Notes

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Phytochemical Survey of Malaysian Plants Preliminary Chemical and Pharmacological Screening.

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Many Malaysian plants that have been used in medicine by the local people are described in Burkill's "Dictionary of the Economic Products of the Malay Peninsula."[1] Further information on Malaysian medicinal and poisonous plants can be found in "Malay Poisons and Charm Cures,"[2] "On Chinese Medicine,"[3] "Malay Village Medicine,"[4] and "The Medical Book of Malayan Medicine."[5]

Douglas and Kiang,[6] Kiang, *et al.*[7] and Amarasingham, *et al.*[8] have recently reported the results of screening Malayan plants for the presence of alkaloids, while Lin[9] has investigated the nature of certain Malaysian dart poisons.

In this paper we report the results of preliminary pharmacological tests carried out on the crude extracts of a number of Malayan and Singapore plants comprising 89 species, 82 genera and 40 families. The extracts were tested for antimicrobial activity, toxicity, and antitumor activity.

We have also taken the opportunity of carrying out spot tests for the presence of alkaloids, phenolic substances, amino acids and peptides, reducing sugars, and acidic and basic substances. These tests were made after the extracts had been subjected to filter paper or thin-layer chromatography.

The experimental methods are described below and the results are summarised in Table I.

Experimental Methods

1. Preparation of Crude Extracts

The plants were collected in Singapore and the west and southwest coast of Malaya from December

*1 Katahira-cho, Sendai (中西香爾, 佐々木慎一).
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*4 Juso-nishinocho, Higashiyodogawa-ku, Osaka (後藤 実, 渡辺順明, 横谷 肇, 松村 親, 富樫 誠).

1) I. H. Burkill: "Dictionary of the Economic Products of the Malay Peninsula," Vol. I and II, Government Printing Press, Singapore, 1935.
2) J. D. Gimlette: "Malay Poisons and Charm Cures," Churchill, 3rd edition, London, 1929.
3) D. Cooper: "Gardens Bulletin," 1929~1930, 6, 1, Government Printing Press, Singapore (reprinred in 1960).
4) I. H. Burkill and M. Haniff: *Ibid.*, 167.
5) Translated by Inche' Ismail Munshi: *Ibid.*, 333.
6) B. Douglas and A. K. Kiang: Malayan Pharmaceutical Journal, **6**, 138 (1957).
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8) D. Amarasingham, N. G. Bisset, A. H. Millard, M. C. Woods: Economic Botany, **18**, 270 (1964).
9) R. C. Y. Lin: Proc. Alumni Assoc., Malaya, **4**, 281 (1951); Brit. J. Pharmacol., **10**, 247 (1955), **12**, 265 (1957). R. C. Y. Lin and G. C. Whittow: *Ibid.*, **15**, 440 (1960).

TABLE I. Results of Screening (50% methanol extracts)

