Natural Product Sciences
6(3) : 131~134 (2000)

Brine Shrimp Toxicity of Fractionated Extracts of Malaysian Medicinal Plants

Mukram M. Mackeen[1], Mohammad N. Khan[2], Zainudin Samadi[3] and Nordin H. Lajis[4]*

[1]Department of Biotechnology, [3]Department of Chemistry, [4]Institute of Bioscience, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia
[2]Department of Chemistry, University of Malaya, 59100 Kuala Lumpur, Malaysia

Abstract – The methanol, dichloromethane, petroleum ether, 90% methanol and 1-butanol fractions of 40 Malaysian medicinal plants belonging to 25 families were tested for brine shrimp lethality. Various parts and fractions of eight (20%) plants, viz. *Annona muricata, Cerbera odollam. Calophyllum inophyllum, Entada phaseoloides, Pithecellobium jiringa, Crotolaria retusa, Morinda elliptica* and *Sellaginella willdenovii* showed very strong toxicity (LC_{50}: <100 ppm). The methanol extract of the seed of *Calophyllum inophyllum* showed exceptionally toxic activity (LC_{50}: 5 ppm).
Key words – Malaysian medicinal plants, brine shrimp, fractionated extracts

Introduction

Malaysia is endowed with rich biodiversity of flora and fauna many of which have not been subjected to scientific investigation. This is reflected, for example, in the large number of species (more than 15,000) of higher plants that have been recorded (Goh *et al.*, 1993). The rich genetic diversity also offers high chemical diversity and therefore, presents a greater possibility of finding new sources of potentially valuable biologically active compounds. In this connection, biological assays are required to evaluate the potency of either extracts or compounds. Although many expensive, high capacity, robust and complicated mechanism-based bioassays are available, they are not suitable as bench-top assays especially in laboratories having limited financial resources and facilities. In order to overcome this problem, inexpensive and convenient bench-top bioassays such as the brine shrimp lethality test may be employed as a preliminary indicator of potential and significant biological activity (Meyer *et al*, 1982). The brine shrimp lethality test has been reported to be useful in predicting biological activities such as cytotoxicity, phototoxicity, pesticidal and trypanocidal activities, enzyme inhibition and ion regulation (Anderson *et al.*, 1991; Fatope *et al.*, 1993; Solis *et al.*, 1993; Zani *et al*, 1995; Ojala *et al.*, 1999). Recently, there has been interest in the brine shrimp lethality assay as a means of detecting ion regulation or ion-channel activity such as that involving Na^+K^+ATPase or calcium channels (Borowitz and McLaughlin, 1992; Watts *et al.*, 1996). Its convenience and reliability, has also made it an efficient technique in performing bioassay-guided isolation (McLaughlin, 1991).

Although there are some reports on the screening of Malaysian plants for various biological activities including brine shrimp lethality, very few of these studies involved fractionated extracts (Sukari *et al.*, 1992; Ali *et al.*, 1996; Mackeen *et al*, 1997a & b; Murakami *et al.*, 2000). Furthermore, the few reports on brine shrimp toxicity were restricted to a small number of plant extracts (Sam *et al.*, 1988; Rahmani *et al.*, 1992). With the view of expanding the present data available on brine shrimp lethality, this paper reports the activity of 200 fractions of 40 Malaysian medicinal plants that were screened for brine shrimp toxicity.

Materials and Methods

Plant materials – The plant materials were obtained from various parts of Malaysia and identified by S. Anthonysamy, Herbarium Unit, Department of Biology, Universiti Putra Malaysia. Their medicinal

*Author for correspondence.

