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Biological Evaluation of Korean Medicinal Plants. II.

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Abstract—Extracts of seventy-two Korean plants were evaluated for their biological activities such as antitumor activities against sarcoma 180, leukemia SN and Ehrlich ascites carcinoma, cytotoxic activities, antimicrobial activities and behavoral observations, and the results are tabulated.

A number of biological evaluations on the plants have been already reported by many laboratories[1~3]. More than seventy Korean plants used as folklorics were previously evaluated in our laboratory[4]. In this successive report, 72 species which belong to 44 families were evaluated.

MATERIALS AND METHODS

The plant extracts were prepared as described previously[4]. Acute toxicity, antitumor activity and antimicrobial activity were measured also as in previous report[4], and general mouse behavior was evaluated according to methods of Irwin[5,6]. The cytotoxic activity test was conducted as the followings.

Cytotoxic activity—The suspension culture method using HeLa-S_3 strain was employed. YLS (yeast extracts, lactoalbumin and 10% horse serum added) medium were used for culture. Fully grown monolayer cells were trypsinized at 37°, then were centrifuged in 1490×g for 3 min. After supernatant was discarded, fresh medium was added into it to wash out residual trypsin. Such washing was repeated twice in cold. HeLa cells were suspended in media (1×10^6 cells/ml). Two different doses of plants extracts were pre-incubated for 10 min at 37° in Dubnoff shaking incubator in open-air. Same volumes of media were added to the control groups. After pre-incubation, all culture tubes were placed in ice-cold bath, then tritiated thymidine(2 μmoles, Sp. Act. 50 ci/mole) were added into both test and control group culture tubes. And cells were incubated for 15 min at 37°. The reaction was stopped by adding 0.5 ml of 50% ice-cold TCA solution. The preci-

Table I—Results of the biological evaluation of plant extracts

Plant name	Date collect.	Part used[a]	Antitumor activity				Cytotoxicity (HeLa, T/C %)		Antibacterial activity[d]		Mouse behavior		Acute toxicity
			Dose (mg/kg, ip)	S-180 T/C (%)[b]	SN-36 T/C (%)	Ehrlich T/C (%)	1×10^{-3} (μg/ml)	1×10^{-2} (μg/ml)	*Staphyl. aureus*	*Escheri. coli*	Activity[f]	Dose range (mg/kg, ip)	>mg/kg, ip
Alismataceae *Alisma plantago* var. *parviflorum*	5/72	tb	250	119	100	105	82	62	In[e]	In	Hp, P(w), Wr	70-600	500
Amarantaceae *Achyranthea japonica*	3/72	rt	125	117	86	101	46	44	In	In	Hp, P(w), Wr	70-600	500
Araceae *Acorus gramineus*	12/72	rz	500	122	98	88	—	88	10	In	Hp(w), SMd	100-900	1000
Araliaceae *Acanthopanax spinorus*	12/74	bk	30	120	94	119	70	34	In	In	SMi, Hp	10-60	60
Aristolochiaceae *Aristolochia manshuriensis*	12/72	st	250	95	112	102	36	26	In	In	nil	70-600	500
Asclepiadaceae *Cynanchum stratum*	12/72	rt	500	88	95	116	40	32	In	In	SMi	100-900	1000
Cynanchum wilfordi	5/72	rz	500	101	89	120	—	—	In	In	Grd, SMd(w)	100-900	1000
Balsaminaceae *Impatiens textori*	10/69	wp	500	117	92	114	—	60	In	In	SMd(w), Hp	100-900	1000
Campanulaceae *Codonopsis lanceolata*	5/72	rt	125	108	88	91	—	—	In	In	SMd(w),Wr,Hp	30-270	250
Platycodon grandiflorum	12/72	rt	125	97	100	123	—	—	In	In	SMd(w)	70-600	500
Caprifoliaceae *Lonicera japonica*	12/72	fl	500	96	109	106	—	—	In	In	Hp(w)	100-900	1000
Caryophyllaceae *Dianthus chinensis*	3/72	fr	250	93	97	103	63	40	In	In	GTd(w), Wr	70-600	500
Melandryum firmum	3/72	wp	250	105	97	115	53	49	In	In	Pe, Rd, Hp	70-600	500
Compositae *Arctium lappa*	3/72	sd	500	108	111	121	61	53	In	12	GTd	100-900	1000
Artemisia princeps pampanini var. orientalis	3/72	lf	125	91	100	93	—	—	In	In	SMd (w), Pe, Wr, GTd	70-600	500
Aster tarfaricus	10/74	rt	500	86	107	112	46	44	In	In	SMd, AG, Grd, Pp, SRd, P, GTd,Rrd,Hp,Wr	100-900	800
Astractylodes japonica	10/69	rz	500	119	88	87	—	—	In	In	Hp	100-900	1000
Cephalonoplos segetum	12/72	rt	500	106	110	97	—	—	In	In	SMd(w) Pe,Hp	100-900	1000