Sample No.	Plant	Part	Yield of extract from powder (%)	Chemical test			Biological test						
							Anti-microbial				Acute toxicity		Antitumor (Ys)
				D	L	Fe	*B. subtilis*	*S. aureus*	*E. coli*	*P. vulgaris*	Estimated LD_{50} mg/kg	Symptom	
	Acanthaceae												
1	*Acanthus ilicifolius* LINN.	L-S	9.4	−	−	+	−	−	−	−	500～1000		−
2		L	27.7	−	−	+	−	−	−	−	>1000		−
3		S	10.0	±	−	−	−	−	−	−	>1000		−
4	*Andrographis paniculata* NEES	W	17.9	+	±	+	+	∓	−	+	>1000		±
5	*Jacobinia coccinea* HIERN	L	19.2	−	±	±	+	+	−	−	>1000		−
6		S	12.8	±	−	+	+	+	−	+	500～1000		±
	Anacardiaceae												
7	*Melanorrhoea woodsiana* SCORT.	L	19.3	±	−	+	+	+	−	+	250～500		±
8		S	16.5	+	−	−	−	−	−	−	>1000		−
	Anisophylleaceae												
9	*Anisophyllea disticha* BAILL.	L	16.4	−	−	±	+	+	+	+	>1000		−
	Anonaceae												
10	*Artabotrys suaveolens* BLUME	L-Fl	7.4	±	−	+	−	−	−	−	62.5～125	b, c	±
11		L	13.3	−	−	+	+	+	+	+	500～1000		±
12		S-L	8.0	−	−	+	+	+	−	−	500～1000		±
13		S	4.6	±	−	+	−	−	−	+	250～500	c	−
14	*Canangium odoratum* BAILL.	L	28.7	±	+	±	+	+	−	−	>1000		−
15		Ba	16.5	+	+	+	+	+	−	−	>1000	b	−
16		S	19.9	±	±	+	−	−	−	−	125～250		−
17	*Desmos chinensis* LOUR.	〃	8.8	±	−	+	+	+	−	−	500	a	−
18		L	19.4	−	−	+	+	+	−	−	500～1000		−
	Apocynaceae												
19	*Allamanda neriifolia* HOOK.	L	12.2	−	±	+	−	+	−	+	>1000		−
20	*Cerbera Odollam* GAERTN.	S	8.7	±	+	−	−	−	−	−	>1000	c	−
21		L-S	16.7	−	+	+	−	−	−	+	>1000		−
22	*Dyera laxiflora* HOOK. f.	〃	13.3	+	+	±	−	−	−	−	>1000		−
23	*Kopsia singapurensis* RIDLEY	S	5.6	+	−	−	−	−	−	−	>1000		−
24	*Plumeria obtusa* LINN.	Ba	14.7	±	+	−	+	+	−	−	>1000		±
25		L-S	14.7	±	−	−	+	+	−	+	>1000	c	−
26	*Rauwolfia perakensis* KING et GAMBLE	S	4.0	+	−	−	−	−	−	−	>1000		−

	Araceae											
27	*Epipremnum giganteum* Schott	Ba	15.9	−	−	−	−	−	−	−	>1000		−
28		S	4.5	±	−	±	−	−	−	−	>1000	b, d	−
	Asclepiadaceae											
29	*Calotropis gigantea* Ait.	S	12.0	+	±	−	−	−	−	−	500～1000		−
30	*Dischidia Rafflesiana* Wall.	P	16.2	±	−	+	+	−	−	−	>1000		−
31		L-S	10.3	+	±	+	+	+	−	−	>1000	b	−
32	*Finlaysonia maritima* Backer	〃	5.7	±	±	±	−	−	−	−	>1000		−
	Campanulaceae											
33	*Isotoma longiflora* Presl.	L-S	19.0	+	−	±	−	−	−	+	>1000		−
34		W	16.5	+	−	+	−	+	−	−	>1000		−
	Celastraceae											
35	*Salacia prinoides* DC.	S	9.0	±	−	±	−	+	−	−	125～250	c	−
36		L	27.9	±	−	+	−	+	−	+	1000	〃	−
	Compositae											
37	*Wedelia biflora* DC.	L-S	28.5	−	−	+	+	+	−	−	250～500	a	−
	Dilleniaceae											
38	*Dillenia indica* Linn.	F	10.8	−	−	±	−	−	−	−	500～1000	b	−
	Ebenaceae											
39	*Diospyros discolor* Willd.	L-S	23.4	+	−	+	+	+	−	+	125～250	d	±
40		S	13.2	−	−	+	+	+	−	+	125～250		±
	Euphorbiaceae											
41	*Bridelia ovata* Decne.	S	1.3	±	+	−	−	−	−	−	>1000		−
42		L	9.1	±	±	−	+	−	−	+	>800		−
43		F	9.6	+	+	+	+	+	−	+	500～1000		±
44	*Croton Joufra* Roxb.	W	9.5	+	−	+	−	−	−	−	500～1000	b	−
45	*Emblica officinalis* Gaertn.	L	15.9	+	±	+	+	+	+	+	500～1000		±
46		S	4.6	+	−	+	+	+	−	+	125～250	a, b, c	±
47	*Euphorbia antiquorum* Linn.	〃	8.0	±	−	−	+	+	−	+	>1000	b	−
48	*Euphorbia Tirucalli* Linn.	〃	8.1	−	+	−	+	+	−	−	1000	a	−
49	*Exocoecaria Agallocha* Linn.	Ba	13.9	−	−	+	‡	+	−	+	15.6～31.2		±
50		L-S	30.0	−	−	±	+	+	−	−	>1000		±
51	*Macaranga triloba* Muell.-Arg.	L	19.4	−	−	+	+	+	+	+	>1000		±
52		Ba	13.9	−	−	±	+	+	−	+	62.5～125		±
53		S	6.9	+	−	±	−	+	−	−	500～1000		±
	Goodeniaceae											
54	*Scaevola frutescensk* Krause	S	22.5	−	+	+	−	−	−	+	>1000		−
55		L-S	19.2	±	−	+	−	−	−	−	>1000		−
	Gramineae											
56	*Lophatherum gracile* Brongn.	S	5.2	−	−	−	+	+	−	−	>1000		−
57		R	3.9	−	−	−	−	−	−	−	>1000		−

