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A Taxonomic Review of Western Australian Plants Screened in KB Cell Culture and Other Bioassays in the Search for New Anticancer Drugs

Richard W. Spjut[1]

ABSTRACT

Antitumor screening data in KB Cell Culture (KB) are presented for extracts of plant samples collected by the author from Western Australia (WA) during Aug–Oct 1981. This includes active ($\leq ED_{50}$ 20 µg/ml) and inactive test results received from Purdue University for 181 samples represented by 127 species in 77 genera. It also includes published and unpublished reports on other WA samples collected largely by the author and two by CSIRO–during the mid 1960's—that were active in KB and other antitumor bioassays from screening done at the Research Triangle Institute (RTI) in North Carolina and at the National Cancer Institute (NCI). These data are summarized by family, genus, species, and plant parts. Fifty-one species in 40 genera are reported active. Although the NCI had screened samples from approximately 35,000 species of plants, 28 genera in 15 families were new discoveries for antitumor activity at the genus level based on a NCI record of all genera that had been screened by Feb 1980. Antitumor activity in Restionaceae and Stackhousiaceae were new at the family level. Root was the most frequently active plant part, especially in Fabaceae and Proteaceae. The most cytotoxic species were *Daviesia podophylla* (Fabaceae) and *Lepidobolus quadratus* (Restionaceae). In view of KB activity in Restionaceae and one species of dryland sedge in the Cyperaceae tribe Schoeneae that showed significant antitumor activity in the L1210 assay, and that essentially all plant-derived anticancer drugs discovered by the NCI were active in L1210, it is suggested that further screening of Schoeneae and the related Restionaceae will lead to discovery of new anticancer drug(s).

INTRODUCTION

This paper reports antitumor screening in KB Cell Culture ("9KB") for 181 of 758 plant samples collected by the author in Western Australia (WA) during Aug–Oct 1981, and it summarizes other antitumor active species collected in WA. The KB assay, a culture of human cancer cells of the nasopharynx in artificial media (Eagle & Foley, 1958; Foley et al., 1958), was routinely employed—among other bioassays (mostly P-388 Leukemia)—by the National Cancer Institute

[1] Richard Spjut, Principal Investigator, World Botanical Associates (WBA), Inc., PO Box 81145, Bakersfield, CA. 93380-1145. The author contributes freely to advance the scientific knowledge in the discovery of new anticancer or other medicinal drugs that may be derived from this report. Neither the WBA nor the author expects to receive any payment or profit from this contribution, except, however, where any royalties are received by others from pursuing the taxonomic leads laid out in this paper, that they should also be equally shared with the author in the same manner as would apply to local practitioners of traditional medicine. The author applied his scientific training and knowledge gained from field experience in the taxonomic selection of plant species and samples in WA. Submitted for peer review Jan 2014, updated May 2014. No response from the NCI Natural Products Group and affiliated chemists. Placed online Oct 24, 2014 as Memoirs of the World Botanical Associates (WBA Memoirs).

(NCI) in preliminary antitumor screening of natural products from 1960 to 1979 (Suffness and Douros, 1979, 1982).

Although the NCI has since employed a 60-cell line for preliminary antitumor screening (Boyd 1992; Boyd & Paull 1995; Cragg 2002), KB screening data reported in this paper may still provide leads to new anticancer drugs, especially where taxonomy indicates there is little or no information on the active compounds as is the case for many WA plants (Spjut 1982, 1985), and when KB activity can be correlated with activity in other antitumor bioassays (Hartwell 1976; Powell & Smith 1980; Cragg & Newman 2005).

Antitumor screening of extracts from samples of an estimated 35,000 plant species (Spjut, 1985) discovered 3,394 species (~10%) active in one or more bioassays (Suffness and Douros 1982). An active species is one from which one or more extracted samples significantly inhibited tumor growth (Geran et al. 1972); the sample(s) and genus associated with the active extract are also regarded active (Spjut & Perdue 1976). Activity in KB is defined by a concentration level of the test substance at which 50% of the cancer growth is inhibited (assumed to be proportional to the amount of protein synthesis), expressed as $ED_{50} \leq 20$ µg/m (Spjut et al. 1986). Additional criteria for a "confirmed active" species are given under material and methods. Not all extracts were screened in KB, approximately 95% judging from my superficial review of one report listing 1,422 extracts and their tumors (Abbott et al. 1967). Compounds isolated from approximately 1% of the active species reached clinical trials (Hartwell 1976; Perdue 1982; Cragg & Newman 2003).

Clinically evaluated antitumor compounds (and their plant sources) discovered from KB screening include bruceantin (*Brucea antidysenterica* J. F. Mill.), camptothecin (*Camptotheca acuminata* Decaisne), ellipticine [*Ochrosia coccinea* (Teijsm.& Binn.) Miq., *Ochrosia moorei* (F. Muell.) F. Muell. ex Benth.], emetine [*Psychotria ipecacuanha* (Brot.) Stokes], homoharringtonine [*Cephalotaxus harringtonia* (Knight ex J. Forbes) K. Koch], maytansine [*Gymnosporia serrata* (A. Rich.) Loes.], nitidine [*Zanthoxylum gilletii* (De Wild.) P.G. Waterman], taxol (*Taxus brevifolia* Nutt.), triptolide [(*Tripterygium wilfordii* Hook. f.); Hartwell 1976; Suffness & Douros 1979, 1982; Perdue 1982), and combretastatins [*Combretum caffrum* (Eckl. & Zeyh.) Kuntze; Spjut (2010)]. KB active plants advanced to clinical trials through fractionation and isolation of the active compounds (in plant samples) that subsequently showed activity in other bioassays, particularly the mouse leukemia assay, L1210 (Hartwell 1976, Table 1, "LE"), or in the case of *Camptotheca* that was initially active in L1210 ("LE"), KB activity was found to be correlated with L1210 activity upon fractionation of *Camptotheca* extracted recollections (Wall et al. 1976). Tubulosine isolated from *Alangium* (Hartwell 1976, Table 20) is biochemically related to emetine (Gupta & Siminovitch 1977; Klausmeyer et al. 2008), which also occurs in *Alangium*, and deoxypodophyllotoxin isolated from various plants, originally *Podophyllum* spp., was active in KB and L1210 (Hartwell 1976). These or related compounds,

including semi-synthetic derivatives, are currently employed, or continue to undergo clinical evaluation, in cancer chemotherapy (Cragg & Newman 2005; Akinboye & Bakare 2011; Pan et al. 2012).

Many KB active compounds that failed to demonstrate clinical activity turned out to be more cytotoxic than specific to inhibiting tumor growth (Hartwell 1976; Suffness and Douros, 1979). This is also evident by the close relationship between KB active species and poisonous plants (Spjut 2005). Nevertheless, the KB active compounds represent a broad spectrum of chemical groups—quinines, cucurbitacins, iridoids, sesquiterpenes, triterpenes, diterpenes, lignans, flavonoids, steroid lactones, quassinoids, ansamitocins, and various alkaloids, but not tannins, saponins, or proteins (Hartwell 1976, Gutiérrez 2007)—that have potential for use in other therapies; examples are nitidine for treating malaria (Bouquet et al. 2012) and neriifolin as an insecticide (McLaughlin et al. 1980).

