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Biological Evaluation of Korean Medicinal Plants

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Abstract—Alcohol extracts of 70 Korean medicinal plants have been tested for their biological activity. Most of the extracts had slight activity only. Some extracts had more pronounced activity. The results are tabulated.

A number of plants of Korea are still used as medicine by the local people. Several phytochemical surveys have been published recently[1-7], however, the medical potentialities of many of these plants have not been fully explored.

In this report, we describe on preliminary biological screening carried out on crude extracts derived primarily from a random selection of commonly occurring native plants.

This survey comprises 70 species, belonging to 61 genera and 35 families, which have been tested for antitumor activity against three different neoplasms *in vivo*, for cytotoxic activity in cell culture, for antimicrobial activity *in vitro*, and for the effect of each extract in a general rat behavior evaluation.

MATERIALS AND METHODS

Preparation of Plant Extracts—Freshly collected, botanically identified plant materials were dried, crushed coarsely and then extracted three times with 95% ethanol at room temperature. The combined filtrates were concentrated under reduced pressure in a water bath of 40° or less to a volume of about 200 ml, deffated extracting with n-hexane and the resulting ethanolic layers were evaporated to dryness. The dried residues were used for the test samples as suspensions in 0.5% carboxymethyl cellulose (CMC) unless otherwise indicated.

Acute Toxicity—The extracts were injected intraperitoneally into two albino mice weighing 18—25g and the minimum dose which killed both the animals within 24hr, was used to give a measure of the toxicity.

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Antitumor Activity—ddD-Mice, weighing 18—25g were used. 0.2 ml of cell suspension in 0.9% saline-glucose solution containing 10^7 ascites tumor cells of either Leukemia SN 36, Ehrlich ascites carcinoma or Sarcoma 180 was transplanted intraperitoneally into each mouse. The control group of animals in a given experiment consisted of 14 mice and the experimetal group, 7 mice. The materials to be tested were administered once a day by intraperitoneal injection, commencing the day after tumor transplantation, until the test animal died, the control group was received only 0.5% CMC.

The mean survival time of each group was calculated according to the formula indicated in the protocols of the Cancer Chemotherapy National Service Center.[8]

An increase in survival time of 25%, or more, compared to that of the control, was taken as being significant. In order to establish initial results as valid, all extracts which produced 25% or greater prolongation of survival time, were retested twice. Extracts which failed to give greater than a 25% activity in both retests were considered an negative, with respect to anti-tumor activity.

Cytotoxic Activity—Assay was performed by the monolayer culture method against HeLa-S3 strain. Cells were inoculated into YLE (yeast extract, lactoalbuminhydrolyzate and Earle's balanced salt solution) medium supplemented by 20% bovine serum and then incubated at 37° untill the monolayer of cell sheets was formed. Fully grown cells were removed from the culture bottle with trypsin-versene solution and resuspened in complete medium. After 3 ml of cell suspension (10^6 cells/ml) were incubated in a series of 15ml screwcap tubes, at 37° for 24hr, the original medium was removed and 3ml of fresh medium containing the extract were added. All samples were tested in serial tenfold dilutions resulting in final concentrations of 3 to 300μg/ml of medium. After incubation for 72hr, growth rate of cells was calculated from protein content which was measured by the method of Oyama and Eagle[9] using bovine serum albumin as a reference. The extracts showing greater than 50% inhibition of cell growth at the concentration of 30 μg/ml of medium, were considered active, and retested twice in order to establish the validity of the initial test results.

Antimicrobial Activity—The extracts were tested for the inhibitory effect on the growth of *Escherichia coli* and *Staphylococcus aureus*, using filter paper disk method. Nutrient broth agar was employed for the culture medium. All extracts tested were dissolved in 70% ethanol. Disks (Toyo filter paper No. 50, 6mm in diameter), moistened with known amounts of samples were dried in room temperature asceptically and placed on the inoculated agar plate. Following incubation at 37° for 24hr, the radius of zone of inhibition by test material was measured in millimeters. Extracts showing any degree of inhibition by visual inspection at the amount of 500μg/disk or less were considered rated active.