	Gnetaceae												
58	*Gnetum latifolium* Blume	L	11.4	±	−	+	−	−	−	+	>1000		±
59		S	18.4	−	−	+	+	+	−	+	>1000		±
60		Wd	19.0	+	±	+	−	−	−	+	>1000		−
	Guttiferae												
61	*Mesua ferrea* Linn.	L	15.8	±	−	+	+	+	−	−	500～1000	a	−
	Labiatae												
62	*Leucas zeylanica* R. Br.	W	12.4	±	−	±	−	+	−	−	>1000		−
	Leguminosae												
63	*Acacia auriculaeformis* A. Cunn.	R-Ba	14.3	±	−	+	+	++	−	+	500～1000	a	−
64		L	23.8	±	−	±	+	+	−	−	250～500	b	±
	Leguminosae (contd.)												
65	*Andira surinamensis* Splitg.	Fl	27.8	+	±	−	−	−	−	−	500～1000	b	±
66		L	13.7	−	−	+	+	−	−	−	125～250	a	±
67	*Cassia siamea* Lam.	〃	7.9	−	+	+	+	+	−	+	>1000		−
68		Ba	12.7	−	+	±	−	−	−	−	>1000		−
69	*Crotalaria striata* DC.	L-F	5.1	+	+	+	+	−	−	−	>1000	d	−
70	*Delonix regia* Rafin.	L	18.7	−	+	+	−	−	−	−	>1000		±
71	*Indigofera hirsuta* Linn.	W	6.8	−	±	+	−	−	−	−	31.2～62.4	b	±
72	*Mimosa sepiaria* Benth.	Ba	19.2	+	−	−	+	+	−	+	250～500	a, b	±
73		L	21.9	−	+	±	+	+	+	+	>1000		±
74	*Pithecellobium jiringa* Prain	S	8.2	+	−	+	+	+	−	−	125～250		−
75	*Swartzia pinnata* Willd.	〃	3.2	+	±	±	−	−	−	−	250～500	c	−
	Loganiaceae												
76	*Fagraea fragrans* Roxb.	L	15.1	−	+	−	−	−	−	+	>1000		−
77	*Gelsemium elegans* Benth.	S	7.5	+	−	−	+	+	−	−	25	a	−
	Loranthaceae												
78	*Loranthus pentandrus* Linn.	L-S	16.7	−	−	+	−	+	−	+	62.5～125	a, b	−
	Lythraceae												
79	*Lagerstroemia speciosa* Pers.	F	10.1	−	−	+	−	−	−	+	500～1000		±
80	*Lawsonia inermis* Linn.	L-S	18.4	−	−	+	++	++	−	+	500～1000	a	±
	Malpighiaceae												
81	*Galphimia glauca* Cav.	S	1.5	±	−	+	−	−	−	−	500	a	±
82		L	22.4	−	+	+	+	+	−	−	500～1000		±
	Malvaceae												
83	*Sida rhombifolia* Linn.	S	2.5	±	−	±	−	−	−	−	>1000	d	−
84		L-S	4.5	±	−	+	−	−	−	−	>1000		−
	Meliaceae												
85	*Carapa guianensis* Aubl.	L	22.7	−	±	+	+	−	−	−	31.2～62.5		±
86		Ba	24.5	±	−	+	−	+	−	+	500～1000		±
87		F	6.7	−	−	+	+	+	−	−	500～1000		±
88		Se	14.1	+	−	+	−	−	−	−	>1000		±

