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Biological Activity of Some Malaysian Plant Extracts

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ABSTRAK

Ekstrak yang diperoleh daripada tiga tumbuh-tumbuhan Labiate Malaysia, termasuk Ocimum sanctum, Mentha arvensis *dan* Ortoshipon staminea, *telah dikaji keaktifan biologinya. Pecahan mudah meruap daripada setiap tumbuhan telah dipisahkan dan komponen utamanya telah dicirikan dengan kromatografi gas (KG), kromatografi gas-spektrum jisim (KG–SJ) dan resonans magnet nukleus (RMN). Kesan daripada setiap pecahan mudah meruap dan sisa ekstrak terhadap keaktifan antibakteria dan antifungi, keaktifan keracunan serangga dan penghalangan percambahan biji benih dikaji.*

ABSTRACT

Extracts obtained from three Malaysian Labiatae plants, including Ocimum sanctum, Mentha arvensis *and* Orthoshiophon staminea, *were investigated for their biological activity. The volatile fraction of each plant was isolated and the major components were characterized by gas chromatography (GC), gas chromatography-mass spectrometry (GC–MS), and nuclear magnetic resonance (NMR). The antibacterial and antifungal activity, insecticidal activity and inhibition of the germination of seeds of the volatile fraction and residue were studied.*

INTRODUCTION

Malaysia has about 10,000 species of seed plants and 1000 species of seedless plants (Latif, 1985). Of these at least 15% have been claimed to have some medicinal uses (Latif *et al.*, 1984). The last decade has witnessed a resurgence of interest and research in plant products, especially in those which show some biological activity. Active phytochemical screening programmes have been carried out by Carrick *et al.* (1968); Chan and Teo (1969, 1972); Chan *et al.* (1977). More recently, survey on alkaloid, saponin and triterpenes/steroid contents of plants collected from various parts of West and East Malaysia have been conducted (Rahmani *et al.*, 1985; Lajis *et al.*, 1985). Several studies on the pharmacological screening of biologically active ingredients in plants have also been reported (Nakanishi *et al.*, 1965; Yeoh, 1983). This paper discusses the result of antibacterial and antifungal activity test, insecticidal activity test and inhibition of the germination of seed test of three Malaysian Labiatae plant extracts.

MATERIALS AND METHODS

Extraction

The air-dried leaves of each plant were immersed in methanol for several days, filtered and extracted with ethyl acetate. The volatile fraction was steam-distilled to separate the essential oil, while the residue was extracted with ethyl acetate. The oil of each species was purified by High Performance

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Liquid Chromatography (HPLC) (in case of *O. santum* and *M. arvensis*) and analyzed by CG–MS to characterize the major components. The extract (oil and residue) of each plant was then subjected to bioassay.

Bioassay

Antifungal and Antibacterial Test. Different concentrations of oil and residue of each extract were tested against *Colletotrichum lagenarium* (Passerini) Ellis et Halsted, *Pyricularia oryzae* Cavara, *Cochliobolus miyabeanus* (Ito et Kuribayashi) Drechsler ex Dastur, *Trichophyton mertagrophytes* (Robin) Blanchard and *Bacillus subtilis* (Ehrenberg) Cohn by using the standard Petri dish method. All the fungi and a bacterium used in the tests are from a stock culture of the Pesticide Research Institute, Kyoto University. A pulp disc (8 mm diameter) impregnated with sample (at specific concentrations) was put into fungal or bacterial culture in agar medium in a Petri dish and left for 24 hours at 24°C (for fungi) and 37°C (for bacteria). Benzylcornium chloride was used as the control, and the size of the clear zone around the disc was compared as shown in Table 1.

Inhibition of the Seed Germination Test. Rice seeds (*Oryza sativa,* variety 'tanginbouzu'), which lack the gibberellin synthesis pathway were used for seed growth and root elongation test. Ten rice seeds were placed on filter paper soaked with plant extract (solution containing 0.5 mg and 5 mg of sample) inside a bottle (35 mm i.d. and 100 mm high). Water (1 ml) was added to the bottle, covered and kept in the dark at 30°C for 3 days. Seeds grown with gibberellin solution were used as control. The germination of each seed was determined by observing the appearance of both shoot and root.

Insecticidal Activity Test. The insecticidal activity of each plant extract was tested against German cockroach (*Blattella germanica* L.) (first instar nymph) and red bean weevil (*Callosobruchus chinensis* L.) (adult). The extract (10 mg) in ethyl acetate was sprayed onto the bottom of cylindrical flasks (area of 96.8 cm^2) and evaporated off the solvent. The test insects (about 20) were kept in the treated flasks and monitored for 72 hrs. to observe for mortality of the insects. The control test was carried out without sample treatment.