Animal Behavior—A hypocratic screening method described by Mälone, *et al.*[10] was applied. Two untrained non-fasted albino-rats (both sexes) in the weight range of 150-210g were administered with extracts intraperitonelly and placed in a bottomless observation

rink (65×54×8cm). The behavior of the rats in this unfamilliar environment was observed by rating procedures and recorded on the standardised word sheet of 30 symptoms. The extracts that showed pronounced activities similar to those of standard compounds, were considered active.

RESULTS AND DISCUSSION

The biological screening results were tabulated in Table I. Of these extracts tested, several extracts gave initial results suggestive of antitumor activity, however, subsequent replicate retests failed to confirm the initial activity. Although nine extracts showed initial results of cytotoxic activity, only two extracts, *Eupatorium lindleyanum* and *Convallaria keiskei*, gave positive results on replicate testing. It was interesting that *Pueraria thunbergii* and *Ampelopsis brevipedunculata* were found to cause detatchment of cell layer from the culture bottle.

The antimicrobial screening has uncovered that several plant extracts have some degrees of activity. *Artemisia capillaris* and *Crysanthemum lavendulaefolium* look most promising as containing potent antimicrobial substances.

In rat behavior evaluations, 55 extracts exhibited a number of different activities. Autonomic activities were shown in 27 kinds of the extracts. Nine of them, such as *Celosia cristata, Alnus japonica, Albizzia julibrissin, Pueraria thunbergii, Sohpora angustifolia, Sanguisorba officinalis, Picrasma ailanthoides, Lycium chinense* and *Styrax japonica* seem worthy of further investigations. Autonomic activities with CNS depresant activities were shown in 17 extracts, out of which *Akeiba quinata, Lithospermum erythrorhizon, Elaeagnus crispa var. typica, Actaea spicuta var. nigra, Styrax japonica, Artemisia viridissima* and *Carthamus tinctorius* showed activities in non-toxic doses. Autonomic and CNS stimulant activities were shown in 4 extracts and autonomic, CNS stimulant and CNS depressant activities in 3 extracts.

From only these results, it is too early to say that biological properties of plants which have a local reputation as medicinals are fully explored. It is possible that ethanol-insoluble active substances are present in a plant and are not extracted by procedure utilized. Volatile, biologically active substances would also not be detected in this screening procedure since the alcohol extracts were concentrated *in vacuo*. Further, the defatting of ethanol-soluble extractives with n-hexane could remove some biologically active principles. The possibility of antagonistic effects due to the heterogeneous character of constituents in the extracts should also be considered. However, the selection of plants for detailed chemical and biological studies would be guided by these results.

Table I—Results from the biological screening of plant extracts.

Plant names	Date coll.	Plant[a] part	Antitumor activity S-180 Dose mg/kg	S-180 T/C[b]	SN36 Dose mg/kg	SN36 T/C	Ehrlich Dose mg/kg	Ehrlich T/C	HeLa T/C	Antibacterial activity[e] *St. aureus*	Antibacterial activity[e] *E. coli*	Rat behavior Activity[g]	Rat behavior Dose range mg/kg	Acute toxicity mg/kg, ip
Amarantaceae														
Celosia cristata	10/69	wp.	150	121.6	150	85.0	150	109.2	81.0	In[f]	0.1	My, Rd	300-800	1000
Araceae														
Arisaema robustum	10/69	wp.	500	104.2	500	96.5	500	120.1	93.0	In	In	Rd	500-1000	1000<
Araliaceae														
Aralia cordata	10/70	rt.	500	100.0	500	85.0	500	92.0	117.0	0.5	In	i	500-1000	1000<
Tetrapanax papyriferum	10/70	br.	500	109.9	500	134.8[c]	500	113.0	112.0	In	In	i	500-1000	1000<
Betulaceae														
Alnus japonica	11/69	fr.	75	86.6	75	112.5	75	114.5	103.0	0.1	5	My, Rd	300-800	1000<
Borraginaceae														
Lithospermum erythrorhizon	11/69	rt.	150	96.3	150	109.0	150	87.5	71.0	In	0.5	My, Pp, Rd	300-800	1000
Caprifoliaceae														
Viburnum sargentii f. intermedium	10/70	wp.	1000	74.0	1000	97.0	1,000	101.6	97.0	In	In	i	500-1000	1000<
Chenopodiaceae														
Kochia scoparia	9/70	lv.	500	90.1	500	107.5	500	88.2	90.0	In	In	Riw	500-1000	1000<
Commeliaceae														
Commelia communis	9/69	wp.	500	100.7	500	111.7	500	96.0	78.0	In	In	My, Rd, SGlw	500-1000	1000<
Compositae														
Artemisia capillaris	9/69	wp.	300	114.2	300	96.0	300	104.4	98.0	0.1	0.1	i	500-1000	1000<
Artemisia viridissima	10/69	lv.	500	97.5	500	122.8	500	101.3	87.0	5	0.5	Dh, Ppw	300-800	1000
Aster spathulifolius	10/70	wp.	500	120.0	—	—	500	74.5	81.0	In	In	Riw	500-1000	1000<
Carthamus tinctorius	10/70	fl.	500	104.2	500	96.8	500	96.3	62.0	0.5	In	Dh, My, Tr	300-500	1000<