The antitumor active species from WA will be summarized by family/genus/species, plant parts, estimated number of species in each genus screened, number of extracts screened and active in the genus, geographical distribution of the genus, and pharmacological data on related active species. This summary will include not only the KB test results received from Purdue University but also antitumor active species reported by the RTI (Wall et al. 1987, unpublished KB actives, RTI 3-cell line actives), and by the NCI in their 60-cell line (published and unpublished), and it will further include plant samples obtained by Commonwealth Scientific Industrial Research Organization (CSIRO) in the mid 1960's that were screened by the NCI in KB and in other assays (Collins et al. 1990; CPAM 1977, 1982; USDA National Agricultural Library Archival Records, ARS 'Active Books,' Australia).

MATERIALS AND METHODS

The author's WA plant collections were obtained under a cooperative agreement between the NCI and the United States Department of Agriculture (USDA)/Agricultural Research Service (ARS) with a WA scientific license issued to the author as a collecting permit.

Rationale for Field Work in Western Australia

I visited Western Australia (WA) to conduct field work based on the botanical novelty and diversity that a systematic sampling of its flora would provide to the NCI antitumor screening program (Spjut 1982, 1985). From review of vegetation surveys of WA, it was estimated that one-third of all genera that commonly characterize the WA vegetation would be new the NCI screen. Sand heath (Kwongan) communities are exceptionally diverse; for example, Beard (1969) in a vegetation survey of the Boorabbin area stated in his description of "scrub heath" that "[t]his formation is by far the richest floristically of any of those in the locality and is also without any definite and consistent dominants so that it is not at present possible to characterize

associations within it." About 100 constituent species—all perennials, mostly shrubs—were listed by Beard (1969); he further commented that his list was incomplete.

The WA flora contains 11,196 indigenous species (FloraBase 2013) in 1,607 genera and 212 families; within the Mediterranean South-West Region, an area about ½ the size of Texas; 49% of approximately 8,000 species are endemic (Hopper & Gioia 2004). Additional subspecies and varieties bring the total known WA taxa to 12,307, and several thousand more species have yet to be described (FloraBase 2013). California, also within a Mediterranean climate—by comparison—has ~4,839 indigenous species with an additional 727 subspecies and varieties in 895 genera; 24% of the species are endemic (Beard et al. 2000; Hickman 1993). The diversity and uniqueness of the WA flora is exceeded only by that of the Cape Region in southern Africa; its Mediterranean flora lies within a smaller area—about ¼ in size—where 69% of ~9,100 indigenous species in 992 genera are endemic (Goldblatt & Manning 2002; Goldblatt et al., 2005).

Samples of plants occurring in seasonal dry tropical forests, brushlands, woodlands and semi-desert scrub have shown a higher incidence of antitumor activity than samples from temperate and tropical forests where precipitation is more uniformly distributed throughout the year, especially seen by root, stem-bark and fruit samples (Spjut unpbl. 1979, 1989, 2010).

This does not mean that plants in different floristic regions (Spjut 1985) growing under a relatively similar climate will have the equivalent chemical diversity in screening for novel antitumor compounds; historical factors are also important. Much of the California flora, for example, is viewed as an assemblage of species from different paleovegetation types that adapted to a gradual decline in summer precipitation during the Neogene, while species that did not adapt perished (Axelrod 1975). Geologic uplift and cyclic changes in precipitation and temperature that occurred since the onset of the glacial period (Pliocene) have fragmented California species populations from which many localized endemic species and subspecies evolved only minor morphological differences without "drastic changes in the genetic system"—as suggested for species hybrid complexes of *Arctostaphylos* and *Ceanothus*—and many "saltational" annual species (Raven 1977). Thus, one may expect such neoendemics to retain the secondary metabolites of their closest ancestral taxa with perhaps only minor variation as analogs of biologically active compounds.

The southwestern WA flora; by contrast, exhibits more endemism at higher taxonomic levels (families, tribes, genera, sections as result of repeated radiation events in many taxonomic groups over a longer period (Johnson & Briggs 1981). Xeromorphic features in WA plants are attributed more to evolution on poor nutrient soils rather than to adaptation to a Mediterranean climate (Barlow 1981; Johnson & Briggs 1981; Hopper and Goia 2004). Nevertheless, the woody fruits in many genera of Proteaceae and Myrtaceae, and the diverse "underground storage organs" seen in many families of WA plants (Pate and Dixon 1982; Dodd et al. 1984) are indicative of a long evolutionary history for surviving drought and fire. For example, *Banksia* (Proteaceae) species

produce massive follicones (Spjut 1994, folliconum) that require heat to open them before seeds disperse; a single follicone may weigh more than a kg, and numerous follicones are often produced on a plant. This huge investment of the plant's resources to reproduction would seem to be a product of adaptation to a relatively stable climate—characterized by a well-marked dry season—in a vegetation type subjected to frequent fire. Adaptation to drought and fire is seen in numerous other WA species that sprout from lignotubers and from shoot buds that lie protected (from fire) under stem-bark, and by fruits that seed underground as exemplified in the Restionaceae *Alexgeorgea* (Carlquist 1976).

Species growing on impoverished sandy soils in relatively drier climates, in contrast to those on nutrient rich substrates in temperate regions, may also compete more for water and nutrients by chemical defense (Harlev et al. 2012) against herbivores, insects, and neighboring plant species in which allelopathy (Willis 2007) would include endophytic and mycorrhizal interactions. The chemical diversity in the North American desert and Mediterranean Australian plants is obvious to an experienced collector by the variety of detectable odors when collecting samples, especially so in the odiferous dominant families Asteraceae in southwestern North America and the Myrtaceae in southwestern Australia. Their biologically active compounds are frequently sesquiterpene lactones and other essential oils, respectively, that have been reported mostly from the aerial parts of plants (Padovan et al. 2013).

However, secondary metabolites in roots of perennial plants appear less known in semi-arid regions because it seems that collectors have either not collected root, or because weight requirements for their extraction have not been practical for their collection. For example, in an NCI report (Abbott et al. 1967), root samples appear <1% among 1,422 extracts screened of plants collected during 1964–1966 from New Guinea, Queensland, New South Wales, Victoria, Tasmania, New Zealand, Uruguay, California, Texas, New Jersey, Maryland, Virginia, North Carolina, Tennessee, South Carolina, Georgia, Florida, and Ethiopia. Although roots from my experience are less often odiferous, that of *Prosopidastrum mexicanum* (Dressler) Burkart (Fabaceae), a Mimosoideae low growing shrub endemic to the Northern Vizcaíno Desert of Baja California, ranks perhaps among the most noxious; upon slashing its root in gathering a sample, it releases a foul odor that permeates the atmosphere over a distance of approximately 500 m, the odor continuing to be obnoxious for a week before finally dissipating upon drying out of the sample. Arctiopicrine and artemisiifolin are examples of sesquiterpene lactones isolated from samples of whole plants of Malta star thistle (*Centaurea melitensis* L., Asteraceae), active in P388 Leukemia (Hartwell 1976), whereas a potent flavonoid, (-)-catechin, found in the root of the related knapweed (*Centaurea stoebe* L) reportedly caused neighboring plants to self destruct (Bais et al. 2003); however, its allelochemical action has been called into question (S. Duke et al. 2009), while (-)-catechin is also recognized as a major ingredient in green tea that may act as a cancer preventive agent (Wang 2000). Flavopiridol, a synthetic flavonoid—based on a naturally occurring flavonoid extracted from stem-bark of *Amoora rohituka* (Roxb.) Wight & Arn. (Meliaceae)—causes

apoptosis in non-small lung cancer cells; it is currently under clinical trials for treating cancer (Takada et al. 2008; Pan et al. 2012).

