Pertanika 14(1), 41-44 (1992)

Toxicity Studies of Plant Extracts on Insects and Fish

MOHD. ASPOLLAH SUKARI, MAWARDI RAHMANI,
ABD. RAHMAN MANAS AND SHOZO TAKAHASHI*
Department of Chemistry, Faculty of Science and Environmental Studies
Universiti Pertanian Malaysia
43400 UPM Serdang, Selangor Darul Ehsan, Malaysia

**Pesticide Research Institute, Faculty of Agriculture*
Kyoto University, Kyoto 606, Japan.

Key words: Insecticidal activity, piscicidal activity, toxicity studies, medicinal plants, essential oils, alkaloid-containing plants.

ABSTRAK

Kajian ketoksikan beberapa ekstrak tumbuh-tumbuhan tempatan terhadap serangga dan ikan telah dilakukan. Pecahan mudah meruap beberapa ekstrak tumbuhan telah dipisahkan dan komponen utamanya telah dicirikan. Keaktifan keracunan terhadap serangga secara sentuhan dan kesan ketoksikan terhadap ikan telah dikaji.

ABSTRACT

Toxicity studies of several local plant extracts on insects and fish were carried out. The volatile fraction of some of plant extracts was isolated and the major components were characterized. The contact insecticidal activity and the toxicity test of the plant extract on fishes were studied.

INTRODUCTION

Natural products have always played a major role in the development of organic chemistry. The various structural types of natural products contribute not only to new findings or pose challenging synthetic problems but also provide hope that they may become the basis for new biologically active substances of commercial significance.

Biological testing has played an important role in toxicity studies of plant extracts. There are numerous bioassay studies on plant extracts. The first of such work involving some local plants was reported as early as 1965 (Nakanishi *et al.*), Our previous investigation revealed that some local Labiatae plant extracts exhibit antifungal, antibacterial and insecticidal activities (Sukari and Takahashi 1988). This study was conducted as part of the research to study the biological activity of local plant extracts. The report discusses the contact insecticidal activity test of the plant extracts against mung bean weevil (*Callosobruchus maculatus*) and the toxicity test of certain plant extracts on two species of fish, *Tilapia mossambica* and *Labistes reticulates.*

MATERIALS AND METHODS

Contact Insecticidal Activity Test

The plant samples selected for the test consisted of the leaves of *Mentha arvensis* (Labiatae), *Eugenia caryophyllus* (Myrtaceae) and *Decaspermum momtanum* (Myrtaceae), and the rhizomes of *Cymbopogon citratus* (Gramineae). The air-dried sample of each plant was immersed in methanol for a few days, filtered, rotary-evaporated and extracted with ethyl acetate. The extracts were steam-distilled to obtain the essential oils, which were analysed by gas chromatography and spectroscopic methods to characterize the major components. The residue of the distillation was again extracted with ethyl acetate. The insecticidal activity test was carried out on the essential oil and residue of each of the plant samples.

Filter paper, sprayed with extract of the sample (1 and 2 mg in ethyl acetate) was placed inside a cylindrical flask (area of 23.8 cm^2). The flask was put aside to evaporate off the solvent. The test insects, *C. maculatus* (about 20 adults for each test) were placed in the treated flask and

monitored for 24 h to observe the mortality. The control test was performed without sample treatment.

Piscicidal Activity Test

The fishes used in the bioassays were *Labistes reticulates* (Cyrinodontiadae) and *Tilapia mossambica* (Cichlidae). They were bred in concrete tanks and were acclimatised in the container for a few days prior to the testing. Ten *L. reticulates* (average length of 2.5 cm) and five *T. mossambica* (average length of 7.0 cm) were placed in small plastic aquariums (19.0 x 11.5 cm) containing 1.6 and 4 l of water, respectively. Tap water was aerated before being used as dilution water. The fishes were not fed for 24 h before the testing and throughout the test period of 72 h.

The plant samples used consisted of the leaves and flowers of *Ocimum sanctum* (Labiatae) and the leaves of *Isotoma longiflora* (Campanulaceae), *Lindera pipericarpa* (Lauraceae) and *Antidesma tomentosum* (Euphorbiaceae). One hundred g of plant material was blended in 1000 ml of water, filtered and squeezed through a muslin cloth. The concentration of this stock solution was 100,000 ppm, from which five test solutions of 5,000, 1,000, 500, 100 and 10 ppm were prepared.

Fish mortalities were observed regularly within the period of 72 h. The results were expressed in terms of LC_{50} (Rahmani *et al.* 1989). There were two replicates for each treatment and the control experiment was carried out without a sample extract.

Gas chromatography (GC) was conducted using a Shimadzu 9A chromatograph on a capillary column (PEG 20 M, 0.2 mm i.d., 25m long) equipped with a flame ionization detector (FID). The column temperature was programmed 70-225°C at 3°C/min. Helium was used as the carrier gas at a flow rate of 50 ml/min.