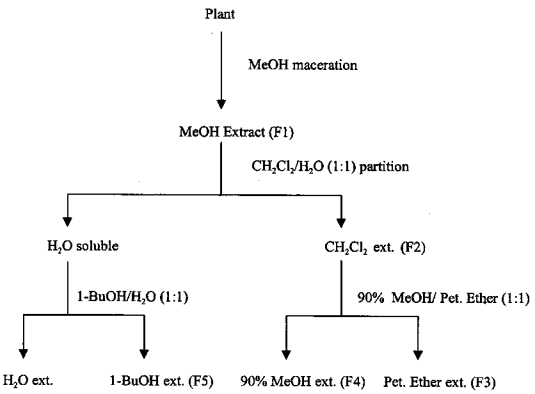

Fig. 1. Extraction scheme.

use was established by reference to Burkill (1966).

Extraction – Varying amounts of fresh plant material (chopped or ground) were soaked in methanol for two days. For each sample, extraction was carried out thrice. The methanol extracts of each sample were combined and concentrated under reduced pressure. Solvent partitioning was then performed as shown in Fig. 1.

Brine shrimp lethality assay – The assay was performed as described previously (Meyer *et al.*, 1982) using brine shrimp (*Artemia salina* Leach) nauplii. The eggs (Gold Eagle, USA) were placed in brine and hatched within 48 h. Each extract (200 mg) was dissolved in 20 ml methylene chloride: methanol (1:1) to prepare a stock solution of 10 mg/ml. From the stock solution, 500, 50 and 5 μl were transferred in triplicate to vials and the solvents were allowed to evaporate. After evaporation, 5 ml of brine was added to each vial to prepare concentrations corresponding to 1000, 100 and 10 ppm. Ten shrimp nauplii were added to each vial (30 shrimps per concentration). The number on survivors at each concentration was recorded and the LC_{50} values ($p<0.05$) were calculated using the Finney computer programme.

Experimental

The LC_{50} values of the methanol, dichloromethane, petroleum ether, 90% methanol and 1-butanol extracts of the 40 medicinal plant species from 25 families screened for brine shrimp lethality are shown in Table 1. Solvent partitioning, as shown in Fig. 1, was carried on all plant species although the initial methanol extracts of some plants were inactive (LC_{50} >1000 ppm). This was done with the purpose of detecting toxicity, if present, after fractionation of the extracts. For convenience, the level of lethality was classified as follows: very strong (LC_{50}<100 ppm), strong (100 ppm<LC_{50}<500 ppm), moderate (500 ppm<LC_{50}<750 ppm), weak (750 ppm<LC_{50}< 1000 ppm) and inactive (LC_{50}>1000 ppm). From Table 1, the methanol extracts of the seeds of *Annona muricata* (LC_{50}: 35 ppm), *Cerbera odollam* (LC_{50}: 32 ppm) and *Calophyllum inophyllum* (LC_{50}: 5 ppm), the bark of *Entada phaseoloides* (LC_{50}: 52 ppm) and the fruits of *Crotolaria retusa* (LC_{50}: 99 ppm) exhibited very strong toxicity. The methanol extract LC_{50} values of *Annona muricata* and *Cerbera odollam* seeds were comparable to that of the positive control, potassium dichromate (LC_{50}: 30 ppm) whereas the seeds of *Calophyllum inophyllum* were six-fold stronger. However, none of the methanol extracts of the other plant parts of *Annona muricata* and *Calophyllum inophyllum* were active.

Strong toxicity was found in the methanol extracts of *Asclepias curassavica* (LC_{50}: 466 ppm), *Cuscuta australis* (LC_{50}: 215 ppm), the stems of *Leucaena glauca* (LC_{50}: 472 ppm), the fruits of *Melia azedarach* (LC_{50}: 206 ppm), the vine of *Tinospora crispa* (LC_{50}: 173 ppm), the roots of *Morinda elliptica* (LC_{50}: 140 ppm) but not in the fruits and leaves, *Selaginella willdenovii* (LC_{50}: 227 ppm) and *Vitex trifolia* var. *ovata* (LC_{50}: 347).