Other historical factors relative to phytochemical diversity are islands that may have once existed off southwestern WA during the Cretaceous (Hopper and Goia 2004), which could have provided refuge for plants that might otherwise have been extirpated by climate change on the continental mainland. A cooler ocean near land moderates extreme fluctuations in temperature and evaporation as I have observed in semi-desert vegetation from coast to inland areas of Baja California (Spjut 1996). Also, Australia is thought to have become drier during the Tertiary as the Antarctic ice cap developed (Tyndale-Biscoe 2005), and Northern Hemisphere floras since the Pliocene appear to have been diminished more by glaciers than those in the Southern Hemisphere (Hopper & Gioia 2004).

A notable distinction to the WA flora is the relative paucity of succulent life forms and annuals that account for a large proportion of the floras in the semi-deserts of western North America and of the Karoo bush of southern Africa. Subtropical steppe and woodland regions that occupy much of Central Australia, extending into WA, have only about 2,000 species with little life form specialization other than "spinifex" grass (Beard 1981). The flora of Baja California, by contrast, contains a more diverse assemblage of life forms, among which are the arboreus cacti [e.g., *Pachycereus pringlei* (S. Wats.) Britt. & Rose], columnar trees clothed with spiny branchlets (e.g., *Idria columnaris* Kellogg) and obese trees (e.g., "elephant trees," *Bursera microphylla* A. Gray, *Pachycormus discolor* Coville ex Standl.). They and others originate from different vegetation-floristic elements—transmontane coniferous forests, California chaparral, Sonoran Desert thorn-succulent scrub, subtropical bushlands and coastal evergreen scrub—all of which contribute to approximately 2,700 species in Baja California (Wiggins 1980), occurring within a much smaller area, about 1/40 of that of Central Australia.

The antiquity of the WA flora is further seen in the geographical distribution and phytochemical diversification of the monocot family Restionaceae as it relates to the breakup of Gondwana (Good 1964; Schuster 1976; Johnson & Briggs 1981; Williams et al. 1998; Hopper & Goia 2004; Bohm 2009). The Gondwana ties between the WA and the African Cape floras are also evident in the dicot families Ericaceae, Fabaceae, Rutaceae, Proteaceae, and excluding grasses (Poaceae), both regions are more diverse in monocots than most other floristic regions (Good 1964; Harborne 1979; Spjut 1985; Goldblatt & Manning 2002; Hopper & Gioia 2004).

Strobel et al. (2004) recognized in their rationale for plant selection in the search for new anticancer chemicals that: "Plants that are endemic, having an unusual longevity, or that have occupied a certain ancient land mass, such as Gondwanaland, are also more likely to lodge endophytes with active natural products than other plants." Thus, the WA flora—having evolved morphologically over millions of years to an increasingly drier climate and alleopathically in competing for limited resources of water and soil nutrients—would seem to be a good botanical source for finding new anticancer drugs, especially in the highly diverse Kwongan communities.

Collection Strategy

Selection of plant samples was based on taxonomy, which included preparation for field work. A review was made of the taxonomic characteristics of families and genera that comprise the WA vegetation, and also their phytogeographical patterns of distribution within WA. This does not mean that all species in the entire flora were reviewed, because from experience, only the most common 10% are economical to collect for a biodiversity screening program, while also recognizing that with increasing effort one could reasonably collect 50% of the species (Spjut 1985). Vegetation studies that describe species composition and their frequency of occurrence indicate the genera and species likely to be collected.

Phenology was reviewed to identify the optimum time to conduct field work—which is when most plants are in flower. Additionally, species in large genera such as *Acacia* and *Eucalyptus*—that are commonly found cultivated outside Australia—were not collected (except for one species of *Acacia*, Spjut 1982). In the field, selection of species further required assessing whether they occurred in sufficient numbers to yield 1.5 kg dry weight without impacting the local species populations. Most species were shrubs <50 cm high consisting of simple to branched wiry stems < 2 cm diam. with small evergreen leaves. Consequently, it was often necessary to collect more than one individual of a species, and to decide whether it was feasible to obtain samples of separate plant parts, usually root (rt) and aerial parts; the latter represented by either woody or herbaceous stems along with attached sclerophyllous leaves. The presence of flower and/or fruit were also noted in sample descriptions, routinely abbreviated on bags and on shipping lists (e.g., st-lf-fl-fr). An effort was made to collect stem-bark (sb) and fruit (fr) samples whenever feasible. Occasional separate samples of woody-stem (with bark, ws-sb) or twig (tw), leaf (lf), flower (fl) or fruit (fr) were obtained.

This systematic approach (Spjut 1982, Spjut et al. 1992) emphasized collecting a greater diversity of plant samples at the genus level than otherwise might be obtained based strictly on ethnobotany (Spjut & Perdue 1976), or a random methodology that results in a greater disproportionate representation of samples in fewer genera (Spjut 1985). Despite the taxonomic limitations (Spjut 1985) and weight requirements for a sample (1.5 kg), 758 samples were obtained from 375 species; thus, on average two samples were obtained from each species. These were represented by 180 genera, 60 of which were new to the NCI screen (Spjut 1982). One sample of a moss (*Thuidium*), and one of a red alga were among the 758 samples.

Drying of samples

Samples were air dried in cotton bags in which each sample was stuffed. To facilitate drying samples were transported on vehicle roof tops while collecting and traveling from one location to another, and upon return to Perth they were hung in green houses for further drying (Spjut 1982).

Voucher specimens: Identification and distribution

Voucher (herbarium) specimens were prepared for all species collected, however, sample parts from one that was missing a voucher (*Eremophila* sp., SPJ-7070) were removed for identification. A duplicate set of all other vouchers was left at PERTH in October 1981, herbarium acronyms cited are according to Index Herbariorum (1952–). Voucher specimens were initially identified by the author and subsequently reviewed by botanists at PERTH. Duplicates were then distributed to curators at the National Arboretum (NA, 1982), the U.S. National Herbarium (US, 1982), and to the University of Wisconsin at Oshkosh (OSH, 1996).