Crysanthemum lavendulaefolium	10/69	wp.	500	122.2	500	113.6	500	109.0	105.0	0.1	0.1	Mi, Rd	500-1000	1000<
Eupatorium lindleyanum	5/70	wp.	500	82.8	500	80.8	500	118.0	40.0	In	In	—	—	1000<
Ixeris dentata	5/70	wp.	1000	89.2	1000	86.6	1000	111.1	101.0	In	In	Ag,Mi,MAdw	500-1000	1000<
Petasites japonicus	11/69	fl.	300	90.8	300	129.0[c]	300	85.0	91.0	0.5	In	Rd	500-1000	1000<
Siegesbeckia pubescens	10/69	lv.	150	90.3	150	85.0	150	100.8	84.0	In	In	Mi, Pp, Rd	500-1000	1000<
Siphonostegia chinensis	10/70	wp.	1000	117.6	1000	88.4	1000	99.2	106.0	In	0.1	i	500-1000	1000<
Syneilesis palmata	10/69	wp.	300	85.0	300	93.7	300	86.4	80.0	5	In	Rdw	500-1000	1000<
Xanthium strumarium var. japonicum	11/69	sd.	150	97.7	150	117.0	150	92.7	28.0[c]	In	In	My, Pp, Rd	500-1000	1000<
Elaegnaceae														
Elaeagnus crispa var. typica	10/69	st.	500	111.0	500	100.3	500	119.9	90.0	In	In	Mi, Rd	300-800	1000<
Euphorbiaceae														
Galarhaeus esula	8/70	wp.	300	115.0	300	65.1	300	122.5	135.0	In	In	i	500-1000	1000<
Hyperaceae														
Hypericum ascyon var. genuinum	8/68	wp.	500	96.4	500	98.4	500	113.0	105.0	In	0.5	My	500-1000	1000<
Juglandaceae														
Platycarya strobilacea	10/69	lv.	150	100.0	150	98.5	150	113.0	45.0[c]	In	In	My. Rd	500-1000	1000<
Labiatae														
Amethystanthus excisus	9/70	wp.	1000	93.6	1000	102.7	1000	94.0	105.0	In	In	AG, Rdw	500-1000	1000<
Melampyrum setaceum var. genuinum	9/71	wp.	—	—	500	77.7	500	90.3	100.0	In	In	My,Slw,Trw	500-1000	1000<
Nepeta cataria	9/69	wp.	300	88.4	300	100.9	300	93.2	44.0[c]	0.5	In	My	500-1000	1000<
Perilla frutescens var. acuta	9/69	wp.	500	74.6	500	82.2	500	76.6	96.0	In	In	i	500-1000	1000<
Teucrium japonicum	9/69	wp.	500	103.0	500	89.6	500	101.8	104.0	—	—	My, Rd, Tr	500-1000	1000<
Lardizabalaceae														
Akebia quinata	10/66	lv.	150	94.3	150	91.3	150	112.8	108.0	In	0.5	My,Pp,Rdw	300-800	1000<
Leguminosae														
Aeschynomene indica	10/70	wp.	1000	117.6	1000	94.1	1000	90.3	119.0	In	In	i	500-1000	1000<
Albizzia julibrissin	10/69	lv.	50	91.1	50	94.9	50	98.1	78.0	In	In	Rdw, Hpw	300-800	1000<

Table I—Continued.

