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Screening of Crude Drugs Used in Nepal for Nematocidal Activity on the Larva of *Toxocara canis*[1)]

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Hot water extracts of 160 crude drugs used in Nepal were tested for their *in vitro* nematocidal activity on the second-stage larva of the dog roundworm, *Toxocara canis*, which is a common pathogenic parasite in larva migrans. Twenty-two of them showed a potent nematocidal activity (RM = 0, after 24 h incubation), and the activities of "Bojho" (rhizome of *Acorus calamus* L.) and "Pan ko Jara" (rhizome of *Alpinia galanga* SWARTZ) were particularly strong (RM = 0, after 6 h incubation). The active principle of Pan ko Jara was identified as [*S*]-1′-acetoxychavicol acetate.

Keywords——nematocidal activity; larva; *Toxocara canis*; Toxocariasis; anthelmintic; larva migrans; *Acorus calamus*; *Alpinia galanga*; Zingiberaceae; 1′-acetoxychavicol acetate

The disease caused by the migration of larvae of animal parasites into human tissues such as liver, lungs, heart, brain, *etc.* is called larva migrans,[2)] and in recent years, such diseases have become a serious problem in public health. It is characterized by persistent hypereosinophilia, hypergammaglobulinemia, hepatomegaly and pneumonitis, and in some very serious cases, though rare, it can be fatal.[3)] Of a number of the causative parasites of this disease, *Toxocara* spp. are particularly important.[4)] As the immunodiagnostic tests have become to be more widely used,[5)] the number of reports on Toxocariasis has increased and more than 1,900 cases were reported in the world by 1981.[4)] In Japan, 37 cases were reported by 1986.[6)]

Although diethylcarbamazine and levamisole were reported to be useful for Toxocariasis,[7)] the effect is not satisfactory and no other anthelmintic is known to be effective against the parasitic diseases caused by nematodes in host tissues.

To find new anthelmintic agents which are effective against the parasites living in tissues, we conducted an *in vitro* screening test of crude drugs used in traditional medicines, in which samples were tested for their nematocidal activity on the larva of the dog roundworm, *T. canis*, a common pathogenic parasite of larva migrans. In our previous work, 160 Ayurvedic crude drugs available in Sri Lanka were tested by this screening system,[8)] of which 29 showed strong nematocidal activity. In the present study, crude drugs used in Nepal were tested by the same screening system.

Materials and Methods

Crude drugs——All the crude drugs used for the screening experiment were purchased from A.K. Shakya & N.K. Shakya (Kathmandu, Nepal, 1986).

Preparation of crude drug extracts——Each crude drug (10 g) was cut into small pieces and extracted with water (100 ml × 2) on a boiling water-bath for 2 h. The solution was filtered through a cotton plug and the filtrate was concentrated to a small volume under reduced pressure, and then lyophilized.

Test solution——Each lyophilized powder was dissolved in 0.75% saline at a concentration of 10 mg/ml. When there was much insoluble material to make the observation of larvae difficult, the solution was diluted with saline to 5 mg/ml. If the solution was still to turbid, the insoluble material was removed by centrifugation.

Assay method and evaluation of nematocidal activity——The assay was done as described in the previous paper[9)]

and the nematocidal activity was evaluated by the RM value. A smaller RM value indicates a stronger nematocidal activity: when all the larvae die, this value is 0. Minimal lethal concentration (MLC) was defined as the lowest concentration giving the RM value 0 after 24 h incubation.