Identifications of many voucher specimens have since been revised as a result of continuing taxonomic studies of the WA flora; the updated taxonomy is applied in this paper. For instance, voucher specimens of *Spjut & Edson 6991* for two samples belonging to the family Restionaceae—were identified by the author as "*Lepidobolus* sp.," "undescribed" (Fig.1). The voucher at PERTH was subsequently given a "ms" (unpublished) name, *L. quadratus* by B. G. Briggs & L.A.S. Johnson in June 1984 (PERTH 1146904; Atlas of Living Australia, Australia Virtual Herbarium, Nov 2013), but not until 30 years after its collection was the species described and its name effectively published (Briggs et al. 2012). Another example is the voucher *Spjut & Edson 6994* for two samples identified as *Daviesia quadrilatera* Benth. (Fabaceae); the species was considered problematic by Crisp (1984) who clarified its taxonomy by recognizing a new species, *D. podophylla*, to which the samples belong. Extracts from samples of both *L. quadratus* and *D. podophylla* are reported herein to have shown significant KB activity.

Extraction and KB Screening

Extraction—Details on the extraction procedure can be found in Abbott et al. (1967), Statz and Coon (1976), Wall et al. (1987), Cragg et al. (1993), and McCloud (2010). For the 758 samples collected by the author, a methylene chloride extract (CH_2Cl_2) was prepared from 500–1500 g of ground plant material sequentially extracted at room temperature with a 1:1 mixture of ethanol (EtOH) and CH_2Cl_2 (and water (H_2O) to produce both organic solvent and aqueous extracts, but only the CH_2Cl_2 extract was screened). Samples obtained by CSIRO during the mid 1960's were extracted either by 95% aqueous ethanol, or two separate solvent extracts were prepared, one ethanol, the other water (Abbott et al. 1967).

KB Cell Culture—Most WA samples were screened in KB cell culture. The methodology for KB screening is described in Geran et al. (1972), and briefly summarized in Spjut et al. (1986).. It should be noted that "confirmed" KB activity in the NCI screening program required three separate tests (Perdue 1982), referred to as a two-stage testing system (Abbott et al. 1967). A plant extract that exhibited minimum activity level of $\leq ED_{50}$ 30 µg/ml in the first test was retested, but then had to show $\leq ED_{50}$ 20 in a second test. If this latter activity level was met, then a new extract was prepared from the same sample for a third test, which had to show at least ≤

Fig. 1. ***Lepidobolus quadratus***. Top: photograph by Richard Spjut at the time of collection, name published by Briggs et al. (2012). Bottom: Label applied to voucher specimen.

PLANTS OF WESTERN AUSTRALIA

Lepidobolus sp. (undescribed)

Between Moore & Hill Rivers on the Northern Sand Plains: 23.8 km. north of south junction of Dandaragan Road with Brand Highway; 5.4 km. south of Cataby Roadhouse. Road construction track off to the east side; low scrub on gentle slopes.

Perennial, rounded, 15 to 30 cm. high, with quadrangular stems. Fairly common in this area.

COLLECTOR R. Spjut & C. Edson DET.: R. Spjut
NO. 6991 DATE 04 September 1981

Voucher for sample collected by Agricultural Research Service, U. S. Department of Agriculture, for the anti-cancer screening program of the Cancer Chemotherapy National Service Center, National Cancer Institute.

Fig. 2. Page 130 from Cumulative Plant and Animal Materials (CPAM 1977). Note entry *Caustis dioica* from Western Australia. NSC (extract) number followed by code "C" for confirmed active, July 1967, collected Oct 1965. PL indicates sample of whole plant collected; an aqueous-ethanol extract from this samples was prepared. 3LE indicates extract confirmed in LE (L1210), 3 is a code for an in vivo assay. Codes in the right two columns are for extractors/investigators and suppliers.

CONFIRMED PLANT AND ANIMAL MATERIALS
CPAM REPORT --- PLANTS IN BOTANICAL ORDER

NSC NO	MC CODE	DATE OF MC CODE	TEST SYSTEMS		DIST CODES	SUPPLIER ID
B099742	C	67/11	C 3P421 6711 CATHARANTHUS, LONGIFOLIUS (PICH.) PICH. RT, ALKALOID FRACTION (B) MADAGASCAR (2400A) , /65,	APOCYNACEAE	140F 422E	C-LO-R-(B)
B099704	C	65/09	C 9KB5 6509 S 3PS31 7211 CATHARANTHUS, PUSILLUS AUTHORITY UNKNOWN PL, (CRUDE AIKALOID) FRACTION INDIA (1200B) , /62,	APOCYNACEAE	140F 422E	CP-B
B099752	C	74/03	U 3P421 7204 C 3PS31 7403 CATHARANTHUS, ROSEUS AUTHORITY UNKNOWN *(WHITE FLOWERING) LF (DEFATTED), ETOH FLA. (1109E) , /64,	APOCYNACEAE	140F 114N	C-64-43
B099761	C	67/11	C 3P421 6711 S 3PS31 7111 S 9PS5 7609 CATHARANTHUS, TRICHOPHYLLUS (BAK.) PICH. PX, FRACTION (B1) (ALKALOID) MADAGASCAR (2400A) , /65,	APOCYNACEAE	140F 422E	CT-AP-B1
B670843	DR	67/09	C 9KB5 6507 CAULOPHYLLUM, THALICTROIDES (L.) MICHX. ST LF FL (FRESH), AQ/ETOH N.C. (1132E) , 05/63,	BERBERIDACEAE	501A DATA CHANGE NOMENCLATURE 09/77 NO CHANGE NOMENCLATURE 09/77	PR-5868
B621568	C	67/07	C 3LE21 6707 CAUSTIS, DIOICA R.BR. PL, AQ/ETOH AUSTRALIA (W.A.) (0800C) , 10/65,	CYPERACEAE	667A	
B803302	C	74/04	C 9KB5 7404 CAYAPONIA, BURAEAVI COGN. ST LF FR, AQ/ETOH COLOMBIA (0600F) , 07/72,	CUCURBITACEAE	532A	PR-30630

09/30/77 PAGE 130

ED_{50} 20 to be considered a "confirmed active" (Perdue 1982). These criteria were changed in November 1965 to ≤ ED_{50} 15, 10, 10 µg/ml, then reinstated in May 1972 to the criteria before November 1965, ≤ ED_{50} 30, 20, 20 µg/ml (Perdue 1982). Wall et al. (1987) considered an extract active in KB if ≤ ED_{50} 20 µg/ml, but reported only those species that showed ≤ ED_{50} 10 µg/ml using fewer dilutions and tubes than the NCI protocol described in Geran et al. (1972).

The KB actives for WA plant extracts screened by the NCI during 1966 and 1967, which were collected in WA through CSIRO (Collins et al. 1990), are based on $\leq ED_{50}$ 15, 10, 10.