	Meliaceae (Contd.)											
89	*Swietinia macrophylla* King	L	20.6	−	−	+	−	+	−	−	15.6～31.2	±
90		F	6.7	±	+	±	−	+	−	+	>1000	−
91		Se	16.2	+	+	±	−	−	−	−	>1000	±
	Menispermaceae											
92	*Coscinium wallichianum* Miers	S	8.4	+	−	±	+	+	−	−	125～250 a	−
93	*Fibraurea chloroleuca* Miers	L	10.2	+	±	+	−	−	−	−	>1000	±
94		Ba	18.8	−	−	+	−	+	−	−	31.2～62.5 c	±
	Moraceae											
95	*Sloetia elongata* Koord.	L	13.6	±	±	+	−	−	−	−	500～1000 a, d	−
	Myristicaceae											
96	*Knema Hookeriana* Warb.	L-S	7.1	−	±	±	−	−	−	−	500～1000	±
	Myrsinaceae											
97	*Ardisia elliptica* Thunb.	L	19.5	−	−	+	+	+	−	−	125～250 d	±
98		Ba	24.2	+	−	+	−	+	−	+	250～500	±
99	*Maesa ramentacea* Wall.	L-F	18.6	±	+	+	+	+	−	−	250～500 b	−
100		S	5.8	±	−	±	+	+	−	+	500～1000 〃	±
101	*Myrsine capitellata* C. B. Clarke	Ba	10.2	±	−	±	+	+	−	+	500～1000 a	±
	Myrtaceae											
102	*Eugenia javanica* Lam.	S	4.0	−	−	+	−	−	−	−	>1000	±
103		L	7.8	−	−	+	+	+	−	−	31.3～62.5 a, b	±
	Nepenthaceae											
104	*Nepenthes arbo-marginata* Lobb.	L-S	13.1	±	−	+	++	+	−	+	250～500	±
	Orchidaceae											
105	*Dendrobium crumenatum* Sw.	W	3.8	+	+	±	+	+	−	−	>1000 c	−
	Rubiaceae											
106	*Gardenia carinata* Wall.	L	27.7	±	−	+	++	++	−	+	1000	−
107		S	12.1	−	−	±	++	+	−	−	>1000	−
108		Ba	20.7	+	+	+	++	+	−	−	>1000	−
109	*Genipa americana* Linn.	L-S	13.9	±	+	±	−	+	−	−	>1000	−
110		F	27.9	−	−	+	+	+	+	+	>1000	±
	Rubiaceae (Contd.)											
111	*Hedyotis capitellata* Wall.	L-S	13.2	±	−	+	−	+	−	+	500～1000 b	−
112		S	8.0	+	+	−	−	+	−	−	500～1000	−
113	*Lucinaea morinda* DC.	L-S	13.5	−	−	+	+	+	−	−	500	−
114		V	13.4	±	+	±	+	+	+	+	31.3～62.5	−
115	*Morinda citrifolia* Linn.	L	39.7	−	+	+	−	+	−	−	>1000	−
116		F	21.2	−	+	±	−	+	−	−	>1000	−
117	*Mussaenda glabra* Vahl.	L	5.9	±	−	+	−	−	−	−	250～500 d	±
118	*Randia densiflora* Benth.	F	22.0	−	+	+	−	−	−	−	500～1000 a	±
119		L	27.7	−	+	+	−	−	−	−	125～250 b	±
120		S	11.0	+	+	−	−	−	−	−	>1000	±