Compositae													
Chrysanthemum indicum	10/69	wp	250	110	104	104	79	49	In	In	SMi(w), Hp	70-600	500
Chrysanthemum sibiricum	12/72	wp	500	95	105	100	38	32	In	In	GTd, Hp (w)	100-900	1000
Inula helenium	12/72	rt	500	117	100	91	—	—	In	In	SMd, Pp	100-900	1000
Inula japonica	12/72	fl	500	89	107	93	67	49	In	In	Hp	100-900	1000
Taraxacum platycarpum	9/74	wp	500	122	104	133[c)]	72	60	In	In	Hp (w)	100-900	1000
Convolvulaceae													
Cuscuta chinensis	12/72	sd	500	102	89	121	—	—	In	In	nil	100-900	1000
Cornaceae													
Cornus officinalis	12/72	fr	500	112	98	106	—	—	10	10	SMd (w)	100-900	1000
Crassulaceae													
Orostachys japonicus	12/72	wp	500	87	97	97	—	59	In	In	Hp, SMd (w)	100-900	1000
Cruciferae													
Rhapanus sativus var.raphanistroides	3/72	sd	500	98	96	91	87	62	In	In	Hp (w), SMd (w)	100-900	1000
Cyperaceae													
Cyperus amuricus var. laxus	8/71	wp	500	116	98	86	—	—	In	In	nil	100-900	1000
Cyperus rotundus	2/74	tb	500	112	115	131[c)]	37	32	In	In	nil	70-600	500
Scirpus martimus	5/72	tb	250	86	92	111	48	36	In	In	GTd, Gr, Wr, Hp	70-600	500
Discoreaceae													
Discorea batatas	17/72	rz	500	113	89	98	—	—	In	In	SMd, Hp, Pp, Wr	100-900	1000
Equisetaceae													
Equisetum hiemale f.genuium	12/72	wp	250	120	107	100	6	27	In	In	SMd, Wr	70-600	500
Euphorbiaceae													
Euphorbia fischerianum	12/72	rt	125	94	108	94	74	56	10	10	SMi (w), GTd, Hp	30-300	250
Iridaceae													
Belamcanda chinensis	12/72	rz	500	100	109	107	74	55	In	In	SMd, Pp	100-900	1000
Labiatae													
Lenonurus japonicus	7/71	wp	500	91	90	92	—	—	In	12	SMd (w)	100-900	1000
Prunella vulgaris	3/72	wp	500	123	100	125[c)]	77	47	In	In	Hp (w)	100-900	1000
Scutellaria baicalensis	5/72	rt	500	93	99	128[c)]	84	59	10	In	SMi, SRi, Wr	100-900	1000
Lauraceae													
Machilus rimosa var. Thunb.	12/72	bk	500	113	93	113	35	28	In	In	SMd, Pe, Pp, Hp	100-900	1000
Leguminosae													
Astragalus membranaceus	5/72	rt	500	100	123	107	49	18	In	In	nil	100-900	1000
Gleditschia japonica var. koraiens	12/72	fr	500	117	105	95	—	—	In	.	SMd,Pp(w),Hp (w)	100-900	1000