The NCI and USDA records on antitumor active plants—The NCI reported new active extracts from preliminary screening in computer generated printouts at monthly intervals. These were also included annually in a cumulative record on "Confirmed Active Plant and Animal Materials" ("CPAM"). These printouts were sorted by plants and animals and alphabetically by genus/species. Each active extract entry included the extract number, tumor data codes, supplier codes, collection date, assay(s), date of confirmed activity but not the actual test values, the plant part, species and family names (e.g., Fig. 2). Data for each active species from the monthly reports were entered on a form by technicians at the USDA ARS lab to track recollections and associated procurement activities (level of priority, phone calls, correspondence, publications, etc.). These forms, known as "active sheets," one for each active plant part (e.g., Fig. 3), were filed into three-ring binder books organized by country (foreign recollections) and state (domestic recollections); a copy of the "Australia" "Active Book" (USDA 1960–1982) was reviewed , and two CPAM records were reviewed for this paper, one printed 30 Sep 1977, and the other 31 Mar 1982 (CPAM 1977, 1982). The former CPAM included all antitumor active extracts in the NCI program at the time; the latter limited to those not dropped from further investigation, which essentially included all species found active since Sep 1977 (to 1982).

Preliminary screening results on active species were generally not published except in experimental studies such as evaluation of extraction procedures (Statz and Coon 1976; Wall et al. 1987), comparative evaluation of bioassays (Suffness et al. 1988), distribution of activity among taxonomic groups (Barclay and Perdue 1976) and on specific groups of organisms such as bryophytes (Spjut et al. 1986). Screening data on inactive species were published in "Cancer Research Supplements" (e.g., Abbott et al. 1967, Fig. 4).

Isolation of the active agents routinely required recollections in quantities of 5 kg or more (dry weight); but active compounds have been isolated from less material since 1982 (Cragg 2002). Active compound(s) were isolated through fractionation guided by bioassay results, the most economical was the KB assay (Wall et al. 1976). Other bioassays were also employed in conjunction with isolation of pure active compounds to further evaluate pharmacological potential for drug development in cancer chemotherapy (Hartwell 1976; Suffness and Douros 1979; Kinghorn et al. 2003). Information on the results of subsequent antitumor screening is mentioned if known.

Distribution of WA plant samples for antitumor screening—Not all of the 758 samples collected by the author in WA (Spjut 1982) have been accounted for in extraction and screening. Following NCI termination notice to the ARS of screening natural products in October 1981—while the author was conducting field work in Australia—arrangements were made to extract all the WA samples at RTI (Spjut 1982). Some extracts were screened in KB by Monroe Wall's group at the RTI. Other extracts and their samples were sent by the RTI to the School of

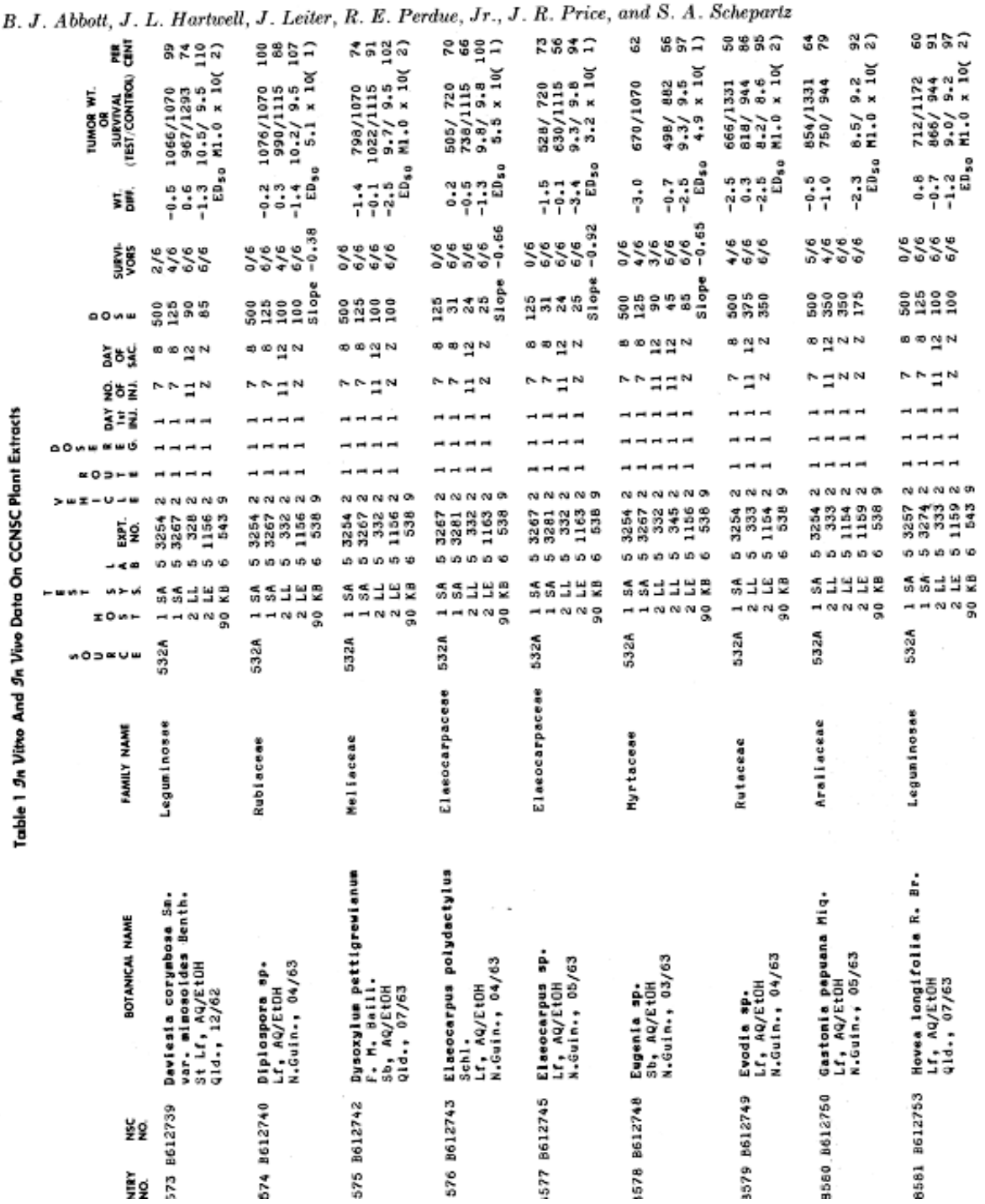
Fig. 3. Page from USDA ARS record on the procurement of recollections of antitumor active plants. In Australia "Active Book" (USDA National Agricultural Library, Special Collections). Record of *Olearia muelleri* created by staff at the USDA ARS New Crops Research Branch, who later were reorganized under the Medicinal Plant Resources Laboratory. Notations: Jonathan Hartwell and Matthew Suffness, Chiefs of the Natural Products Branch in Bethesda MD. "Released" in reference to Dr. Hartwell having determined through CSIRO, or directly from the chemist (Dr. Jeffries), that Dr. Jeffries had no further interest in the plant. Dr. Hartwell then assigned a medium priority ("M") for recollection, later cancelled by Dr. Suffness, probably because the active agent was subsequently determined to be centauredin, a sesquiterpene lactone that reportedly had weak cytotoxic action against KB (Jefferies et al. (1974), and was thus not likely to be developed as a new anticancer drug,.