Gas Chromatography (GC)

Gas chromatography was conducted using a Hewlett Packard chromatograph on a capillary column (OV – 101, 0.3 mm i.d., 25 m long) equipped with a flame ionization detector (F1D). The column temperature was programmed from 80° to 240°C at 5°/min. Helium was used as the carrier gas at a flow rate of 50 ml/min.

Gas chromatograph-mass spectrometry (GC–MS)

Mass spectra were measured using a mass spectrameter HITACHI M–90 combined with a gas chromatograph.

High Performance Liquid Chromatography (HPLC)

The oils of *O. sanctum* and *M. arvensis* were purified by HPLC on silicic acid column (Nucleosil 8 mm x 30 cm) eluting with 6% ether in hexane (5 ml/min), and the major components were separated.

Nuclear Magnetic Resonance (NMR) spectra were recorded on a JEOL FX–9OQ for solutions in $CDCl_3$ with TMS as internal standard.

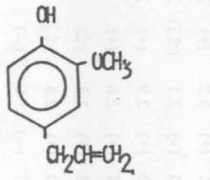
Infrared (IR) analysis was performed as thin films on a Beckman IR Acculab 7 spectrophotometer.

RESULTS AND DISCUSSION

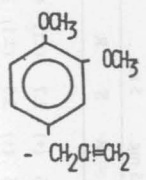
GC–MS analysis of essential oil of *O. sanctum* showed the presence of methyleugenol (2) as the main constituent (86% of the steam-distillate), eugenol (1) (0.5%) and β–caryophyllene (3) (3%) by comparison of their retention time (capillary GC on OV–101) with the authentic samples. The presence of methyleugenol (2) in *O. sanctum* and its attractiveness to 9 species of *Dacus* has already been reported (Tan, 1982). The steam distillate of *O. sanctum* containing methyleugenol is a good source of *Dacus* attractant.

Similar investigations on the soil of *M. arvensis* have resulted in the characterization of piperitenone oxide (4) as the major component (*ca* 87% of the volatile) on the basis of its ^1H NMR and IR data, which were in agreement with the literature (Yukawa and Ito, 1973). Among many cultivars of *M. arvensis,* there are many different varieties that produce menthol, menthone, piperitenone oxide and piperitone oxide as the major terpene compound (Ikeda *et*

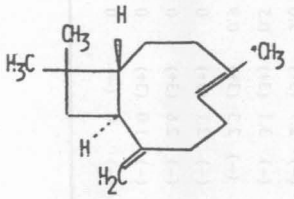
al., 1971). Malaysian *M. arvensis* belongs to a variety which produce piperitenone oxide as the major terpene but the origin of the variety was not identified. From the volatile fraction of *O. staminea*, methyleugenol (2) (3%) eugenol (1) (8.3%) and β– caryophllene (3) (2.2%) were confirmed as the essential oil constituents. Other constituents of the oil were left unidentified.



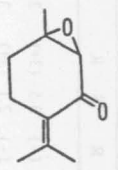
EUGENOL (1)



METHYLEUGENOL (2)



β-CARYOPHYLLENE (3)



PIPERITENONE OXIDE (4)

The results from antibacterial and antifungal assay shown in Table 1 indicate that *O. sanctum* and *M. arvensis* extracts exhibit some degree of inhibitory activity to *T. mertagraphytes* and *B. subtilis* growth at the concentrations above 10^4 ppm. On the other hand, no effect was observed on three other organisms used in the test. *Ocimum gratissimum* which contains high amount of eugenol (1) (81.24% of essential oil) has been shown to be fungitoxic to betelvine pathogenic fungi (*Alternaria alternata, Colletotrichum capsici* and *Sclerotium rolfsii*) at the concentration of 100 ppm (Tripathi *et al.*, 1985). This has been attributed to the presence of eugenol as the main component.

The results in Table 2 show that essential oil from *M. arvensis* strongly inhibited the germination of rice whereas oil from *O. santum* showed a slight activity at the higher concentration. Inhibitory activity of piperitenone oxide and piperitone oxide toward lettuce seed germination has been reported (Shimizu, 1986). The inhibitory activity of *M. arvensis* essential oil in the present study could most probably be due to the presence of piperitenone oxide too. A parallel experiment has demonstrated that piperitenone oxide separated from oil of *M. arvensis* inhibited the germination of *Phaseolus aureus* seed (Yusof, 1986).

