[CANCER RESEARCH 43, 5775–5777, December 1983]

Plants with Potential to Enhance Significant Tumor Growth[1]

Mary E. Caldwell[2] and Willis R. Brewer

Pharmaceutical Sciences Department, College of Pharmacy, University of Arizona, Tucson, Arizona 85721

ABSTRACT

Of 20,000 plant extracts submitted to the National Cancer Institute antitumor screens from 1960 to 1980, over 95% exhibited inadequate tumor-inhibiting activity or promoted tumor growth. Of these, 50 extracts representing 42 species showed significant levels of tumor growth enhancement compared to controls on the basis of tumor weights when the extracts were administered to test and control animals dosed equally on the basis of implanted tumor weights.

Because of the continuing threat of environmentally induced or promoted cancer in the human population, the species identified in this report are deemed worthy of studies designed to quantitate the risks of contact by botanists, hikers, plant hobbyists, and field workers. Even more fundamental, studies of these plants could provide knowledge of new compounds under the influence of which tumor growth is enhanced. Further studies might also reveal the mechanisms of this tumor enhancement.

INTRODUCTION

The authors initiated a National Cancer Institute research contract for the University of Arizona College of Pharmacy in 1960, for the purpose of facilitating the evaluation of antitumor properties of plants found growing in the Southwestern United States and in Northwestern Mexico. During the next 20 years, 2,500 species were collected, processed, and 20,000 extracts were prepared by the research staff for submission to the CCNSC[3] for screening in mice and other animals against 7 types of tumor implants. One thousand of the extracts exhibited tumor-inhibiting properties. For example, the seeds of *Caesalpinia gilliesii* (*Leguminosae*), the yellow flowering desert bird of Paradise, was reported out of the primary screen with a weight of tumor in treated animals compared to the weight of tumor in control animals percentage value of 14, corresponding to a 5WM Walker carcinosarcoma 256 (i.m.) tumor reduction of 88%. Approximately 19,000 of the extracts lacked adequate tumor-inhibiting properties in the primary screen to be considered for further antitumor investigation. Among these there were 300 extracts which exhibited prominent tumor growth enhancement, beginning at the 100% weight increase of test tumors over controls. Fifty of them exhibited significant activity in the range of 175 to 466% increases.

[1] This work was supported by Contract Fund NCI-NO1-CM-33750 from the National Cancer Institute. Additional support was provided by the Elsa U. Pardee Foundation, the Arizona Division of the American Cancer Society, and the University of Arizona Alumni Fund.

[2] To whom correspondence should be addressed, at Department of Pharmaceutical Sciences, University of Arizona College of Pharmacy, Tucson, Ariz. 85721.

[3] The abbreviation used is: CCNSC, Cancer Chemotherapy National Service Center.

Received August 2, 1982; accepted September 1, 1983.

MATERIALS AND METHODS

Plant Collections. Early in 1960, plant species which were collected were identified in the field by individuals with native healing experience in localities spread from the southern portions of California, Arizona, New Mexico, and Texas through the coastal, desert, and mountainous areas of Northwestern Mexico. These plant identities were corroborated by taxonomists of the University of Arizona Herbarium, where permanent plant mountings were deposited for future reference. Collections varied from 2 to 25 kg. By the end of the first year, it became the practice to collect other species found in the vicinity of those used in folk medicine by the native healers.

Extractions. With smaller collections, the whole plant was shade dried and subjected to various extraction procedures. Larger collections were subdivided into plant parts, depending upon the morphology of the species, and then extracted. A part of each sample was subjected to a hot, aqueous infusion process which represented the folk medicine procedure of preparing a "tea." This filtered aqueous extract was lyophilized and stored frozen. Each sample was also subjected to a 50:50 ethanol:chloroform maceration, filtration, and a concentration procedure yielding a semisolid which was stored frozen. Aqueous extracts (A) and ethanol:chloroform extracts (B) were shipped by mail to the assigned facilities of the CCNSC for antitumor screening against tumor implants in experimental animals. Extracts were further fractionated, as indicated by test results received from CCNSC, when further delineation of the active chemical constituents was believed essential. These fractions were indicated by the letter F. Isolated constituents prepared directly from the original plant material were identified by the letter D (see Table 1).

Screening. The crude extracts and fractions described above were mailed frozen to CCNSC laboratories, where they were received by staff members, thawed, suspended in appropriate media, injected into experimental animals or embryonated egg tissue bearing solid tumor implants, which were weighed at implant and at sacrifice, and compared with controls, all in accordance with the protocols of the CCNSC (1). Results of the statistically interpreted data were reported to the authors by means of Screening Data Summary forms and were also reported in *Cancer Research* as the extracts were eliminated from further investigation because of tumor enhancement or lack of adequate tumor inhibition (1). Approximately 19,000 were eliminated in the primary screen by this procedure. Among them, only 300 were prominent in terms of tumor growth enhancement. Of these, only 50 provided significant levels of growth of the tumor implants in the test period of 8 to 12 days of treatment begun not less than the third day following implant.