NAME: Olearia muelleri (Sond.) Benth. '70
EXTRACTOR: 667A released
FAMILY: Asteraceae
DISTRIBUTION:
Genus:
Species:
Priority: M
STATUS: Date:
ONS:
Preliminary Active:
Confirmed Active: IC-KB, 1/66
ACTIVE SAMPLE:
Content: Tw-lf Source: Australia (W.A.)
Date of Collection: 10/64
Number:
B-657824
RECOLLECTION:
PR No.: Content: Date: Source:

Hartwell memo 9-12-74: needs 50-100# collection
On Weiser list 4-13-76.
Suffness 11-23-77: Cancel.

Pharmacy and Pharmacal Sciences at Purdue University, chaired by John Cassady. The WA samples and corresponding extracts at Purdue University were later retrieved by Thomas McCloud (pers. comm.), Manager of the NCI extraction screening facility at Frederick,

Fig. 4. Page from Abbott et al. (1967, Table 1): Example of plant species, plant parts, extracts and bioassays employed by the NCI in the preliminary screen.



B. J. Abbott, J. L. Hartwell, J. Leiter, R. E. Perdue, Jr., J. R. Price, and S. A. Schepartz

Table 1 *In Vitro* And *In Vivo* Data On CCNSC Plant Extracts

NTRY NO.	NSC NO.	BOTANICAL NAME	FAMILY NAME	SOURCE CODE	HOST SYS.	LAB	EXPT. NO.	VEHICLE CODE	ROUTE CODE	DAY 1st INJ.	NO. OF INJ.	DAY OF SAC.	DOSE	SURVIVORS	WT. DIFF.	TUMOR WT. OR SURVIVAL (TEST/CONTROL)	PER CENT
573	B612739	Daviesia corymbosa Sm. var. mimosoides Benth. St Lf, AQ/EtOH Qld., 12/62	Leguminosae	532A	1 SA	5	3254	2	1	1	7	8	500	2/6			
					1 SA	5	3267	2	1	1	7	8	125	4/6	-0.5	1066/1070	99
					2 LL	5	328	2	1	1	11	12	90	6/6	0.6	967/1293	74
					2 LE	5	1156	2	1	1	2	2	85	6/6	-1.3	10.5/ 9.5	110
					90 KB	6	543	9								ED_{50} M1.0 x 10(2)	
574	B612740	Diplospora sp. Lf, AQ/EtOH N.Guin., 04/63	Rubiaceae	532A	1 SA	5	3254	2	1	1	7	8	500	0/6			
					1 SA	5	3267	2	1	1	7	8	125	6/6	-0.2	1076/1070	100
					2 LL	5	332	2	1	1	11	12	100	4/6	0.3	990/1115	88
					2 LE	5	1156	2	1	1	2	2	100	6/6	-1.4	10.2/ 9.5	107
					90 KB	6	538	9					Slope	−0.38		ED_{50} 5.1 x 10(1)	
575	B612742	Dysoxylum pettigrewianum F. M. Bail. Sb, AQ/EtOH Qld., 07/63	Meliaceae	532A	1 SA	5	3254	2	1	1	7	8	500	0/6			
					1 SA	5	3267	2	1	1	7	8	125	6/6	-1.4	798/1070	74
					2 LL	5	332	2	1	1	11	12	100	6/6	-0.1	1022/1115	91
					2 LE	5	1156	2	1	1	2	2	100	6/6	-2.5	9.7/ 9.5	102
					90 KB	6	538	9								ED_{50} M1.0 x 10(2)	
576	B612743	Elaeocarpus polydactylus Schl. Lf, AQ/EtOH N.Guin., 04/63	Elaeocarpaceae	532A	1 SA	5	3267	2	1	1	7	8	125	0/6			
					1 SA	5	3281	2	1	1	7	8	31	6/6	0.2	505/ 720	70
					2 LL	5	332	2	1	1	11	12	24	5/6	-0.5	738/1115	66
					2 LE	5	1163	2	1	1	2	2	25	6/6	-1.3	9.8/ 9.8	100
					90 KB	6	538	9					Slope	−0.66		ED_{50} 5.5 x 10(1)	
577	B612745	Elaeocarpus sp. Lf, AQ/EtOH N.Guin., 05/63	Elaeocarpaceae	532A	1 SA	5	3267	2	1	1	7	8	125	0/6			
					1 SA	5	3281	2	1	1	7	8	31	6/6	-1.5	528/ 720	73
					2 LL	5	332	2	1	1	11	12	24	6/6	-0.1	630/1115	56
					2 LE	5	1163	2	1	1	2	2	25	6/6	-3.4	9.3/ 9.8	94
					90 KB	6	538	9					Slope	−0.92		ED_{50} 3.2 x 10(1)	
578	B612748	Eugenia sp. Sb, AQ/EtOH N.Guin., 03/63	Myrtaceae	532A	1 SA	5	3254	2	1	1	7	8	500	0/6			
					1 SA	5	3267	2	1	1	7	8	125	4/6	-3.0	670/1070	62
					2 LL	5	332	2	1	1	11	12	90	3/6			
					2 LL	5	345	2	1	1	11	12	45	6/6	-0.7	498/ 882	56
					2 LE	5	1156	2	1	1	2	2	85	6/6	-2.5	9.3/ 9.5	97
					90 KB	6	538	9					Slope	−0.65		ED_{50} 4.9 x 10(1)	
579	B612749	Evodia sp. Lf, AQ/EtOH N.Guin., 04/63	Rutaceae	532A	1 SA	5	3254	2	1	1	7	8	500	4/6	-2.5	666/1331	50
					2 LL	5	333	2	1	1	11	12	375	6/6	0.3	818/ 944	86
					2 LE	5	1154	2	1	1	2	2	350	6/6	-2.5	8.2/ 8.6	95
					90 KB	6	538	9								ED_{50} M1.0 x 10(2)	
3580	B612750	Gastonia papuana Miq. Lf, AQ/EtOH N.Guin., 05/63	Araliaceae	532A	1 SA	5	3254	2	1	1	7	8	500	5/6	-0.5	854/1331	64
					2 LL	5	333	2	1	1	11	12	350	4/6	-1.0	750/ 944	79
					2 LE	5	1154	2	1	1	2	2	350	6/6			
					2 LE	5	1159	2	1	1	2	2	175	6/6	-2.3	8.5/ 9.2	92
					90 KB	6	538	9								ED_{50} M1.0 x 10(2)	
8581	B612753	Hovea longifolia R. Br. Lf, AQ/EtOH Qld., 07/63	Leguminosae	532A	1 SA	5	3257	2	1	1	7	8	500	0/6			
					1 SA	5	3274	2	1	1	7	8	125	6/6	0.8	712/1172	60
					2 LL	5	333	2	1	1	11	12	100	6/6	-0.7	866/ 944	91
					2 LE	5	1159	2	1	1	2	2	100	6/6	-1.2	9.0/ 9.2	97
					90 KB	6	543	9								ED_{50} M1.0 x 10(2)	

Maryland (Natural Products Support Group), and subsequently screened for ant-HIV and antitumor activity.