Plant names	Date coll.	Plant[a] part	Antitumor activity S-180 Dose mg/kg	S-180 T/C[b]	SN-36 Dose mg/kg	SN-36 T/C	Ehrlich Dose mg/kg	Ehrlich T/C	HeLa T/C	Antibacterial activity[e] *St. aureus*	Antibacterial activity[e] *E. coli*	Rat behavior Activity[g]	Rat behavior Dose range mg/kg	Acute toxicity mg/kg, ip
Gleditschia officinalis	10/69	fr.	500	93.7	500	104.0	500	92.9	78.0	In	In	AG, Mi, Rd	500-1000	1000<
Lespedeza bicolar var. typica	10/71	wp.	500	97.7	500	68.0	500	114.8	80.0	In	In	Mi, Rdw	500-1000	1000<
Lespedeza cunneata	9/71	lv.	300	102.5	300	97.8	300	122.5	91.0	In	In	i	500-1000	1000<
Lespedeza cyrtobotrya	10/70	lv.	1000	102.5	1000	92.0	1000	103.5	94.0	0.1	In	My, Rdw	500-1000	1000<
Lespedeza maximowicz	10/70	wp.	500	99.5	500	93.3	500	101.0	85.0	5	In	Mi, Rd	500-1000	1000<
Pueraria thunbergii	10/69	sd.	300	92.6	300	134.5[c]	300	89.7	82.0	0.5	In	i	500-1000	1000<
Pueraria thunbergii	10/69	rt.	300	92.1	300	91.9	300	100.8	ND[d]	0.5	In	My,AGw,Rdw	300-800	1000<
Sophora angustifolia	9/69	rt.	300	97.6	300	95.7	300	98.6	62.0	0.5	0.1	Rd	300-800	1000<
Liliacaee														
Convallaria keiskei	5/69	wp.	300	123.0	300	120.5	300	122.8	47.0	0.5	5	Hpw, NAd	500-1000	1000<
Menispermaceae														
Cocculus trilobus	10/71	wp.	—	—	300	111.6	300	86.3	82.0	In	5	My	500-1000	1000<
Nyctaginaceae														
Mirabillis jalapa	10/69	wp.	500	118.5	500	121.5	500	112.0	103.0	In	In	Mi, Rdw	500-1000	1000<
Orchidaceae														
Gastrodia elata	8/69	rt.	1000	116.7	150	133.9[c]	1000	112.0	110.0	In	In	Rd	500-1000	1000<
Phyllanthaceae														
Securinega suffruticosa	10/70	lv.	1000	107.2	500	136.5[c]	1000	99.0	102.0	In	In	i	500-1000	1000<
Polygonaceae														
Persicaria flaccida	9/69	wp.	500	97.0	500	105.9	500	89.7	73.0	In	In	My, Rdw	500-1000	1000<
Persicaria thunbergii var. coreana	9/69	wp.	500	85.7	500	85.5	500	97.6	100.0	In	5	Mi, Ppw, Rd	500-1000	1000<
Persicaria viscosa	6/70	wp.	1000	97.9	1000	89.3	1000	85.5	97.0	In	In	i	500-1000	1000<
Polygonum aviculare	6/69	wp.	300	97.0	300	126.1[c]	300	94.8	41.0[c]	In	In	Pw, Rd	500-1000	1000<
Polypodiaceae														
Pteridium aquilinum var. glabrum	9/69	wp.	500	106.3	500	126.2	500	104.4	100.0	In	In	My, Rd, Tr	500-1000	1000<