Lemnaceae *Spirodela polyrhiza*	3/72	wp	250	95	92	91	—	58	In	In	SMi	70-600	500
Liliaceae *Anemarrhena asphodeloides*	5/72	rz	125	100	88	109	14	35	In	In	SMi	70-600	500
Asparagus lucidus	5/72	tb	500	99	109	113	—	—	In	In	SMd, Pp, Hp(w)	100-900	1000
Lilium auratum	12/72	rz	250	108	88	100	62	53	In	In	SMd, Pp, Hp	70-600	500
Liriope platyphylla	5/72	tb	500	99	103	119	66	58	In	In	SMd (w)	100-900	1000
Polygonatum japonicum	2/74	rz	500	98	108	89	53	58	In	In	Wr.	70-600	500
Smilax china	3/72	rz	250	86	96	88	48	33	In	In	Wr, Hp	70-600	500
Malvaceae *Malva alitoria*	12/72	sd	500	88	96	88	—	—	In	In	nil	100-900	1000
Phytolaccaceae *Phytolacca americana*	9/71	rt	250	93	97	94	—	—	In	In	SMd, Hp	70-600	500
Plantaginaceae *Plantago asiatica*	3/72	sd	500	96	120	119	—	—	In	In	SMd, GTd	100-900	1000
Polygonaceae *Polygonum cuspidatum*	12/72	rz	250	95	92	106	39	23	In	In	SMd, Pp, Hp, Wr	100-900	1000
Polygonum multiflorum	5/72	rz	500	99	97	113	—	—	In	In	Wr, SRi, SMi	100-900	1000
Rheum undulatum	2/73	rz	250	91	100	109	36	35	10	In	SMd, Pp, Hp, Wr	100-900	1000
Primulaceae *Lysimachia davurica*	7/70	wp	500	91	111	107	—	—	In	.	nil	100-900	1000
Ranunculaceae *Aconitum japonicum*	3/72	rt	500	118	95	108	58	50	In	In	SMi, GTd, Hp	1-6	10
Aconitum koreanum	12/72	tb	125	110	96	90	—	—	10	10	SMi	30-300	2500
Cimicifuga heracileifolia	3/72	rz	250	106	110	97	36	36	In	In	nil	70-600	500
Paeonia albiflora var. typica	2/74	rt	125	87	89	108	—	—	In	In	nil	70-600	500
Rosaceae *Craegus pinnatifida var.* mayor	3/72	fr	250	117	103	96	—	73	In	In	nil	70-600	500
Rubus corenus	12/72	fr	250	95	88	88	44	42	In	In	SMd, GTd, Hp, Wr	100-900	1000
Rubiaceae *Gardenia jasmenoidies*	12/72	fr	500	103	110	114	—	—	In	In	SMd	100-900	1000
Rutaceae *Citrus aurantium amora*	3/72	pc	250	120	100	100	—	—	In	In	nil	70-600	500
Scrophulariaceae *Scrophularia oldhami*	12/72	rt	500	104	106	120	—	—	In	In	nil	100-900	1000

Solanaceae *Lycium chinensis*	5/72	fr	250	95	90	102	—	60	In	In	nil	70–600	500
Taxaceae *Torreya nucifera*	12/72	fr	250	95	90	102	—	60	In	In	nil	70–600	500
Typhaceae *Typha orientalis*	3/72	fl	500	109	93	101	42	38	In	In	nil	100–900	1000
Umbelliferae *Angelica davurica*	12/72	rt	500	100	108	113	63	59	In	In	SMi	100–900	1000
Angelica gigas	5/72	rt	500	86	93	119	78	48	In	In	nil	70–400	500
Cnidium officinale	5/72	rz	500	95	118	119	—	71	In	In	Pp, Hp	100–900	1000
Peucedanum japonicum	7/71	wp	500	88	105	89	—	—	In	In	nil	100–900	1000
Valerianaceae *Patrinia scabiosifolia*	2/72	rz	125	102	103	88	—	—	In	In	Hp	30–300	250
Vitaceae *Ampelopsis japonica*	12/72	rz	500	97	89	97	—	—	In	In	nil	100–900	1000