121	*Randia macrantha* DC.	S	3.4	+	+	−	−	−	−	−	>1000		−
122		L	18.9	+	±	+	+	−	−	+	>1000		−
123	*Randia spinosa* Poir.	S	10.0	±	+	−	−	−	−	−	62.5～125		±
124	*Timonius Wallichianus* Valet.	L-S	9.4	±	+	−	−	−	−	−	125～1000	a	±
125	*Uncaria longifolia* Merrill	〃	17.0	±	+	+	+	+	−	−	62.5～125	b, c	±
126	*Uncaria pteropoda* Miq.	L	2.5	−	−	+	−	−	−	−	>1000		−
127		R	1.6	+	−	−	−	−	−	−	>1000	a	−
128		S	9.8	+	−	+	+	−	−	−	15.6～31.2		±
129		Se	11.1	+	±	±	−	+	−	−	31.2～62.5		−
130	*Uncaria Roxburghiana* Korth.	Wd	26.1	−	+	−	+	+	−	+	62.5～125		±
	Rutaceae												
131	*Evodia Roxburghiana* Benth.	L	11.5	−	−	+	+	+	−	+	>1000		±
132		Ba	11.3	−	±	+	+	+	−	+	62.5～125	d	−
133	*Glycosmis pentaphylla* Correa	L-S	15.3	+	−	−	+	−	−	−	>1000		−
134		S	4.0	±	−	±	−	−	−	−	>1000		−
135	*Murraya paniculata* Jack	L	30.0	+	−	+	+	+	−	−	>1000		−
136		Ba	12.0	+	±	−	−	−	−	−	>1000		−
	Sapotaceae												
137	*Achras Sapota* Linn.	S	11.5	+	−	+	−	+	−	−	250～500		±
138		L	17.8	±	−	+	+	+	−	+	250～500		±
	Soranaceae												
139	*Lycium chinense* Mill.	S	3.4	+	±	±	−	−	−	+	>1000		−
140	*Solanum torvum* Sw.	L-S, F	11.1	+	±	±	−	−	−	−	>1000	b	−
141		S	3.6	±	+	+	+	+	−	−	>1000		±
	Sterculiaceae												
142	*Cola nitida* Schott et Endl.	S	2.8	±	−	−	−	−	−	−	31.2～62.5		±
143		L	11.8	−	−	−	−	−	−	−	250～500	d	±
	Tiliaceae												
144	*Grewia tomentosa* Juss.	L	9.5	±	±	+	−	+	−	−	>1000		−
145		S	5.7	+	−	+	+	+	−	−	62.5～125	c	−
	Turneraceae												
146	*Turnera ulmifolia* Linn.	W	8.9	−	−	±	−	+	−	−	250～500	d	−
	Verbenaceae												
147	*Avicennia alba* Blume	L	25.0	±	±	+	+	+	−	−	>1000		−
148		S	14.1	+	±	±	±	±	−	−	>1000		−
149		F	53.6	−	+	+	+	+	−	−	>1000		−
150	*Clerodendron laevifolium* Blume	S	14.3	+	±	±	−	+	−	−	>1000		−
151		L-S	25.8	±	±	+	+	+	−	−	>1000	b	−

Plant Part. L: leaves; S: stems; L-S: leaves and stems; Wd: wood; W: whole; Ba: barks; R-Ba: root barks; Fl: flowers; P: pitchers; F: fruits; Se: seeds; V: vines.
Reagent. D: Dragendorff; L: Liebermann-Burchard; Fe: $FeCl_3$.
Symptoms. a: tonic and clonic convulsion; b: diarrhoea; c: tail erection; d: exophthalmus

1961 to March 1962. Whenever possible, herbarium specimens were prepared and deposited at Herbaria in Research Laboratories, Takeda Chemical Industries, Ltd. The various parts of each plant were air dried and ground to a fine powder, and a 300 g. portion of the powdered plant material was extracted three times with 700 ml. of 50% (v/v) aqueous methanol by refluxing for one hour on a water bath kept at 80°. The combined filtrates were concentrated *in vacuo* to dryness to give the crude extract.

2. Preliminary Chemical Tests

a) Filter paper chromatography. About 5 μl. of a solution of the crude extract in methanol (ca. 5 mg./ml.) was applied on Toyo Roshi filter paper No. 51 (7 cm. × 30 cm.) at 2 cm. intervals. After drying, the chromatograph was developed according to the method of Sasakawa,[10] by employing a 4:1:5 mixture of butanol–acetic acid–water.

Each specimen was appiled to separate strips for spraying with the following reagents: (i) Dragendorff (alkaloids); (ii) 2% aqueous $FeCl_3$ solution (phenolics); (iii) ninhydrin (amino acids and peptides); (iv) aniline hydrogen phthalate (reducing sugars); (v) 0.04% aqueous ethanol solution of bromocresol green (acidic and basic substances).