As for the insecticidal activity, the contact toxicity test indicated that application of 10 mg each of the essential oil from *O. sanctum* and *M. arvensis* showed 100% mortality in *B. germanica* and *C. chinensis* within 24 hrs, (about 20 insects were used in each test). On the other hand, the oil from *O. staminea* and the residues of the three plant extracts investigated did not indicate any toxicity effect on the test insects.

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TABLE 1.

Antifungal and antibacterial assay of plant extract at various concentrations.

Fungus & Bacterium	Concentration (ppm)																			
	Ocimum sanctum							*Mentha arvensis*								*Orthoshiphon staminea*				
	Oil				Residue			Oil				Residue				Oil			Residue	
	$5x10^2$	10^3	10^4	10^5	$5x10^2$	10^3	10^4	$5x10^2$	10^3	10^4	10^5	$5x10^2$	10^3	10^4	10^5	$5x10^2$	10^3	10^4	$5x10^2$	10^3	10^4
Trichophyton mertagrophytes	–	–	*	±	–	–	*	–	–	*	*	–	–	*	*	–	–	*	–	–	*
Bacillus subtilis	–	–	±	++	–	–	±	–	–	±	+	–	–	+	++	–	–	–	–	–	–

– : clear zone was not observed. ± : Clear zone less than 10 mm (width). + : Clear zone *ca.* 10 mm (width). ++ : Clear zone *ca.* 15 mm (width).

* : Not tested.

TABLE 2.

Seedling growth (S, cm) and root elongation(R) of *Oryza sativa* grown on plant extract.

Control				*O.sanctum*								*M. arvensis*								*O. staminea*							
AcOEt		GA-3		Oil				Residue				Oil				Residue				Oil				Residue			
				0.5 mg		5 mg		0.5 mg		5 mg		0.5 mg		5 mg		0.5 mg		5 mg		0.5 mg		5 mg		0.5 mg		5 mg	
S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R
2.5	(3+)	15.5	(3+)	0.4	(+)	0	(–)	2.3	(3+)	3.2	(3+)	0	(–)	0	(–)	3.5	(3+)	2.5	(2+)	3.6	(+)	2.7	(±1)	4.0	(3+)	3.1	(2+)
2.3	(3+)	16.1	(3+)	1.7	(+)	0	(–)	2.6	(3+)	2.4	(3+)	0	(–)	0	(–)	2.1	(3+)	3.2	(2+)	3.5	(+)	3.5	(±1)	3.6	(3+)	3.1	(2+)
2.2	(3+)	16.0	(3+)	0.6	(+)	0	(–)	2.4	(3+)	2.8	(3+)	0	(–)	0	(–)	2.4	(3+)	2.9	(+)	3.5	(+)	3.2	(±1)	2.9	(3+)	2.9	(2+)
2.6	(3+)	15.0	(3+)	0.7	(+)	0	(–)	2.9	(3+)	2.4	(3+)	0	(–)	0	(–)	2.7	(3+)	3.0	(2+)	3.1	(+)	3.2	(+)	2.8	(3+)	2.9	(2+)
2.7	(3+)	16.0	(3+)	0.8	(+)	0	(–)	2.7	(3+)	2.8	(3+)	0	(–)	0	(–)	3.1	(3+)	0.5	(+)	3.0	(+)	3.1	(±1)	2.7	(3+)	2.5	(2+)
2.7	(3+)			0.9	(+)	0	(–)	3.0	(3+)	2.1	(3+)	0	(–)	0	(–)	2.3	(3+)	0.9	(–)	2.0	(+)	2.9	(+)	2.4	(3+)	2.0	(–)
2.8	(3+)			0.6	(+)	0	(–)	2.6	(3+)	2.3	(3+)	0	(–)	0	(–)	2.1	(3+)	0	(–)	1.8	(+)	2.6	(+)	0	(–)	1.0	(+)
3.0	(3+)			0.8	(+)	0	(–)	2.8	(3+)	2.6	(3+)	0	(–)	0	(–)	2.6	(3+)	0	(–)	1.2	(+)	2.5	(±)	0	(–)	0	(–)
2.7	(3+)			0	(–)	0	(–)	2.9	(3+)	2.7	(3+)	0	(–)	0	(–)	1.0	(3+)	0	(–)	0	(+)	2.5	(±)	0	(–)	0	(–)
3.2	(3+)			0	(–)	0	(–)	2.4	(3+)	2.5	(3+)	0	(–)	0	(–)	0	(–)	0	(–)	0	(–)	0	(–)	0	(–)	0	(–)

(3+) : Clear elongation observed.

(–) : No elongation observed.

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