RESULTS

Table 1 identifies the extracts or fractions of the species which produced significant levels of tumor enhancement, expressed as a mean percentage of tumor weight of treated mice compared with controls. These levels are reported in 3 columns beginning with the 175% level (well above the minimum primary screen cut-off level for elimination of the extract from further tumor inhibiton testing). The second column begins with the 185% level, and the third and most important column begins with the 195% level. The 13 extracts falling in the third column represent the highest level of tumor growth enhancement. The B extract

Table 1

Effect of plant extracts on growth of transplantable tumors

Plant: scientific names/common names	Family	Plant parts and extracts[a]	Tumor identification and dosage (-m or -s[b])	Tumor evaluation (maximum T/C%[c])		
				175–184%	185–194%	195% or above
Abies concolor (Gordon et Glendinning) Hoopes/ silver fir, white fir	Pinaceae	FO21	7D1-m		188	
	Pinaceae	FO23	7D1-m	184		
	Pinaceae	FO62	7D1-s			224
Abronia villosa Wats./sand verbena	Nyctaginaceae	PL-A	8H1-s		194	
Aesculus californica (Spach.) Nutt./California buckeye	Hippocastinaceae	LF-ST-B	3FV-s		185	
Agave americana L., var. *marginata* Trel./variegated century plant	Amaryllidaceae	LF-B	8H1-s	181		
Alnus arguta (Schlecht) Spach./alder	Betulaceae	BK-A	5WM-m	177		
Alnus firmifolia Fernald/alder	Betulaceae	FO09	3FV-m	175		
	Betulaceae	FO20	7D1-m			286
Alnus oblongifolia Torr./alder	Betulaceae	LF-ST-A	3CA-s	175		
Artemisia ludoviciana Nutt. subsp. *albula* (Wooton) Keck/wormwood sagebrush	Compositae	PL-A	8H1-m	175		
Caesalpinia pulcherrima L. Sw./red bird of Paradise, dwarf poinciana	Leguminosae	SD-pods-B	8H1-m		194	
Calandrinia ciliata (Ruiz et Pavon) DC./red maids	Portulacaceae	PL-B	3FV-s	176		
Cercis occidentalis Torr./western redbud	Leguminosae	PX-A	8H1-m	180		
Chenopodium fremontii Wats./goosefoot pigweed	Chenopodiaceae	PL-B	3SA-m		192	
Cucurbita foetidissima H.B.K./buffalo gourd	Cucurbitaceae	RT-B	3SA-m		194	
Cucurbita foetidissima H.B.K./buffalo gourd (fresh plant)	Cucurbitaceae	PX-B	FV			247
Cucurbita foetidissima H.B.K./buffalo gourd (dried plant)	Cucurbitaceae	PX-B	FV			314
Cyperus globulosus Aubl./flat sedge, nut sedge	Cyperaceae	FL-LF-ST-B	3FV-m	179		
Eriogonum jamesii Benth./wild buckwheat, antelope sage	Polygonaceae	PL-A	7D1-m		192	
Erythrina breviflora DC./coral tree	Leguminosae	FL-LF-ST-A	7D1-m	183		
Escontria chiotilla (Weber) Ross/chiotilla	Cactaceae	ST-B	8H1-B		187	
Gaillardia pinnatifida Torr./blanket flower	Compositae	PX-A	3CA-s		190	
Heracleum lanatum Michk./common cow parsnip	Umbelliferae	FR-LF-ST-A	3CA-m	182		
Jatropha macrorhiza Benth./jatropha	Euphorbiaceae	FL-LF-ST-A	7D1-m		186	
	Euphorbiaceae	FO36	7D1-m			209
	Euphorbiaceae	F103	5WA-m	181		
Koeberlinia spinosa Zucc./Crucifixion Thorn	Koeberliniaceae	FL-ST-A	3CA			206
Laphamia dissecta Torr./rock daisy	Compositae	PL-A	8H1-m	184		
Lepidium virginicum L./pepper grass	Cruciferae	DOO6	5WA-m	178		
Maclura pomifera Schneid./Osage orange	Moraceae	FL-LF-ST-B	3CA-s	182		
Mammillaria arizonica Engelm./Arizona pincushion	Cactaceae	PL-A	8H1-m	180		
Pectis cylindrica (Fern.) Rydb./no common name	Compositae	PL-A	3CA-s		191	
Penstemon barbatus (Cav.) Roth, var. *torreyi* (Benth) Keck/beard tongue	Scrophulariaceae	PL-A	8H1-m		192	
Phrygilanthus sonorae (S. Wats) Standl./no common name	Loranthaceae	PL-A	8H1	183		
Potentilla fruticosa L./bush cinquefoil	Rosaceae	FL-LF-R	3CA			212
Proboscidea sinaloensis Van Eselt./devil's claw	Martyniaceae	PL-A	3FV-m	180		
Prunus cerasifera Ehrh./cherry plum	Rosaceae	FO13	5WA-m	181		
Quercus wislizenii A.DC., var. *frutescens* Engelm./interior live oak	Fagaceae	LF-ST-A	3FV			208
Rhododendron occidentale (T. et G.) Gray/western azalea	Ericaceae	LF-ST-B	3FV-s	177		
Rhus laurina Nutt.	Anacardiaceae	FR-LF-ST-A	3FV-m		192	
Rhus microphylla Engelm./laurel sumac	Anacardiaceae	LF-ST-B	3SA-m		194	
Rosa hybrid, Red Talisman rose	Rosaceae	FO15	7D1-m			207
	Rosaceae	FO16	7D1-m	181		
Salix irrorata Anderss/willow	Salicaceae	LF-ST-B	7D1-m	184		
Sapindus saponaria L., var. *Drummondii* (Hook et Arn.) L. Benson/western soapberry	Sapindaceae	BK-A	7D1			201
Scutellaria tesselata Epling/skullcap	Labiatae	PL-B	8H1-s			195
Solanum bicolor Willd./nightshade	Solanaceae	PX-A	7D1-m			220
Thevetia peruviana Schum./yellow oleander	Apocynaceae	FR-B	5WM			466
Yucca thornberi McKelvey/Thornber yucca	Liliaceae	FR-A	8H1-s	191		