However, since most samples gave positive reactions with the ninhydrin, aniline hydrogen phthalate, and bromocresol green reagents, the results of these nonspecific reagents are omitted from Table I.

b) Thin-layer chromatography. Moist silica gel was spread over a glass plate (7 cm. × 20 cm.) which was then heated at 110° for 1 hr. About 5 μl. of the sample solution (25 mg./ml.) was applied in small spots at 2 cm. intervals and 3 cm. from one end of the plate. The plate was developed for about 40 min. with a mixture of 1:1 chloroform–methanol until the solvent front was 2 cm. from the top. After drying, the plate was sprayed with the following reagents: (i) Dragendorff reagent (alkaloids) and (ii) Liebermann–Burchard reagent (steroids).

3. Antimicrobial Tests

Each crude extract was dissolved in 30% (v/v) aqueous methanol, and the solutions were diluted to a concentration of 10 mg./ml. The inhibition of growth of the bacterium or fungus was assayed according to the agar streak-dilution method.[11] The strains of bacteria and fungi used were as follows:

No. 1 *Bacillus subtilis* PCI 219
No. 2 *Staphylococcus aureus* FDA 209P
No. 3 *Escherichia coli* IFO 3044
No. 4 *Proteus vulgaris* IFO 0337
No. 5 *Aspergillus niger* IFO 6342
No. 6 *Penicillium luteum* IFO 6345
No. 7 *Mucor spinescens* IFO 6350
No. 8 *Candida albicans* IFO 0601

Bacteria Nos. 1～4 were incubated on agar medium at pH 6.0 at 33° for 18 hr., while the fungi Nos. 5～8 were incubated on sucrose–potato–agar at pH 6.0 at 25° for 3 days. However, the results on the four fungi are not listed in the Table because almost all samples had no effect (see Conclusions).

The results of the assays are expressed by the following notations: Inhibition of growth at concentrations of 1～4.9 mg./ml. of the crude extract is expressed by (++); at concentrations of 5～10 mg./ml., (+); and no inhibition noticed at concentrations of 10 mg./ml., (−).

4. Toxicity Tests

Four-week old male mice (CF_1 strain) were employed. The crude extracts were made into 20 mg./ml. Acacia gum emulsions, which were injected intraperitoneally into three mice, the dosage being 0.5 ml./10 g. The symptoms were observed after 5～7 hr. and any deaths within 72 hr. were noted. When this dosage (equivalent to 1000 mg./kg.) proved fatal, the tests were repeated with different mice using a stepwise reduction of the dose to one half, one fourth, and so on, until no death was observed within 72 hr.

5. Antitumor Activity

Yoshida sarcoma cells (0.1 ml. or 10^7) were transplanted into the abdominal cavity of five-week old Donryu rats, each weighing about 100 g. The crude plant extracts were made into 200 mg./ml. Acacia gum emulsions, which were injected intraperitoneally (dosage, 0.5 ml./100 g. or 1000 mg./kg.) three days after the transplantation. If the rats died within 48 hr. of injection, the dosage was decreased in sequence as mentioned above.

After 6, 24, and 48 hr. of injection, a droplet of tumor ascites was aspirated off with a glass capillary. The Giemsa-stained smear was checked under a microscope, and morphological changes were followed.

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The extracts giving distinct cellular changes are assigned (+); those giving slight cellular changes (±); and those giving negative results (−).

Conclusion

The results are summarized in Table I and are discussed briefly below.

1. Chemical Tests

Almost all samples gave positive reactions with the ninhydrin, aniline hydrogen phthalate and bromorcresol green reagents. The results obtained with these reagents are considered non-specific and do not appear in the Table.

Forty-six samples showed a positive reaction towards the Dragendorff reagent. Those giving an especially strong reation were leaves, stems and whole plant of *Isotoma longiflora* PRESL (Campanulaceae), and the bark of *Coscinium wallichianum* MIERS (Menispermaceae).

Thirty-seven samples gave a positive Liebermann-Burchard reaction. The strongest reaction was observed with the stems of *Cerbera odollam* GAERTN. (Apocynaceae).

