. <i>Derris elliptica</i>	Leguminosae	0.078
5. <i>Glycosmis pentaphylla</i>	Rutaceae	8.5
6. <i>Piper nigrum</i>	Piperaceae	1.65
7. <i>Polygala monticola</i>	Polygalaceae	21
8. <i>Polygala paniculata</i>	Polygalaceae	65
9. <i>Stemona tuberosa</i>	Stemonaceae	26
10. <i>Tabernaemontana divaricata</i>	Apocynaceae	85

SCHEME 1

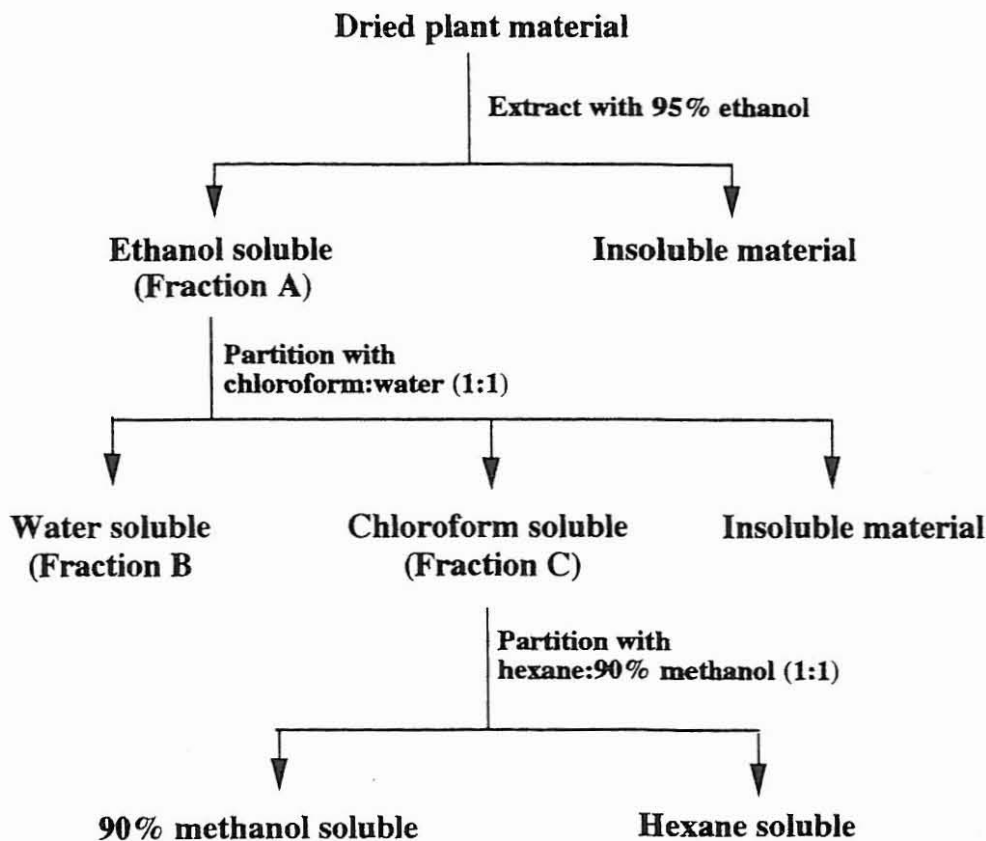


TABLE 2.
Bioactivity of partitioned plant extracts using brine shrimp method.

Plant material	Fractions	LC ₅₀ (ppm)
1. <i>Azadirachta indica</i>	Ethanol extract	23
	Chloroform soluble	12
	Water soluble	700
2. <i>Derris elliptica</i>	Ethanol extract	0.078
	Chloroform soluble	0.058
	Water soluble	275
3. <i>Piper nigrum</i>	Ethanol extract	1.65
	Chloroform soluble	1.20
	Water soluble	550
4. <i>Polygala paniculata</i>	Ethanol extract	65
	Chloroform soluble	12
	Water soluble	3400

carried out on these two plants because their chemical constituents were well established. *D. elliptica* contains toxic rotenone (1) (Fukami and Nakajima 1971). *P. nigrum* contains active piperine (2), pellitorine (3) and other unsaturated isobutylamides (Miyakado *et al.* 1983). However, partitioning of these extracts (Scheme 1) together with *Azadirachta indica* (mambu or neem) and *Polygala paniculata* with chloroform: water (1:1) demonstrated the effectiveness of this bioassay in detecting the presence of bioactive compounds and also enriching the target compounds. The results of this partitioning are shown in Table 2, In all cases the active compounds are concentrated only in the chloroform extracts. The chloroform fraction may be further partitioned with hexane: 90% methanol (1:1), thus further enriching the extracts with bioactive compounds.

One of the plants investigated in detail for controlling insect populations is *Glycosmis pentaphylla*. The crude extract of the plant was found to be very active against brine shrimp and from this an active quinazolone alkaloid, arborine (4), was isolated and tested against fruit-fly (*Dro-*

sophila melanogaster) (Kawazu *et al.* 1977, 1989). Arborine completely retarded the larval growth which leaves the larvae and pupae small and abnormal in shape. The minimum inhibitory effect of the first instar was 0.8 mg/2 g diet (Ahmad and Rahmani 1991). The activity against the brine shrimp bioassay may be used as a convenient indicator for toxicity to invertebrate insect pests as shown in the above results.

MATERIALS AND METHODS

Plant materials were air dried before use. A herbarium specimen of each sample has been deposited at the herbarium, Department of Biology, UPM. The shrimp eggs were provided by En. Cheah Sin Hock, Faculty of Fisheries and Marine Science, UPM, and kept refrigerated.

Preparation of brine shrimp larvae

Sea water was placed in a small rectangular container divided into two compartments by a perforated dam. Shrimp eggs were placed in one compartment and covered with a dark cloth. The other compartment was left uncovered to have a source of light (or sunlight) to which the larvae would be attracted. The eggs were allowed to hatch and mature for 48 hours before testing was carried out. After this 10 newly-hatched larvae were placed in vials containing the sample to be assayed (50 larvae for each dilution). The number of survivors was monitored 24 hours later, percentage mortality was calculated and LC₅₀ obtained.

Preparation of extracts

Dried ground plant material (50 g) was soaked in 95% EtOH overnight, filtered and concentrated to dryness to give a dark coloured viscous solid extract (Fraction A). A stock solution was prepared by dissolving 20 mg of the extract in 2 ml solvent. From this solution, 500, 250, 50, 25 and 5 µl were transferred to testing vials corresponding to 1000, 500, 100, 50 and 10 ppm respectively. The solvent was evaporated under nitrogen and warmed to 50°C using an analytical evaporator. 5 ml of sea water was added to each vial and the experiment was carried out in five replicates (50 shrimps per dilution). For very active extracts, lower range of concentration was prepared using similar procedures.

Partition of the extracts

A portion of Fraction A was partitioned between chloroform : water (1:1) and the water solubles

were taken to dryness and labelled Fraction B (Scheme 1). The chloroform solubles were dried and evaporated to give Fraction C. Both fractions were tested against brine shrimp using a similar procedure to that above. The active chloroform solubles were further partitioned with hexane : 90% methanol and each was tested against brine shrimp.

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