TABLE I. Nematocidal Activity of Traditional Medicines of Nepal on Second-stage Larva of *Toxocara canis* (10 mg/ml)

No[a)]	Local name	Botanical origin[b)]	Part	Family	RM value		
					3 h	6 h	24h
N 80	Aankha Paar	*Calotropis gigantea* L.	leaf	Asclepiadaceae	100	100	99
N190	Agur	*Vepris* sp.	lignum	Rutaceae	100	70	0
N 19	Ajamoda	*Apium graveolens* L.	fruit	Umbelliferae	92	87	33*
N160	Ajayapal	*Croton tiglium* L.	seed	Euphorbiaceae	100	100	33
N 26	Akarkala	*Spilanthes oleracea* JACQ.	root	Compositae	100	100	60*
N 17	Amala	*Emblica officinalis* GAERTN.	fruit	Euphorbiaceae	100	98	43
N 13	Anar ko Bokara	*Punica granatum* L.	pericarp	Punicaceae	100	100	71
N137	Arel Beej	*Ricinus communis* L.	seed	Euphorbiaceae	100	100	35
N117	Ashok Bokara	*Saraca indica* L.	bark	Leguminosae	100	100	91*
N181	Ashok Phool	*Saraca indica* L.	flower	Leguminosae	100	100	72*
N 38	Asogandha	*Withania somnifera* DUNAL	root	Solanaceae	100	100	58
N 65	Asuro	*Justicia adhatoda* L.	whole plant	Acanthaceae	100	90	63
N175	Atibala	*Ipomoea* sp.	seed	Convolvulaceae	100	100	53
N 64	Atisa		rhizome		100	100	88
N 58	Bakuchi	*Psoralea corylifolia* L.	seed	Leguminosae	99	42	0
N 71	Balu ko Jara	*Sida acuta* BURM. f. DIELS	whole plant	Malvaceae	100	100	23
N104	Ban Lasoon	*Lilium nepalense* D. DON	bulb	Liliaceae	100	100	69
N 5	Bansa Lochan	*Bambusa bambos* L.	silicone	Bambusaceae	100	98	71
N129	Barahi Kanda	*Dioscorea deltoidea* WALL.	rhizome	Dioscoreaceae	100	100	82*
N 3	Barro	*Terminalia belerica* ROXB.	pericarp	Combretaceae	100	100	62
N 34	Bayubidanga	*Embelia ribes* BURM f.	fruit	Myrsinaceae	100	100	72*
N 11	Bel ko Chana	*Aegle marmelos* CORR.	fruit	Rutaceae	100	100	61
N 2	Bethe	*Chenopodium album* L.	fruit	Chenopodiaceae	100	100	93
N142	Bhaki Amilo	*Rhus semialata* MURRAY	seed	Anacardiaceae	100	100	50
N163	Bhargi	*Clerodendrum indicum* KTZE	root bark	Verbenaceae	100	100	70
N188	Bhoj Patra	*Betula utilis* D. DON	bark	Betulaceae	100	100	17*
N143	Bhoota Kesa	*Selinum tenuifolium* WALL.	root	Umbelliferae	100	100	100
N156	Bhringiraj	*Wedelia calendulacea* LESS.	whole plant	Compositae	100	100	50
N133	Bidarikanda	*Convolvulus micranthus* ROEM.	root	Convolvulaceae	100	100	88
N 82	Bihin	*Solanum indicum* L.	whole plant	Solanaceae	100	100	38
N124	Bijaya Sala	*Pterocarpus marsupium* ROXB.	lignum	Leguminosae	95	56	3*
N 35	Bikhama	*Aconitum palmatum* D. DON	rhizome	Ranunculaceae	100	100	81
N 74	Bojho	*Acorus calamus* L.	rhizome	Araceae	17	0	0*
N187	Bramhi	*Celtis australis* L.	whole plant	Ulmaceae	100	100	47
N116	Chabo	*Piper chaba* HUNTER	whole plant	Piperaceae	67	38	0
N 94	Chameha Jara		root		100	100	70
N 79	Chamsur	*Lepidium sativum* L.	seed	Cruciferae	100	100	50
N159	Chandmaruwa	*Rauwolfia serpentina* BENTH.	root	Apocynaceae	100	100	67*
N147	Chhatiwan	*Alstonia scholaris* R. BR.	bark	Apocynaceae	100	100	58
N112	Chiraito	*Swertia* sp.	whole plant	Gentianaceae	100	100	59*
N 39	Chitu Jara	*Plumbago zeylanica* L.	whole plant	Plumbaginaceae	100	100	93
N113	Chulthi Amilo	*Rheum emodi* WALL.	petiole	Polygonaceae	99	97	2
N 93	Chutro	*Berberis aristata* DC.	bark	Berberidaceae	100	95	39*
N141	Dam Paate		whole plant		100	100	38
N 72	Danti	*Baliospermum montanum* MUELL.	lignum	Euphorbiaceae	100	100	85*
N127	Dariya		seed		100	100	74*
N185	Deva Daru	*Cedrus deodara* LOUD.	bark	Pinaceae	100	96	5*
N 49	Dhanyaro Phool	*Woodfordia fruticosa* KURZ	flower	Lythraceae	100	100	0
N131	Drona Puspa	*Leucas cephalotes* SPRENG.	whole plant	Labiatae	100	100	77*
N 40	Gaja Pipal	*Piper* sp.	fruit	Piperaceae	100	100	63*