Abies concolor (Gordon et Glendinning) Hoopes

BK-A	FO21	Resin type compound from benzene extraction
BK-A	FO23	Benzene fraction
BK-A	FO62	Methyl ethyl ketone-soluble fraction of the water-soluble portion of benzene fraction

Alnus firmafolia Fernald

FL-LF-ST	FO09	Hot water fraction
FL-LF-ST	FO19	Hot water fraction after extraction with benzene and chloroform

Table 1—*Continued*

Plant: scientific names/common names	Family	Plant parts and extracts[a]	Tumor identification and dosage (-m or -s[b])	Tumor evaluation (maximum T/C%[c]) 175–184%	185–194%	195% or above
Jatropha macrorhiza Benth.						
PL	FO36	Non-polar eluates from an alumina column chromatography of a defatted chloroform extract				
PL	F103	Methanol-soluble portion of a defatted chloroform extract				
Lepidium virginicum L.						
FR-LF-RT-ST	DO06	Water portion of a defatted ethanol extract after partition between chloroform and water				
Prunus cerasifera Ehrh.						
LF-ST	FO13	Water portion of a defatted ethanol extract after partition between chloroform and water				
Rosa hybrid var. Red Talisman						
FL-LF-ST	FO15	Tannin portion of a water extract				
FL-LF-ST	FO16	Non-tannin portion of a water extract				

[a] Plant parts: PL, entire plant; FL, flowers; FR, fruit; LF, leaves; ST, stems; RT, root; PX, growth above ground; BK, bark; SD, seeds.
Extracts: A, crude, lyophilized aqueous; B, alcohol:chloroform, concentrated to a semisolid consistency; FO/DO, fractions of crude extracts, as follows:
[b] Dose assay: multiple, tumor identification-m; single dose, tumor identification-s.
Tumors: 3SA (Sarcoma 180); 3CA (Adenocarcinoma 755); 7D1 (adenocarcinoma of duodenum); 3FV (solid Friend virus leukemia); 8H1 (HS1, human sarcoma); 5WM (Walker carcinosarcoma 256, i.m.); 5WA (Walker carcinosarcoma 256, s.c.).
[c] T/C%, weight of tumor in treated animals compared to the weight of tumor in control animals (expressed as precentage). Only maximum T/C% is given.

of the fruit of *Thevetia peruviana* tested with implanted Walker carcinomasarcoma 256 by intramuscular administration in mice produced a mean tumor weight of treated mice compared with controls of 466% tumor enhancement in the primary screen, the highest recorded result. The collections were made from the abundant plantings of the yellow oleander scattered about the campus of the University of Arizona.

DISCUSSION

The species reported as possessing constituents which may result in tumor enhancement on introduction into experimental animals may also possess possible tumor-enhancing activity on absorption through the skin of those in the human population who come in contact with these plants. Gardeners, students, botanists, weed cutters, campers, and plant enthusiasts may be exposed to such hazards. It seems appropriate to invite the attention of other investigators to the desirability of determining the risks involved in various types of contact with these species by plant workers. Furthermore, the more fundamental interest in these plants, that of determining the nature of the active phytochemical compounds capable of enhancing tumor growth, should be pursued by investigators. Knowledge of the mechanisms by which these compounds enhance growth may be of great significance.

ACKNOWLEDGMENTS

The authors are grateful to Dr. Charles T. Mason, Jr., Director of the University of Arizona Herbarium, for authentication of species; to Robert Barr, the staff collector; and to Richard Wiedhopf, for crude extract processing and record keeping.

REFERENCES

1. Abbott, B. J., Leiter, J., Hartwell, J. L., Caldwell, M. E., Beal, J. L., Perdue, R. E., Jr., and Schepartz, S. A. Screening data from the Cancer Chemotherapy National Service Center screening laboratories. XXXIV. Plant extracts. Cancer Res., *26* (Part 2): 761–928, 1966.