The RTI and the Purdue University screened many WA plant samples in the KB assay during 1982–1985. The RTI, under the direction of Monroe Wall, initially reported KB activity for nine species to the USDA ARS Plant Exploration and Plant Taxonomy Laboratory (PETL) via

correspondence, Oct 1983, requesting assistance for their recollections; however, PETL was unable to provide personnel for travel to WA. Consequently, the author—at the request of the Chief of the PETL—provided information on where in WA each of the active species could be recollected in 25 kg or more based on his memory of where each active species occurred in abundance. However, no recollections were obtained at that time. Subsequently, Monroe Wall published their reports on KB active species that included not only the previous nine species but additional species from WA and 13 from India, China, and Madagascar (Wall et al. 1987) originally supplied under the former USDA/ARS cooperative agreement.

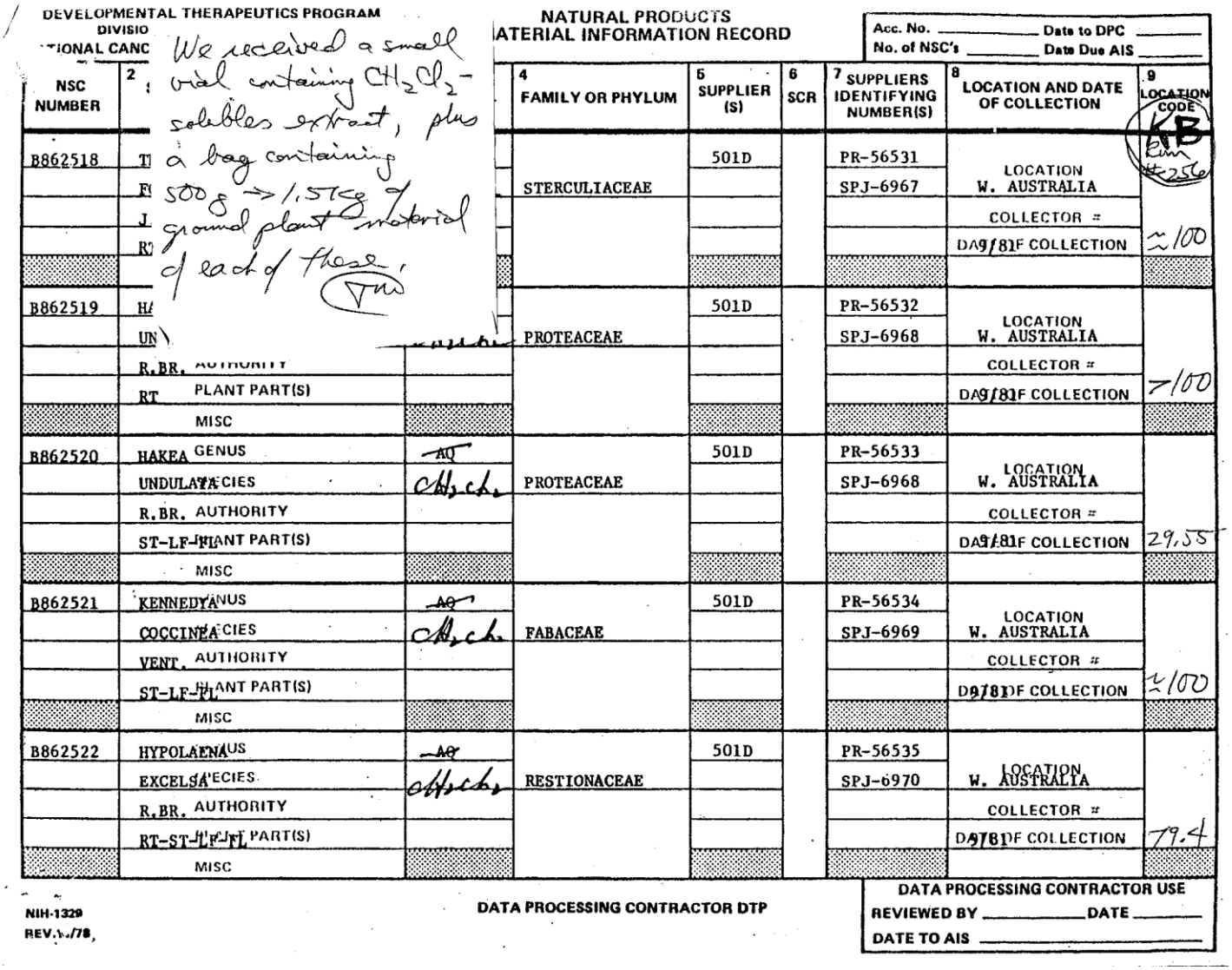
Since 1985, the NCI redeveloped their preliminary antitumor screening program of natural products, which led to a request for recollections of three WA species in Proteaceae. Samples from two of the WA species requested earlier by Monroe Wall (*Grevillea excelsior* root, *Isopogon scabriusculus* root) were again requested and subsequently obtained by contract sources in WA through the World Botanical Associates (WBA), which was a partnership formed by the author in Laurel Maryland in May 1983 to supply novel botanical products for medical research. The recollections were of aerial parts, woody stems and twigs, not the root, however.

The results of the WA samples screened in KB by Purdue University were sent to the author via correspondence dated1 July 1986 by Thomas McCloud, who at the time was a graduate student under John Cassady. The KB test results were noted for each sample on a "Natural Products Material Information Record" (form NIH-1329). This included the NSC (extract) number, taxonomy (species name and authority, family name), plant parts, USDA accession (PR) number (PR-56531–56870), and collector's number (SPJ for Spjut (SPJ-6967–7220); there were five records to a page, 68 pages total (e.g., Fig. 5). A total of 340 WA samples were listed, but KB results were reported for only 181 samples. A ‘post-it’ note from Thomas McCloud stated that the Purdue University had received from the RTI the methylene chloride extracts (CH_2Cl_2) and corresponding ground plant material, 500–1500 g. A hand written circled notation under “Location code” stated KB Run #256. The Purdue University findings were not immediately published pending a full accounting of all WA samples screened. However, since this accounting seems no longer obtainable, the KB test results for 181 of the 758 samples are presented herein. This includes the inactive as well as the active species unlike the aforementioned reports by the RTI (Wall et al. 1987). The WA samples also reported active by the NCI and the RTI are collectively summarized. A discussion on the significance of the findings follows.