Moderate and weak activities were shown by the methanol extracts of *Equisetum debile* (LC_{50}: 535 ppm), the leaves of *Ricinus communis* (LC_{50}: 942 ppm), *Lawsonia inermis* (LC_{50}: 616 ppm), *Phyllagathis rotundifolia* (LC_{50}: 611 ppm), the seeds of *Melia azedarach* (LC_{50}: 550 ppm) and *Premna foetida* (LC_{50}: 832 ppm). The rest of the methanol extracts in Table 1 were inactive.

The methanol extracts of *Annona muricata, Calophyllum inophyllum, Asclepias curassavica, Cuscuta australis, Equisetum debile, Ricinus communis, Entada phaseoloides, Leucaena glauca, Crotalaria retusa, Lawsonia inermis, Phyllagathis rotundifolia, Melia azedarach, Tinospora crispa, Premna foetida* and *Vitex trifolia* var. *ovata* showed loss of activity upon fractionation suggesting the possibility of synergism. On the other hand, marked increase in toxicity was observed in the dichloromethane (LC_{50}: 231 ppm) and 1-butanol (LC_{50}: 250) fractions of *Pithecellobium jiringa* and *Hedyotis herbacea*, respectively. The toxicity of *Pithecellobium jiringa*

Table 1. Brine shrimp lethality of fractionated extracts

No.	Plant Species	Family	Plant Part	LC_{50} (ppm)
1.	*Andrographis paniculata* Nees	Acanthaceae	whole	>1000 (Fa)
2.	*Asystasia intrusa* Blume	Acanthaceae	whole	>1000 (Fa)
3.	*Annona muricata* Linn.	Annonaceae	(a) seed	35 (F1)
		Annonaceae	(b) pericarp	>1000 (Fa)
4.	*Cerbera odollam* Gaertn.	Apocyanaceae	seed	32 (F1); 28 (F2); 278 (F5)
5.	*Asclepias curassavica* Linn.	Asclepiadaceae	whole	466 (F1)
6.	*Cleome icosandra* Linn.	Capparidaceae	whole	>1000 (Fa)
7.	*Cuscuta australis* R. Br.	Convolvulaceae	whole	215 (F1)
8.	*Ipomoea pes-caprae* R. Br.	Convolvulaceae	whole	>1000 (Fa)
9.	*Equisetum debile* Roxb.	Equisetaceae	whole	535 (F4)
10.	*Euphorbia tirucalli* Linn.	Euphorbiaceae	leaf	>1000 (Fa)
11.	*Ricinus communis* Linn.	Euphorbiaceae	(a) leaf	942 (F1)
			(b) seed	>1000 (Fa)
12.	*Calophyllum inophyllum* Linn.	Guttiferae	(a) leaf	>1000 (Fa)
			(b) bark	>1000 (Fa)
			(c) fruit	>1000 (Fa)
			(d) seed	5 (F1)
13.	*Hyptis suaveolens* Poit.	Labiatae	whole	>1000 (Fa)
14.	*Ocimum santum* Linn.	Labiatae	whole	>1000 (Fa)
15.	*Lindera pipericarpa* Boerl.	Lauraceae	bark & leaf	>1000 (Fa)
16.	*Entada phaseoloides* Merr.	Leguminosae	bark	52 (F1)
17.	*Pithecellobium jiringa* Prain	Leguminosae	pod	>1000 (F1); 231 (F2); 110 (F3); 86 (F4); >1000 (F5)
18.	*Hevea brasiliensis* Muell. Arg.	Leguminosae	bark & seed	>1000 (Fa)
19.	*Leucaena glauca* Benth.	Leguminosae	(a) stem	472 (F1)
			(b) leaf	>1000 (Fa)
20.	*Crotalaria retusa* Linn.	Leguminosae	(a) fruit	99 (F1)
			(b) leaf	>1000 (Fa)
21.	*Desmodium umbellatum* DC.	Leguminosae	leaf	>1000 (Fa)
22.	*Loranthus ferrugineus* Roxb.	Loranthaceae	leaf	>1000 (Fa)
23.	*Lawsonia inermis* Linn.	Lythraceae	leaf	616 (F1)
24.	*Phyllagathis rotundifolia* Blume	Melastomaceae	whole	611 (F1)
25.	*Melia azedarach* Linn.	Meliaceae	(a) leaf	>1000 (Fa)
			(b) fruit	206 (F1)
			(c) seed	550 (F1)
26.	*Tinospora crispa Diels*	Menispermaceae	vine	173 (F3)
27.	*Pteridium aquilinum* Kuhn	Polypodiaceae	frond	>1000 (Fa)
28.	*Morinda citrifolia* Linn.	Rubiaceae	fruit & stem	>1000 (Fa)
29.	*M. elliptica* Ridl.	Rubiaceae	(a) root	140 (F1); 65 (F2); 263 (F3); 27 (F4), 66 (F5)
			(b) fruit & leaf	>1000 (Fa)
30.	*M. umbellate* Linn	Rubiaceae	stem, fruit & bark	>1000 (Fa)
31.	*Mitragyna speciosa* Korth.	Rubiaceae	leaf	>1000 (Fa)
32.	*Hedyotis herbacea* Linn.	Rubiaceae	whole	>1000 (Fa); 250 (F5)
33.	*H. corymbosa* Lamk.	Rubiaceae	whole	>1000 (Fa)
34.	*H. capitellata* Wall.	Rubiaceae	leaves	>1000 (Fa)
35.	*Clausena excavata* Burm.	Rutaceae	leaves	>1000 (Fa)
36.	*Selaginella willdenovii* Baker	Selaginellaceae	whole	227 (F1) 33 (F5)
37.	*Datura metel* Linn.	Solanaceae	whole	>1000 (Fa)
38.	*Premna foetida* Reinw.	Verbenaceae	whole	832 (F1)
39.	*Vitex trifolia* var. *ovata* Mak.	Vitaceae	whole	347 (F1)
40.	*Curcuma aeruginosa* Roxb.	Zingiberaceae	rhizome	>1000 (Fa)
	Potassium dichromate (positive control)			30