Eighty-five samples responded positively towards ferric chloride. The samples giving a strong reaction were leaves of *Diospyros discolor* WILLD. (Ebenaceae), leaves of *Macaranga triloba* MUELL.-ARG. (Euphorbiaceae), roots and bark of *Acacia auriculaeformis* A. CUNN. (Leguminosae), bark of *Carapa guianensis* AUBL. (Meliaceae), leaves of *Fibraurea chloroleuca* MIERS (Menispermaceae), leaves of *Mussaenda glabra* VAHL (Rubiaceae), and fruits of *Genipa americana* LINN. (Rubiaceae).

2. Antimicrobial Activity

The highest antimicrobial activity observed was (++), *i.e.* growth inhibition as concentrations of 1~4.9 mg./ml., in the samples shown in Table II.

TABLE II. Plant Extracts Having (++) Effect against Microbes

Active against *Bacillus subtilis*
Exocoecaria agallocha LINN. (Euphorbiaceae) (bark)
Lawsonia inermis LINN. (Lythraceae) (flower)
Nepenthes arbo-marginata LOBB. (Nepenthaceae) (leaves and stems)
Gardenia carinata WALL. (Rubiaceae) (leaves, stems, bark)
Avicennia alba BLUME (Verbenaceae) (stems) Active against *Staphylococcus aureus*
Acacia auriculaeformis A. CUNN. (Leguminosae) (root and bark)
Lawsonia inermis LINN. (Lythraceae) (leaves and stems)
Gardenia carinata WALL. (Rubiaceae) (leaves)
Avicennia alba BLUME (Verbenaceae) (leaves)

The only samples showing (+) activity (*i.e.*, at concentrations of 5~10 mg./ml.) against fungi are listed in Table III. None were active against *Asp. niger*.

TABLE III. Plant Extracts Having (+) Effect against Fungi

Stems of *Coscinium wallichianum* MIERS (Menispermaceae) : *P. luteum, C. albicans.*
Bark of *Fibraurea chloroleuca* MIERS (Menispermaceae) : *M. spinescens.*
Stems of *Solanum torvum* SW. (Solanaceae) : *M. spinescens, C. albicans.*

3. Toxicity

About half of the samples had an LD_{50} value exceeding 1000 mg./kg. Nineteen samples having an LD_{50} of less than 125 mg./kg. and, therefore, high toxicity are shown in Table I. The symptoms observed after injections were, in most cases, stretching,

increase of respiration rate, Ataxia, anorexia and crouching with pilo-erection. Besides these, 19 samples caused tonic and clonic convulsion, 21 caused diarrhoea, 12 caused tail erection and 10 caused exophthalmus.

4. Antitumor Activity

None showed conspicuous cellular activity against Yoshida sarcoma. However, 62 samples (41%) showed weak activity, which is represented by (±) in Table I. The cytological effects observed were destruction of tumor cells, appearance of vacuoles in cytoplasma and di- or poly-nuclear cells. A high rate of appearance (53 out of 62 samples) of di-nuclear cells was observed.

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Summary

Preliminary results of the screening of Malaysian and Singapore plants comprising 89 species, 82 genera and 40 families are reported. The extracts were tested for antimicrobial activity, toxicity and antitumor activity, and spot tests have also been carried out for testing the presence of alkaloids, phenolic substances, amino acids, reducing sugars, and acidic and basic substances.

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Preparative Methods of 4-Methoxy-2,6-dinitrophenol.

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The title compound (I) has been reported to be formed by the following methods: (i) the direct nitration[1~3] of 4-methoxyphenol, (ii) the methylation[2,3] of 2,6-dinitrohydroquinone with dimethyl sulfate in a alkaline solution, and (iii) the alkaline degradation[4] of a nitramine (II). However, the nitration (i) is much suffered from a oxidative side-reaction to give a black tar, the methylation (ii) is so difficult because of the instability of 2,6-dinitrohydroquinone in alkaline medium, and the degradation (iii) is not suitable for a practical preparation. We report now two preparative methods.

The first one is the nitration of 2-nitro-4-methoxyphenol (III) which is prepared from 1,4-dimethoxybenzene by the direction of Robinson and Smith.[5] Two step nitration

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