N111 Ghoda Tapre	*Centella asiatica* URB.	whole plant	Umbelliferae	100	100	35*
N 45 Ginyari	*Premna integrifolia* L.	bark	Verbenaceae	100	100	100
N 75 Gokhatu	*Tribulus terrestris* L.	fruit	Zygophyllaceae	100	100	39
N 4 Gorakhamundi	*Sphaeranthus indicus* L.	flower	Compositae	69	48	0
N105 Gucholha		whole plant		100	80	33*
N138 Gulaban Phasa		whole plant		100	100	90*
N 16 Gurujo ko Lahara	*Tinospora cordifolia* MIERS	stem	Menispermaceae	100	100	84*
N125 Haledo	*Curcuma longa* L.	rhizome	Zingiberaceae	90	33	0*
N 8 Harro	*Terminalia chebula* RETZ.	fruit	Combretaceae	100	100	60
N 44 Indrajau ko Bokara	*Holarrhena antidysenterica* WALL.	bark	Apocynaceae	100	100	93
N 1 Isabgol	*Plantago ovata* FORSK.	seed	Plantaginaceae	100	100	100*
N183 Jamuna Beej	*Memecylon angustifolium* WIGHT	fruit	Melastomaceae	94	94	35
N 9 Jangi Harro	*Terminalia* sp.	fruit	Combretaceae	99	99	74*
N103 Jatamasi	*Nardostachys jatamansi* DC.	rhizome	Valerianaceae	97	76	0
N139 Jawani Khawani	*Helicteres isora* L.	fruit	Sterculiaceae	100	100	38
N 47 Jethimadhu	*Glycyrrhiza glabra* L.	root	Leguminosae	100	93	0
N106 Jibanti	*Desmotrichum fimbriatum* BL.	bulb	Orchidaceae	100	100	0
N 36 Kacholha	*Coccus lacca* KERR		Homopteraceae	100	98	39*
N 61 Kafal Bokara	*Myrica esculenta* BUCH.-HAM.	bark	Myricaceae	100	100	91*
N 10 Kakoli		rhizome		100	100	88
N179 Kalo Basak	*Adhatoda vasica* NEES	whole plant	Acanthaceae	82	33	0
N 91 Kalo Haledo	*Zingiber* sp.	rhizome	Zingiberaceae	96	36	0
N 73 Kalo Jira	*Centratherum anthelminticum* WILLD.	fruit	Compositae	100	100	64*
N 31 Kampila		fruit hair		100	100	73*
N132 Kancho Silajit	*Styrax officinale* L.	resin	Styracaceae	100	100	82*
N146 Kankol	*Piper* sp.	seed	Piperaceae	100	100	44
N 81 Kanthakari	*Solanum xanthocarpum* S. & W.	fruit	Solanaceae	100	72	33
N 27 Karanja	*Pongamia glabra* VENT.	fruit	Leguminosae	100	100	80*