Extracts: F1 = MeOH; F2 = CH_2Cl_2; F3 = petroleum ether; F4 = 90% MeOH ; F5 = 1-BuOH; Fa = F1, F2, F3, F4 & F5

was mainly present in the petroleum ether (LC_{50}: 86 ppm) and 90% methanol (LC_{50}: 110 ppm) fractions from dichloromethane. The 90% methanol fraction of *Morinda elliptica* showed the highest toxicity (LC_{50}: 27 ppm) compared to the other fractions. For *Annona muricata*, *Ricinus communis*, *Calophyllum inophyllum*, *Leucaena glauca*, *Crotalaria retusa*, *Melia azedarach* and *Morinda elliptica*, only certain

plant parts were active in the assay.

Our results may now suggest which fractions and parts of the plant species studied may be useful for further work to isolate the active compounds.

Acknowledgements

This work was supported by the IRPA grant 03-02-04-0043 from Universiti Putra Malaysia and the Ministry of Science, Technology and the Environment of Malaysia

References

Ali, A. M., Mackeen, M. M., El-Sharkawy, S. H., Hamid, J. A., Ismail, N. H., Ahmad, F. B. H. and Lajis, N. H. (1996) Antiviral and cytotoxic activities of some plants used in Malaysian indigenour medicine. *PERTANIKA Journal of Tropical Agricultural Science.* **19**, 129-136.

Anderson, J. E., Goetz, C. M., McLaughlin, J. L. and Sufness, M. (1991) A blind comparison of simple bench-top bioassays and human tumour cell cytotoxicities as antitumor prescreens. *Phytochemical Analysis.* **2**, 107-111.

Borowitz, J. L. and McLaughlin, J. L. (1992) Evidence for calcium channels in brine shrimp: diltiazem protects shrimp against cadmium. *Bulletin of Environmental Contamination and Toxicology.* **48**, 435-440.