RESULTS AND DISCUSSION

Insecticidal Activity Test of Plant Extracts

Previous investigations on the oil of *M. arvensis* showed piperitenone oxide to be the major component (*ca* 87% of the volatile) (Sukari and Takahashi 1988). In this study, GC analysis of the essential oil of *E. caryophyllus* showed the presence of eugenol as the major constituent (80.4% of the steam-distillate) by comparing retention time with the authentic sample. The major component was separated by preparative TLC developed with hexane-diethyl ether (7:3), and its IR and ^1H NMR data were in agreement with the data in the literature. Yu and Fang (1981) have reported the presence of eugenol (78.5 - 82.4%) as the major constituent of oil isolated from the leaves of *E. caryophyllus* grown in China.

Similar GC analysis of oil of *C. citratus* has identified citral and geraniol as major components (total of 27.3% of the volatile). Another major peak (*ca* 12%) in the mixture was not identified. Citral was the main component of the essential oil of the same plant species reported by Oliveros-Belardo and Aureus (1977) and Formacek and Kureczka (1982). On the other hand, GC-MS studies on the volatile fraction of the extract of *D. momtanum* have suggested the presence of some monoterpenes and sesquiterpenes, none of which was identified. Further work to analyse the chemical components of these oils is still in progress.

TABLE 1
Essential oils analysis of plant extracts

Plant	Major Components
M. arvensis	Piperitenone oxide
E. caryophyllus	Eugenol
C. citratus	Citral, geranoil
D. momtanum	Monoterpenes, Sesquiterpenes

The results from insecticidal activity test of the plant extracts on *C. maculatus* are shown in Table 2. The contact toxicity test found essential oils of *M. arvensis, E. caryophyllus* and *D. momtanum* to exhibit 100% mortality in *C. maculatus*, whereas oil of *C. citratus* exhibited about 30% mortality at the same concentration. The oils of both *M. arvensis* and *E. caryophyllus* were more toxic to the insects and took a relatively shorter time to achieve 100% mortality compared to the oil of *C. citratus.* (Table 2)

Control experiments using piperitenone oxide and eugenol have demonstrated the same degree of toxicity against the insects for both these compounds (Table 2). These results indicate that the toxicity of the oils of both plant species in the present study could most probably be due to the presence of piperitenone oxide and eugenol in the essential oils. Both these com-

TABLE 2
Toxicity of plant extracts on *Callosobruchus maculatus*

Plant sample	Weight of Extract (mg)	% Mortality	Time (hours)
Mentha arvensis			
Oil	1	100	2
	2	100	1
Piperitenone oxide	1	100	1.5
	2	100	1
Non-volatile	1	0	
	2	0	
Eugenia caryophyllus			
Oil	1	100	4
	2	100	2
Eugenol	1	100	4
	2	100	2
Non-volatile	1	25	24
	2	55	24
Decaspermum momtanum			
Oil	1	100	11
	2	100	11
Non-volatile	1	0	
	2	0	
Cymbopogon citratus			
Oil	1	25	24
	2	35	24
Non-volatile	1	0	
	2	0	
Ethyl acetate (control)		0	

Time = the length of period taken to achieve the mortality of the insects.
Twenty insects were used in each test.

pounds were found to be the major components of the essential oils of *M. arvensis* and *E. caryophyllus*, respectively.

The bioassay on the residue (non-volatile fraction) of the three plant extracts did not indicate any toxicity effect on the test insects. However, the test on the extract of *E. caryophyllus* showed some degree of insecticidal activity within the test period of 24 h.

Piscicidal Activity Test of Plant Extracts

Alkaloid from *Isotoma longiflora* was extracted following the procedure of Arthur and Chan (1963). A few piperidine alkaloids were isolated and characterized. Another alkaloid-containing plant, *Lindera pipericarpa*, was found to contain some aporphine alkaloids (Kiang and Sim 1967). Recent investigations on this plant species have also identified a few other alkaloids, of the same type (Lajis 1989).

The work on *Antidesma tomentosum* has indicated the presence of at least two alkaloids. The major product was identified as a peptide alkaloids, based on its spectroscopic data (Rahmani *et al.* 1989b).

The chemical composition of *Ocimum sanctum*, which is a non-alkaloidal plant, consists of essential oils, organic acids, fat and fatty acids, flavonoids and others (Ponglux *et al.* 1987). Our own investigation on the petroleum extract of the stem bark of this plant species has resulted in the isolation of a few terpenoids, which are still being characterized.

Two species of fish were chosen for this bioassay, namely *Labistes reticulates* (Cyprinodontiadae) and *Tilapia mossambica* (Cichlidae). They were selected because they were available in large quantities and could be handled easily for the testing.

The plant samples used consisted of the extracts of *Ocimum sanctum* (Labiatae), *Isotoma longiflora* (Campanulaceae), *Lindera pipericarpa* (Lauraceae) and *Antidesma tomentosum* (Euphorbiaceae). Isolation and structural studies of their chemical components were carried out in our department.