N178 Kauso	*Mucuna prurita* HOOK.	seed	Leguminosae	100	100	100*
N161 Kawaphachini	*Piper cubeba* L.	fruit	Piperaceae	86	47	0
N140 Khorasani Ajuwaine	*Hyoscyamus niger* L.	seed	Solanaceae	100	100	84*
N135 Koairala Bokara	*Bauhinia variegata* L.	bark	Leguminosae	100	100	90*
N189 Krishna Agur	*Vepris bilocularis* ENGLER	lignum	Rutaceae	35	17	0*
N 37 Kuchila	*Strychnos nux-vomica* L.	seed	Loganiaceae	67	67	52
N 51 Kuma Kuma	*Didymocarpus leucocalyx* C.B. CL.	young leaf	Gesneriaceae	100	80	21*
N 18 Kuta	*Saussurea lappa* CLARKE	root	Compositae	96	77	37*
N 48 Kutaki	*Picrorhiza kurroa* ROYLE	rhizome	Scrophulariaceae	100	97	12
N151 Lal Makhana		seed		100	100	34
N118 Lekha Pangro	*Entada phaseoloides* MERR.	seed	Leguminosae	100	100	71
N 88 Lodha	*Symplocos paniculata* WALL.	bark	Symplocaceae	80	77	57*
N 90 Madanphal	*Xeromphis spinosa* KEAY	fruit	Rubiaceae	100	100	69
N 6 Madise Soup	*Foeniculum vulgare* MILL.	fruit	Umbelliferae	100	100	56
N144 Maharangi	*Onosma echioides* L.	root	Boraginaceae	100	100	79*
N155 Majito	*Rubia cordifolia* L.	root	Rubiaceae	85	18	0
N 23 Majufal	*Quercus intectoria* OLIVIER	gall	Fagaceae	100	100	42
N120 Malakaguni	*Celastrus paniculatus* WILLD.	seed	Celastraceae	99	95	17
N172 Masaparni	*Teramnus labialis* SPENG.	whole plant	Leguminosae	100	100	100*
N 87 Mothe	*Cyperus* sp.	tuber	Cyperaceae	100	97	56
N186 Mudilo		root		67	67	67
N 32 Mugrelo	*Nigella sativa* L.	seed	Ranunculaceae	100	100	38
N169 Nag Kesar	*Mesua ferrea* L.	fruit	Guttiferae	98	83	46
N174 Nagbala	*Sida spinosa* L.	seed	Malvaceae	100	100	77
N108 Neem	*Melia azadirachta* L.	stem	Meliaceae	100	100	52
N 28 Nepali Musali		root		100	100	37*
N 98 Netrabal		whole plant		100	100	64
N 12 Nir Kamal	*Nymphaea stellata* WILLD.	flower	Nymphaeaceae	100	100	39
N109 Nirmasi	*Aconitum* sp.	tuber	Ranunculaceae	100	100	97
N 41 Nisontha	*Ipomoea turpethum* R. BR.	root	Convolvulaceae	100	100	51