Burkill, I. H. (1966) *A dictionary of the economic products of the Malay Peninsula.* Vol. I & II. Crown Agent, London.

Fatope, M. O., Ibrahim, H. and Takeda, Y. (1993) Screening of higher plants reputed as pesticides using the brine shrimp lethality assay. *International Journal of Pharmacognosy.* **31**, 250-254.

Goh, S. H., Soepadmo, E. and Chuah, C. H. (1993) *Phytochemical guide to the Malaysian flora.* University of Malaya Press, Kuala Lumpur.

Meyer, B. N., Ferrigni, N. R., Putnam, J. E., Jacobsen, L. B., Nichols, D. E. and McLaughlin, J. L. (1982) Brine shrimp: a convenient general bioassay for active plant constituents. *Planta Medica.* **45**, 31-34.

Murakami, A., Ali, A. M., Mat-Salleh, K. M., Koshimizu, K. and Ohigashi, H. (2000) Screening for the in vitro anti-rumor-promoting activities of edible plants from Malaysia. *Bioscience, Biotechnology and. Biochemistry.* **64**, 9-16.

Mackeen, M. M., Ali, A. M., El-Sharkawy, S. H., Manap, M. Y., Salleh, , K. M., Lajis, N. H. and Kawazu, K. (1997a) Antimicrobial and cytotoxic properties of some Malaysian traditional vegetables (ulam). *International Journal of Pharmacognosy.* **35**, 174-178.

Mackeen, M. M., Ali, A. M., Abdullah, M. A., Nasir, R. M., Mat, N. B., Razak, A. R. and Kawazu, K. (1997b) Antinematodal activity of some Malaysian plant extracts against the Pine Wood nematode. *Pesticide Science.* **51**, 165-170.

McLaughlin, J. L., Chang, C. J. and Smith, D. L. (1991) Bench-top bioassays for the discovery of bioactive natural products: an update. *Studies in Natural Products Chemistry.* Vol. 9. pp. 383-409. Ed. Atta-ur-Rahman. Elsevier, Amsterdam.

Ojala, T., Vuorela, P., Kiviranta, J., Vuorela, H. and Hiltunen, R. (1999) A bioassay using *Artemia salina* for detecting phototoxicity of plant courmarins. *Planta Medica.* **65**, 715-718.

Rahmani, M., Ismail, H. B. M., Ahmad, F., Manas, A. R. and Sukari, A. (1992) Screening of tropical plants for the presence of bioactive compounds. *Pertanika* **15**, 131-135.

Sam, T. W., Ng, A. S., Cheah, P. B. and Ong, K. S. (1988) Toxicity screening with the brine shrimp (*Artemia salina*) of plant extracts. *Proceedings of the UNESCO Sub-Regional Seminar/Workshop on the Systematic Identification of Natural Products.* Eds. Said, I. M. and Din, L. B., pp. 50-57, Bangi, 13-17 June 1988

Solis, P. N., Wright, C. W., Anderson, M. M., Gupta, M. P. and Phillipson, J. D. (1993) A microwell cytotoxicity assay using *Artemia salina* (brine shrimp). *Planta Medica.* **59**, 250-252.

Sukari, M. A., Rahmani, M., Manas, A. R. and Takahashi, S. (1992) Toxicity studies of plant extracts on insects and fish. *Pertanika*, **15**, 41-44.

Watts, S. A., Yeh, E. W. and Henry, R. P. (1996) Hypoosmotic stimulation of ornithine decarboxylase activity in the brine shrimp *Artemia franciscana. The Journal of Experimental Zoology.* **274**, 15-22.

Zani, C. L., Chaves, P. P. G., Queiroz, R., Oliveira, A. B. de, Cardoso, J. E., Anjos, A. M. G., Grandi, T. S. M. and De Oliveira, A. B. (1995) Brine shrimp lethality assay as a prescreening system for anti-*Trypanosoma cruzi* activity. *Phytomedicine.* **2**, 47-50.

(Accepted July 19, 2000)