

Limitations of a Random Screen: Search for New Anticancer Drugs in Higher Plants¹

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The inherent limitations of a random search of higher plants for novel cancer chemotherapeutic agents are reviewed—the National Cancer Institute's (NCI) Anticancer Screening Program. A graphic summary of plant exploration for the NCI is depicted on a world map showing 58 floristic regions. It is estimated that less than one-half of the world flora is economically feasible for collection. Random screening of approximately 35,000 species has led to guidelines that precluded further screening of all species in 333 genera and another 2,905 species in 1,773 genera. These taxa are reported to represent one-half to two-thirds of the species that characterize vegetation in geographic areas most frequently explored for the NCI. It is estimated that 40,000 untested species of flowering plants are readily available and meet the NCI guidelines for antitumor screening. However, because of apparent diminishing returns from random screening of chemicals in plant genera, it is suggested that a good representation of the diversity in the world flora could be obtained in 10,000 collections, if random sampling follows the phytogeographic outline that is recommended. Modifications to the screening methodology might be geared to an expected point of diminishing returns for discovering novel chemotypes. Additionally, the NCI should continue random screening to increase the development of new anticancer drugs; past screening has generated a tremendous wealth of data. Finally, in this paper, the author proposes to utilize lists representing taxa commonly collected for the NCI to create a manual of worldwide common plants.

In 25 yr, the National Cancer Institute (NCI) screened more than 120,000 plant extracts from 35,000 species for novel anticancer agents. Some promising discoveries are: taxol, indicine-n-oxide, phyllanthoside, and homoharringtonine, isolated from *Taxus brevifolia* Nutt., *Heliotropium indicum* L., *Phyllanthus acuminatus* Vahl, and *Cephalotaxus harringtonia* (Knight ex Forbes) K. Koch, respectively (M. Suffness, pers. comm.).

From 1960 until 1982, about ½ of the plant samples were supplied to the NCI through a cooperative agreement with the Agricultural Research Service (ARS) of the United States Department of Agriculture (USDA). This agreement, expending nearly ½ million dollars annually since 1972, was terminated as a result of widespread 1981 budget cuts of federal programs. Other substantial suppliers were Commonwealth Scientific and Industrial Research Organization (Australia), Central Drug Research Institute (India), National Defense Medical Center (Taiwan), University of Arizona, University of Costa Rica, University of Concepcion (Chile), University of Brazil (Rio de Janeiro), and the University of Hawaii (J. L. Hartwell, pers. comm.).

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The NCI procedure followed a stepwise-collecting and -testing protocol to isolate methodically bioactive chemicals in plants for ultimate evaluation in the treatment of human cancers. Initially, species were randomly collected in small amounts from ½–2 kg dried weight. Samples of roots, bark, twigs, leaves, or any combination of these were submitted to a routine extraction and prescreening procedure (Suffness and Douros, 1979). Extracts from approximately 10% of 35,000 species tested were active, i.e., these extracts significantly inhibited tumor growth, increased the life span of leukemic mice, and/or were cytotoxic in vitro (Geran et al., 1972). Selected active species were then re-collected in large quantities (50–250 kg dried) to isolate the active agent(s). Occasionally, massive samples of one to many tons were needed to supply sufficient amounts of the active agent for preclinical and/or clinical studies.

In this paper, the terms “tested” and “screened” refer to the “prescreen” procedures (Suffness and Douros, 1982). Abbreviations cited for tumors (screens, bioassays, or test systems) follow Hartwell (1976, Table 2), and include: WA (Walker carcinoma 256, rat), SA (Sarcoma 180, mouse), CA (Adenocarcinoma 755, mouse), KB (Human epidermoid carcinoma of the nasopharynx, cell culture), LE (Lymphoid leukemia L-1210, mouse), LL (Lewis lung carcinoma, mouse), and PS (Lymphocytic leukemia P-388, mouse). The duration for which these were employed in the prescreen is shown in Suffness and Douros (1979, Fig. 2); PS and KB were the tumors primarily used since 1969.

“Random” collecting was not entirely random (Spjut and Perdue, 1976). It is broadly defined in this paper as sampling without a preconceived selection of species. This is not to imply that samples were obtained without thought. An initial tendency was to shortcut the discovery process by collecting plants on the basis of folkloric, chemotaxonomic, and climatic relationships, but, as screening experience progressed, there was a tendency to minimize bias in collecting so that promising compounds would not be missed. Guidelines focused more on excluding rather than selecting plant taxa—species in 1971, families to a limited extent from 1972–1975, and, finally, genera in 1979. Taxa were precluded from further screening for 2 reasons: (1) many samples had been tested without yielding significant activity, or (2) active agents had been isolated and it was apparent that continued screening would not lead to isolation of new compounds. Unless a change was made in the screening methodology, there was little to gain from collecting additional species of figs (*Ficus*), for example, because after testing more than 10% of the species in *Ficus*, it was clearly evident that PS activity was infrequent and unlikely to exceed marginal criteria; similarly in milkweeds (*Asclepias*) activity would predictably occur in KB, and the cytotoxic agents were invariably found to be cardenolides. Genera excluded from further screening are listed in this paper (Table 1, 2).