N 92 Okhar ko Bokara	*Juglans regia* L.	pericarp	Juglandaceae	100	100	73*
N 78 Padamchal	*Rheum emodi* WALL.	rhizome	Polygonaceae	100	100	64*
N 14 Pakhan Beda	*Bergenia cilliata* STERNB.	root	Saxifragaceae	74	74	0
N 42 Pan ko Jara	*Alpinia galanga* L.	rhizome	Zingiberaceae	36	0	0
N123 Panch Aunla	*Gymnadenia crassinervis* FINET	root	Orchidaceae	100	100	34
N177 Panyu		bark		100	97	9*
N100 Patali	*Stereospermun tetragonum* DC.	bark	Bignoniaceae	100	87	49
N 68 Pipalamool	*Piper longum* L.	whole plant	Piperaceae	57	17	0
N107 Pitta Papada	*Naregamia alata* W. & A.	whole plant	Meliaceae	100	98	51
N 21 Priyangu	*Aglaia roxburghiana* MIQ.	fruit	Meliaceae	100	100	68
N136 Pudina	*Mentha arvensis* L.	whole plant	Labiatae	100	100	90
N114 Punarnawa		whole plant		100	100	56
N148 Raj Brikshya	*Cassia fistula* L.	seed	Leguminosae	100	100	84
N176 Ramal Gatta	*Nelumbo nucifera* GAERTN.	seed	Nymphaeaceae	100	100	84*
N170 Rasanjan	*Berberis aristata* DC.	resin	Berberidaceae	100	99	50*
N145 Rasna		whole plant		100	100	87*
N 66 Rato Pate	*Ajuga bracteosa* WALL.	whole plant	Labiatae	100	100	41
N158 Rittha	*Sapindus mukorossi* GAERTN.	fruit	Sapindaceae	100	98	62
N 86 Roopkesar		flower		100	100	58
N 50 Rudilo	*Nyctanthes arbor-tristis* L.	stem	Oleaceae	100	100	66
N184 Sajiwan Beej	*Atropha curcas* L.	seed	Euphorbiaceae	100	100	92*
N164 Sal Mishri		fruit		100	95	67
N 67 Salparni	*Desmodium gangeticum* DC.	whole plant	Leguminosae	100	100	72*
N168 Samudra Pheerja	*Sepia esculenta* HOYLE	born	Sepiidae	100	100	100
N157 Samudruphal	*Barringtonia acutangula* GAERTN.	fruit	Lecythidaceae	74	69	38*
N 62 Sanaie Patti	*Cassia angustifolia* VAHL	leaf	Leguminosae	100	100	53
N110 Sankha Puspa	*Leucas* sp.	whole plant	Labiatae	100	100	48*
N 7 Sariwa		fruit		100	100	92
N 96 Satabari	*Asparagus racemosus* WILLD.	root	Liliaceae	100	100	85
N 43 Sathi	*Curcuma zedoaria* ROSC.	rhizome	Zingiberaceae	100	99	33*
N 97 Satuwa	*Paris polyphylla* SMITH	rhizome	Liliaceae	90	69	68
N 25 Seto Khayar		resin		100	100	98*
N 22 Seto Musali		root		100	100	58
N 83 Shobhanjan		fruit		100	100	100*
N 29 Simal Khoto	*Bombax ceiba* L.	gall	Bombacaceae	100	100	92*
N 95 Simal ko Phool	*Bombax malabaricum* DC.	flower	Bombacaceae	100	100	98
N180 Sinwali	*Vitex* sp.	leaf	Verbenaceae	100	100	59
N128 Siplikan	*Crataeva religiosa* HOOK.	fruit	Capparidaceae	100	100	41*
N 54 Srikhanda Dhoolo	*Santalum album* L.	lignum	Santalaceae	100	100	6*
N 85 Sugandhawal	*Valeriana wallichii* DC.	rhizome	Valerianaceae	100	100	72
N152 Sutho	*Zingiber officinale* ROSC.	rhizome	Zingiberaceae	100	100	33*
N 77 Tagar		whole plant		100	100	33*
N 30 Talamakhana	*Hygrophila spinosa* T. ANDERS.	seed	Acanthaceae	100	100	64*
N102 Talispatra	*Rhododendron* sp.	stem, leaf	Ericaceae	98	68	0
N 99 Tatelo	*Oroxylum indicum* KURZ	bark	Bignoniaceae	100	100	86
N 89 Tejmool	*Zanthoxylum armatum* DC.	bark	Rutaceae	100	100	78*
N 69 Tejpat	*Cinnamomum tamala* NEES & EBERM	leaf	Lauraceae	100	100	43
N 15 Thoolo Ausadhi		rhizome		100	100	95*
N 60 Thoolo Pipal	*Piper longum* L.	fruit	Piperaceae	54	17	0*
N122 Timur	*Zanthoxylum armatum* DC.	fruit	Rutaceae	100	38	0
N 76 Tupmalanga		seed		100	100	96
N130 Usir	*Cymbopogon* sp.	whole plant	Gramineae	69	39	0