The bioassays were carried out at specific conditions, as recommended by the American Public Health Association (APHA 1985). The measured values for pH, temperature, dissolved oxygen (DO), and hardness of water were 6.8, 26°C, 7.2 mg/1 and 41 mg/ $CaCO_3$, respectively. The average lengths of *L. reticulates* and *T. mossambica* used in the assay were 2.5 cm and 7.0 cm, respectively.

The mortality of the fish was chosen as the measurable effect to determine the toxicity of the plant extracts. The LC_{50} for each plant sample was determined by plotting the graph of the concentrations of the plant extract against mortality percentage (Rahmani *et al.* 1989b). Any sample extract that gave a LC_{50} value above 5000 ppm was considered non-active. The results are summarized in Table 3.

The choice of the fish species used in the test depended on the availability of the fish at the time of the test. The results obtained from the piscicidal activity test show *I. longiflora* to have the highest toxicity value against *T. mossambica*, followed by *O. sanctum* and *L. pipericarpa*. It was also

TABLE 3

Plant samples	Experimental fish	LC_{50}(ppm)
O. sanctum (leaves)	*T. mossambica*	890
O. sanctum (flowers)	*T. mossambica*	1300
I. Longiflora (leaves)	*T. mossambica* *L. reticulates*	408 non-active
L. pipercarpa (leaves)	*T. mossambica* *L. reticulates*	4675 1565
A. tomentosum (leaves)	*T. mossambica* *L. reticulates*	non-active non-active

noted that the effects of extracts of *I. longiflora* and *L. pipericarpa* toward the two species of fish differed. *L. reticulates* showed more tolerance to the extract of *I. longiflora* than *T. mossambica*. On the other hand, the extract of *L. pipericarpa* showed a higher toxic effect on *L. reticulates* compared to *T. mossambica*. The toxicity effect of *A. tomentosum* on the two species of fish was very low and can be considered as non-active.

ACKNOWLEDGEMENTS

The authors wish to thank Omar Yusof and Shukur Hj. Ahmad for their participation in this project, Isharuddin Misron for technical help and JSPS for their support. This work was supported by IRPA Research Grant No. 50289.

REFERENCES

AMERICAN PUBLIC HEALTH ASSOCIATION. 1985. *Standard Method for the Examination of Water and Wastewater,* p 413.

ARTHUR, H.R. and R.P.K. CHAN. 1963. New Alkaloid from *Isotoma longiflora. J. Chem. Soc.* 750-751.

FORMACEK, V. and K.H. KURECZKA. 1982. *Essential Oils Analysis by Capillary GC and C-13 NMR Spectroscopy,* N. York: Wiley and Sons.

KIANG, A.K. and K.Y. SIM. 1967. Lindcarpine, an Alkaloid from *Lindera pipericarpa, J. Chem. Soc.* C. **4**: 282-283.

LAJIS, N.H., 1989. Structural and Chemical Studies of Some Malaysian Medical Plants, *In* Proc. Unesco Sub-Regional Seminar/Workshop on the Systematic Identification of Natural Products, UKM, 13-17 June 1988, 172-185.

NAKANISHI, K. S-I. SASAKI, A.J. KIANG, J. GOH, H. KAKISAWA, M. OHASI, M. GOTO, J.M. WATANA, H. YOKOTANI, C. MATSUMURA and M. TOGASUI. 1965. Phytochemical Survey of Malaysian Plants. Preliminary Chemical and Pharmacological Screening, *Chem. Pharm. Bull.* **13(7)**: 882-890.

OLIVEROS-BELARDO, L and E. AUREUS. 1977. Essential Oil from *Cymbopogon citratus. In* Int. Congr. Essent. Oils, 7th 1977, 7, 166-168; C.A. 1977, Vol. 92, 37768.

PONGLUX, D., S. WONGSERIPIPATANA, T. PHADUNGCHAROEN, N. RUANGRUNGSRI and K. LIKHITWITAYAWUID. (Eds.). 1987. *Medicinal Plants.* Victory Power Point Corp. Ltd., Bangkok.

RAHMANI, M., T.Y. LEONG and N.H. LAJIS. 1989a. Toxicity Studies of Plant Extracts on Two Species of Fish. *Pertanika* **12(2)**: 189-191.

RAHMANI, M., M.A. SUKARI and H. ISMAIL. 1989b. The Alkaloidal Constituents of Malaysian Plants. *In: Proc. Unesco Sub-Regional Seminar/Workshop on the Systematic Identification of Natural Products,* UKM, 13-17 June 1988. 149-153.

SUKARI, M.A. and S. TAKAHASHI. 1988. Biological Activity of Some Malaysian Plant Extracts. *Pertanika* **11(2)**: 249-253.

YU, J. and H. FANG. 1981. Studies on the Essential Oils of Clove Buds and Clove Leaves. *Shongcaoyao* **12(8)**: 340-342; C.A., 1981, Vol. 96, 139645.

(Received 25 June, 1991)