The random acquisition of higher plant samples was thus carefully guided to avoid duplication of those genera and species already screened. With the exception of excluded plants and those not occurring in sufficient abundance, species were sampled as encountered in selected geographic areas. Additionally, plants in genera not previously tested and others reportedly used for certain medicinal purposes were especially sought out but “random” collecting was not entirely abandoned in lieu of this. These modalities were sometimes combined into an overall strategy

TABLE 1. GENERA WITH 100 OR MORE EXTRACTS SCREENED FOR ANTITUMOR ACTIVITY.^a

Abies—50	Citrus—12	Indigofera—700	Psidium—140
Abutilon—100	Clematis—250	Inga—200	Psychotria—700
Acacia—800	Clerodendrum—400	Ipomoea—500	Quercus—450
Acalypha—450	Clethra—68	Jacaranda—50	Randia—300
Acer—200	Clusia—145	Jasminum—300	Rapanea—200
Aegiphila—160	Coccoloba—150	Jatropha—175	Rhamnus—160
Agave—300	Combretum—250	Juniperus—60	Rhus—250
Albizia—150	Cordia—250	Lantana—150	Rosa—250
Alchornea—70	Cornus—4	Liatris—40	Rubus—250
Allium—450	Crotalaria—650	Linum—230	Rumex—200
Allophylus—190	Croton—750	Litsea—400	Salix—500
Alnus—35	Cryptocarya—250	Lobelia—300	Salvia—700
Aloe—332	Cupania—55	Lonchocarpus—150	Sambucus—40
Amaranthus—60	Cyperus—550	Lonicera—200	Sapium—120
Annona—120	Dalbergia—300	Lupinus—200	Scaevola—100
Ardisia—400	Datura—10	Macaranga—280	Senecio—3,000
Artemisia—400	Derris—80	Manilkara—70	Sida—200
Asclepias—120	Desmodium—450	Maytenus—225	Siparuna—150
Aspidosperma—80	Dioscorea—600	Miconia—700	Sloanea—120
Aster—500	Diospyros—500	Mikania—252	Smilax—350
Astragalus—2,000	Dombeya—350	Mimosa—500	Solanum—1,700
Atriplex—200	Drypetes—200	Morinda—80	Solidago—100
Baccharis—400	Elacocarpus—200	Myrcia—500	Sterculia—300
Bauhinia—300	Erigeron—200	Myrica—35	Strychnos—200
Berberis—450	Erythrina—100	Nectandra—100	Styrax—130
Betula—60	Erythroxylum—250	Ocotea—400	Swartzia—100
Bidens—230	Eucalyptus—500	Oenothera—80	Symplocos—350
Bridelia—60	Euclea—20	Opuntia—250	Syzygium—500
Buddleja—100	Eugenia—1,000	Palicourea—200	Tabebuia—100
Byrsonima—120	Euonymus—176	Passiflora—500	Tabernaemontana—100
Caesalpinia—100	Eupatorium—1,200	Penstemon—252	Tecoma—16
Calliandra—100	Euphorbia—2,000	Persea—150	Tephrosia—300
Calyptanthus—100	Fagara—250	Phoradendron—190	Terminalia—250
Canthium—200	Faramaea—120	Phyllanthus—600	Thalictrum—150
Capparis—250	Ficus—800	Physalis—100	Theobroma—30
Casearia—160	Fraxinus—70	Phytolacca—35	Tibouchina—200
Cassia—600	Garcinia—400	Pinus—100	Tournefortia—150
Cassine—40	Gardenia—250	Piper—2,000	Trema—30
Casuarina—45	Gnidia—100	Pithecellobium—200	Trichilia—300
Ceanothus—55	Grewia—150	Pittosporum—150	Vaccinium—400
Cecropia—100	Guarea—170	Plantago—265	Verbena—253
Celtis—80	Gauteria—250	Pluchea—50	Vernonia—1,000
Centaurea—600	Helenium—40	Podocarpus—100	Viburnum—216
Cestrum—150	Helianthus—110	Polygonum—300	Virola—60
Chenopodium—150	Helichrysum—500	Populus—35	Vismia—35
Chrysanthemum—200	Heliotropium—250	Potentilla—500	Vitex—250
Chrysophyllum—150	Hibiscus—300	Pouteria—50	Vitis—70
Cinnamomum—250	Hypericum—400	Protea—130	Xylopia—150
Cirsium—150	Hyptis—400	Protium—90	Yucca—40
Cissus—350	Ilex—400	Prunus—430	Zanthoxylum—30
Citharexylum—115			
			Total: 201 genera
			58,956 species

^a Numbers following genera are of species in genus (Willis, 1973).

of random collecting that was targeted for geographical areas predetermined to have high concentrations of untested genera and preselected medicinal plants (USDA Memorandum, 1979c).

Retrospective studies on the relationships of antitumor activity with taxonomy