[a] Each sample is referred to by its code number at the Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Kanazawa University. The same samples are also kept in the Museum of Materia Medica, Toyama Medical and Pharmaceutical University.

[b] The botanical sources are identified by the comparison of the appearances of the crude drugs referring to the description in the literature.[11] The genus was definitely determined, although species were not quite clear.

[c] *: 5 mg/ml, #: 1 mg/ml.

Identification of the active principle of "Pan ko Jara"——Cut "Pan ko Jara" (5 g) was extracted under reflux with $CHCl_3$, MeOH, and water (each 100 ml × 2 h × 2 times), successively. The yields and the RM values (0.1 mg/ml, after 24 h of incubation) of the extracts were as follows: $CHCl_3$, 76 mg, 0; MeOH, 197 mg, 0; water, 268 mg, 100. The $CHCl_3$ and MeOH extracts were combined and fractionated by silica-gel column chromatography eluting with benzene (fr. 1, 52 mg), benzene–acetone = 19:1 (fr. 2, 126 mg, fr. 3, 10 mg), and MeOH (fr. 4, 69 mg). The RM values of fr. 1–fr. 4 at a concentration of 0.1 mg/ml were 100, 0, 63, and 95, respectively. The active fraction (fr. 2) was purified by medium pressure liquid chromatography (MPLC) on LiChroprep Si-60 (hexane:acetone = 4:1) to give [*S*]-1′-acetoxychavicol acetate (**1**, 98 mg). GC-MS analysis of the side fractions from MPLC indicated the presence of 1′-acetoxyeugenol acetate, *p*-coumaryl diacetate and coniferyl diacetate.[10)]

[*S*]-1′-Acetoxychavicol acetate (1)[10)]——Colorless oil. IR ($CHCl_3$): 1735 cm^{-1}. UV λ_{max} nm (log ε): 218 (3.95), 261 (2.75). ^{1}H-NMR ($CDCl_3$): δ 2.12 (3H, s), 2.30 (3H, s), 5.22 (1H, d with fine sprittings, J = 10 Hz), 5.28 (1H, d with fine sprittings, J = 17 Hz), 5.97 (1H, ddd, J = 6, 10, 17 Hz), 6.24 (1H, brd, J = 6 Hz), 7.05 and 7.34 (each 2H, ABq, J = 9 Hz). MS m/z (%): 234 (M^+, 2), 192 (43), 150 (67), 132 (100), 131 (33), 121 (16). HRMS m/z: Calcd for $C_{13}H_{14}O_4$ (M^+): 234.0891. Found: 234.0882. $[\alpha]_D^{20}$ − 53° (c = 0.17, EtOH, lit. − 53°).[10a)]

Results and Discussion

1. Nematocidal activity of hot water extracts

Hot water extracts of 160 crude drugs used in Nepal were tested for their *in vitro* nematocidal activity on the second-stage larva of *Toxocara canis* at a concentration of 10 mg/ml or 5 mg/ml (TABLE I). The nematocidal effect of each test material is represented by the relative mobility (RM) value at 3, 6 and 24 h after the start of incubation. The aqueous extracts of 22 crude drugs showed strong (RM = 0, after 24 h incubation) and 17 showed appreciable (0 < RM < 34) activity.

Of these crude drugs which showed potent activity, "Bojho" and "Pan ko Jara" exhibited particularly strong activity (RM = 0, after 6 h incubation). "Bojho" is used as a stomachic and for the treatment of remittent fevers, bronchitis, colic and dysentery of children in Nepal.[11a)] It is also used as an insectifuge. The botanical source of "Bojho" is the rhizome of *Acorus calamus* L. (Araceae).[11a)] In Chinese medicine, it is used as an aromatic stomachic and a related crude drug, the rhizome of *Acorus gramineus* SOLAND, is used as an analgetic and stomachic and also as an anthelmintic.[12)] The botanical origin of "Pan ko Jara" used in the present study is described below.