Primulaceae														
Lysimachia barystachys	8/70	wp.	300	125.0	300	101.8	300	110.3	91.0	5	0.1	MAd, My	500-1000	1000<
Ranunculaceae														
Actaea spicata var. nigra	9/69	rt.	150	101.0	150	113.2	150	100.8	45.0[c]	In	In	Mi,Pw,MAd,Mi	500-1000	1000<
Clematis apiifolia	6/69	wp.	150	102.8	150	95.1	150	106.2	103.0	In	5	Rd	500-1000	1000<
Clematis tubulosa	9/70	lv.	500	85.8	500	86.3	500	110.5	98.0	0.5	In	i	500-1000	1000<
Lycoctonum longicassidatum	8/70	wp.	75	123.7	75	101.8	75	109.4	119.0	In	5	AG,MAiw,Tr	30-100	150
Rhammaceae														
Rhamnus davurica	9/70	lv.	500	93.3	500	105.2	500	101.8	109.0	—	—	i	500-1000	1000<
Rosacceae														
Agrimonia pilosa var. japonica	8/69	wp.	500	104.0	500	96.0	500	123.9	78.0	In	In	Mi	500-1000	1000<
Sanguisorba officinalis	10/69	rt.	500	85.7	500	109.8	500	97.9	96.0	0.5	5	My, Rdw	300-800	1000<
Rutaceae														
Zanthoxylum piperitum	9/69	sd.	500	91.4	500	70.9	500	89.7	24.0[c]	In	0.5	My, MAiw	500-1000	1000<
Scrophulariaceae														
Melampyrum roseum var. typicum	10/70	wp.	1000	116.5	1000	100.2	1000	124.0	89.0	In	In	Mi	500-1000	1000<
Simarubraceae														
Picrasma ailanthoides	10/70	lv.	150	90.9	150	91.3	150	85.4	101.0	In	In	Rdw	300-800	1000<
Solanaceae														
Lycium chinense	8/69	lv.	300	101.0	300	83.0	300	89.3	69.0	In	0.1	My,AG,Rdw	300-800	1000<
Solanum nigrum	8/69	wp.	1000	101.0	1000	86.4	1000	97.3	74.0	In	5	My,Rdw	500-1000	1000<
Styracaceae														
Styrax japonica	10/70	sd.	150	97.0	150	100.9	150	87.2	88.0	5	In	My, Rdw	300-800	1000
Umbelliferae														
Cnidiumoffiicinale	10/69	rt.	150	92.8	—	—	150	105.3	69.0	In	5	MAi,My,SGl	500-1000	1000<
Verbenaceae														
Clerodendron trichotomum	8/69	lv.	500	133.8[c]	300	120.0	300	135.0	25.0[c]	In	In	My,Rdw,Tr	500-1000	1000<
Vitex rotundifolia	9/71	wp.	1000	101.0	1000	86.6	1000	117.7	90.0	In	5	My	500-1000	1000<
Vitaceae														
Ampelopsis brevipedunculata	9/70	lv.	750	86.1	750	86.4	750	99.0	ND[d]	In	0.5	Mi, Cvw	500-1000	1000
Vitis amurensis	9/69	lv.	500	96.9	500	109.0	500	103.2	102.0	5	In	My, Rdw	300-800	1000<

a) wp, whole plant; lv, leaves; sd, seeds; rt, roots; fl, flowers; st, stalks; br, barks; fr, fruits.

b) T/C, $\frac{\text{the mean survival time of the treated group}}{\text{the mean survival time of the control group}} \times 100$

or $\frac{\text{protein content of the treated group}}{\text{protein content of the control group}} \times 100$

c) Negative result after retest.

d) ND, could not be measured (cells were detached from the bottles)

e) Minimal inhibitory amounts of samples, mg in a disk.

f) In, Inactive up to 5mg/disk of sample.

g) AG, abdominal griping; Ag, analgesia; Cv, convulsion; Dh, diarrhea; Hp, hypermia; i, very little activity was seen at the highest dose employed; MAd, decrease of motor activity; MAi, increase of motor activity; Mi, miosis; My, mydriasis; Pp, palpebral ptosis; Rd, decrease of respiration rate; Ri, increase of respiration rate; SGl, screen grip loss; Sl, salivation; Tr, tremor; w, weak.

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