a) bk, bark; fl, flower; fr, fruit;lf, leaf; pc, pericarpium; rt, root; rz, rhizome; sd, seed; st, stalk; tb, tuber; wp, whole plant.

b) T/C, the mean survival time of the treated group over the mean survival time of the control group ×100

c) Negative result after retest.

d) Number indicates the diameter (mm) of inhibited zone with 500 μg/disk of a sample.

e) In, inactive

f) AG, abnormal gait; Al, alertness; BTd, decrease of body tone; Cv, convulsion; Gr, grooming; GTd, decrease of grip tone; Hp, hypothermia; P, passivity; Pp, palpebral ptosis; Rd, decrease in respiration rate; SMd, decrease in spontaneous movement; SMi, increase in SM; SR, startle response, ST, Straub's tail; TRd, decrease in touch response; Tr, tremor; Wr, writhing; (w) in each response indicates "weak" activity in the evaluation.

pitates were extracted four times with 5% ice-cold TCA. The precipitates were solublized in 1ml of tissue solublizer (NCS, Amersham) for 2 hrs at 55°. The samples were counted in 10ml of toluene containing 6 g/*l* PPO, and 75 mg/*l* POPOP.

RESULTS AND DISCUSSION

The results of biological evaluation are shown in Table I. And some parts of the data was reported at the 3rd Asian symposium on medicinal plants and spices[7]. Most extracts appeared to be inactive against the murine tumor models employed. As the one of parameters to determine the cytotoxic activity of crude plant extract, it was employed that the degree of inhibition of thymidine incorporation into HeLa cell DNA were measured. As the data showed, most crude plant extracts exhibited some degree of inhibition of DNA synthesis in HeLa cells.

Amongst the crude plants extracts tested so far, 14 species appeared to show potent (more than 50%) inhibition of DNA synthesis in HeLa cells at two different doses ($1\times10^3\mu$g/ml, and $1\times10^2\mu$g/ml).

Such results imply that further cytotoxic studies like long term monolayer culture of HeLa cells with those crude plant extracts might be warranted. In addition, it should be noted that the cytotoxicity does not necessarily coincide with the antitumor activity. As the data showed in Table I, although most of plant extracts appeared to be cytotoxic, they did not show any antitumor activities in survival studies. In antimicrobial activity test, *Cornus officinalis*, *Euphorbia fischerianum* and *Aconitum koreanum* appeared to be positive against two strains employed. The extracts active against *Staphylococcus aureus* were *Acorus gramineus*, *Scutellaria baicalensis* and *Rheum undulatum*, *Arctium lappa* and *Leonurus japonicus* were active against *Escherichia coli*.

With mouse behavoral studies, *Rubus corenus*, showed CNS depressant activity, the depressant activity with autonomic was shown by *Aster tarfaricus*, and *Machilus remosa* var. Thunb. Other extracts exhibited only weak depressant or stimulant activity. The extracts showing weak CNS stimulant activities were *Acanthopanax spinosus*, *Cyanchum stratum*, *Scutellaria baicalensis*, *Spirodela polyrhiza*, *Anemarrhena asphodeloides*, *Aconitum koreanum* and *Angelica davurica*. Among the seventy-two plants tested, seventeen did not show any significant activity.

With respect to behavoral study, it should be noted that only the alcohol extracts of the plants were prepared and used for the purpose of evaluation. Thus there may exist some possibilities, for instanse, the volatile components not obtained during the extract preparation, could possess some biological activities.

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