Of those crude drugs which showed potent nematocidal activity, four were derived from Piperaceous plants, *i.e.* "Chabo" (whole plant of *Piper chaba* HUNTER), "Kawaphachini" (fruit of *Piper cubeba* L.), "Pipalamool" and "Thoolo Pipal" (whole plant and fruit of *Piper longum* L., respectively). In our previous screening of Sri Lankan crude drugs,[8)] "gammiris" showing a strong nematocidal activity and containing pyrrolidin- and piperidin-amides with relatively long aralkyl chains as the active principles[13)] was also from a Piperaceous plant (fruit of *Piper nigrum* L.; pepper). Since such amide constituents are reported to be contained in many Piperaceous plants, the nematocidal activities of the above Piperaceous drugs may also be attributable to their analogous amide constituents.

2. Botanical origin of "Pan ko Jara"

"Pan" or "Paan" usually refers to *Piper betle* L.[11a)] and "jara" means root in Nepali. Therefore, "Pan ko Jara" means "root of *P. betel*" in Nepali. However, the outward appearance and smell of our sample were very different from those of the root of *P. betle*, and rather resembled those of a rhizome of *Alpinia* sp. (Zingiberaceae), particularly *A. galanga* SWARTZ. Anatomically, the development of bundle sheath and other characteristics are almost identical to those of *A. galanga*, except that "Pan ko Jara" used in this study has no stone cells beneath the epidermis and no typical hair on the epidermis, both of which were reported as the morphological characteristics of *A. galanga* by Konoshima *et al.*[14)] The chemical constituents isolated from this sample are again identical with those reported for the rhizome of *A. galanga*[10b)] (see Materials and Methods). We therefore tentatively concluded that "Pan ko Jara" used in the present study is the rhizome of *Alpinia galanga*, though the confirmative evidence has not been obtained.

Since "Pan" sometimes means the materials for betel chewing in general in Nepal[15)] and the chewing sometimes contains such plant materials as cardamon, fennel, *etc.*, besides betel nuts, we now consider that "Pan ko Jara" means "a root material used for Pan."

3. Identification of the active principle of "Pan ko Jara"

In order to identify the nematocidal principles of "Pan ko Jara," cut rhizomes were extracted with $CHCl_3$, MeOH and water, successively. Since the activity was found only in the organic extracts, the $CHCl_3$ and MeOH extracts were combined and fractionated by silica-gel column chromatography. Further

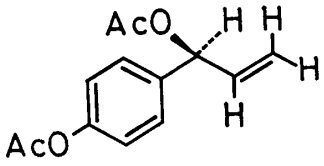

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Chart 1.

purification of the active fraction by MPLC gave a colorless oil as its active principle which was identified as [*S*]-1′-acetoxychavicol acetate (**1**) by the comparisons of the spectral data with those reported.[10] The minimal lethal concentration (MLC) of **1** was 0.4 mM. Since **1** is the major constituent of the organic extracts, the nematocidal activity of "Pan ko Jara" is attributable to **1**.

In tropical countries, relatively frequent cases of larva migrans may be expected. For example, the disease called tropical eosinophilia or eosinophilic lung, known for a long time in tropical and subtropical countries[16] and characterized by persistent hypereosinophilia, is thought to be caused by the infection of the larvae of animal ascarids or filariae.[17] The hepatitis-like disease notified in Palm Island in 1979 was considered to have been caused by the larvae of *Toxocara pteropodis*, normally a parasite on fruit bats.[18]

In the present screening work, extracts of some Piperaceous plants showed a strong nematocidal effect on the larva of dog roundworm. These Piperaceous plants are widely used as spices or luxury in South East Asia. In our previous screenig works, other luxuries and spices such as betel nuts[9] and mace[19] were found to have nematocidal activity. Therefore the daily consumption of these plant materials may have some role in the prevention of the infection of parasites in this region.

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