RESULTS

Table 1 presents KB test results for 181 samples collected in WA from 127 species in 77 genera, arranged alphabetically by family/genus/species names, and by plant parts, which include 42 root (rt), 14 woody-stem (w-stem), 24 twig-leaf (tw-lf), 100 whole plant (pl) or aerial parts minus root (px) of woody plants and herbaceous perennials, and one fasciation (of *Banksia menziesii*, Proteaceae)—an abnormal growth thought to be caused by an endophyte. Twenty-one (16.5%)

Fig. 5. First of 68 pages of Natural Products Material Information Record with post-it note from Thomas Cloud. Columns from left to right: NSC number is extract number, species name, authority and plant parts, type of extract, family name, supplier RTI (501D), USDA accession number (PR) followed by collector's number (SPJ for Richard Spjut), Location, and KB test results.



DEVELOPMENTAL THERAPEUTICS PROGRAM DIVISIO ·TIONAL CANC

NATURAL PRODUCTS ATERIAL INFORMATION RECORD

Acc. No. _____ Date to DPC _____
No. of NSC's _____ Date Due AIS _____

We received a small vial containing CH_2Cl_2-solubles extract, plus a bag containing 500 g → 1,575 g ground plant material of each of these. TMC

NSC NUMBER	2		4 FAMILY OR PHYLUM	5 SUPPLIER (S)	6 SCR	7 SUPPLIERS IDENTIFYING NUMBER(S)	8 LOCATION AND DATE OF COLLECTION	9 LOCATION CODE
B862518	T			501D		PR-56531		KB Rum #256
	F		STERCULIACEAE			SPJ-6967	LOCATION W. AUSTRALIA	
	J						COLLECTOR #	
	R						DAY/MO/YR OF COLLECTION	≃100
B862519	H			501D		PR-56532		
	UN		PROTEACEAE			SPJ-6968	LOCATION W. AUSTRALIA	
	R.BR.	AUTHORITY					COLLECTOR #	
	RT	PLANT PART(S)					DAY/MO/YR OF COLLECTION	≃100
		MISC						
B862520	HAKEA	GENUS		501D		PR-56533		
	UNDULATA	SPECIES	PROTEACEAE			SPJ-6968	LOCATION W. AUSTRALIA	
	R.BR.	AUTHORITY					COLLECTOR #	
	ST-LF-FL	PLANT PART(S)					DAY/MO/YR OF COLLECTION	29.55
		MISC						
B862521	KENNEDYA	GENUS		501D		PR-56534		
	COCCINEA	SPECIES	FABACEAE			SPJ-6969	LOCATION W. AUSTRALIA	
	VENT.	AUTHORITY					COLLECTOR #	
	ST-LF-FL	PLANT PART(S)					DAY/MO/YR OF COLLECTION	≃100
		MISC						
B862522	HYPOLAENA	GENUS		501D		PR-56535		
	EXCELSA	SPECIES	RESTIONACEAE			SPJ-6970	LOCATION W. AUSTRALIA	
	R.BR.	AUTHORITY					COLLECTOR #	
	RT-ST-LF-FL	PLANT PART(S)					DAY/MO/YR OF COLLECTION	79.4
		MISC						

NIH-1329
REV. 5/78,

DATA PROCESSING CONTRACTOR DTP

DATA PROCESSING CONTRACTOR USE
REVIEWED BY _____ DATE _____
DATE TO AIS _____

species were active, 13 (10%) at ≤10 µg/ml (Table 1). Active species by plant parts—from base to the top of plant—were 7 root (16.6%), 1 twig-leaf (4.2%), 14 (13.7%) of the whole plant or aerial portion minus root (pl, px) and one fasciation (*Banksia menziesii*). Root was thus most frequently active plant part. Two species with active root also had active aerial parts, *Daviesia podophylla* (Fabaceae) and *Isopogon linearis* (Proteaceae).

Table 2 includes KB actives from Table 1, those reported by Wall et al. (1987, and unpublished), and other reports of antitumor activity under comments. Essentially all known antitumor active

species that were collected in WA for the NCI screening before 1982 are reported, 51 total. Among the active species of woody plants collected by the author, 24 species were separated into more than one plant part (Table 2) of which 20 showed activity only in one part of the plant: 15 root, 2 twig–leaf (Proteaceae, *Lambertia multiflora*, *Petrophile striata*), 2 fruit (Proteaceae, *Banksia lehmaniana*, *Xylomelum angustifolium*), and 1 fasciation as already mentioned (Proteaceae, *Banksia menziesii*).

Thus root samples from shrubs account for most of the active species in WA woody plants, and appear to have taxonomic significance in the families Fabaceae and Proteaceae. It may be noted that the Proteaceae also had KB activity in aerial parts of four species, but only in two species was activity limited to the aerial parts (*Lambertia multiflora*, *Petrophile striata*).

Relatively few samples of fruit and stem-bark were obtained from WA. Of three fruit samples known to have been screened by Wall et al. (1987), two were active; while no fruit samples were screened at Purdue University.

Table 2 also presents data for the number of extracts screened for each of 40 active genera. These data were obtained from a computer printout of all genera that had been screened (NCI 1980), in which there was a total of 6,494 generic names listed, a summary total indicating 108,699 extracts screened and 4,870 extracts active. Based on these data, 28 new active genera were discovered in the following 15 families (Table 2): Asparagaceae (*Asparagus*), Asteraceae-Cichorieae (*Arctotheca*, *Sonchus*), Celastraceae (*Psammamoya*), Ericaceae (*Andersonia*, *Leucopogon*), Fabaceae (*Daviesia*, *Gastrolobium*, *Hardenbergia*, *Hovea*, *Kennedia*), Geraniaceae (*Pelargonium*), Goodeniaceae (*Lechenaultia*), Haemodoraceae (*Anigozanthos*), Loganiaceae (*Logania*), Myrtaceae (*Calythropsis*, *Verticordia*), Proteaceae (*Banksia*, *Hakea*, *Isopogon*, *Lambertia*, *Persoonia*, *Petrophile*, *Xylomelum*), Restionaceae (*Lepidobolus*), Rhamnaceae (*Pomaderris*), Scrophulariaceae (*Eremophila*), and Stackhousiaceae (*Stackhousia*). Additionally, Restionaceae and Stackhousiaceae were new active families, and appear to be first reports of any biological activity in these families; the Stackhousiaceae, however, might be merged with Celastraceae (Stevens 2001–Angiosperm Phylogeny Website). Table 2 also comments on biological activity in related species and genera; however, the search for chemical and pharmacological data on these taxa was not exhaustive.

The most cytotoxic were *Daviesia podophylla* (Fabaceae) root (0.0076) and *Lepidobolus quadratus* (Restionaceae) whole plant (0.0359). The latter species was represented by separate samples of male and female plants; activity in the female plant sample (0.0359) was ~34 times more potent than that of the male (1.22).

DISCUSSION

The percentages of KB active species for the total number tested, 17% for root of shrubs and 14%, for the entire plant or aerial parts excluding root—usually perennial herbs and semi-woody species but also includes slender-stemmed shrubs—are higher than generally reported for KB

Table 1. KB data for Western Australian Plants screened during 1982–1985 at Purdue University

Family	Species	Collector's #	root	w-stem	twig-leaf	pl or px
[1]Amaranthaceae						
	Ptilotus divaricatus (Gaudich.) F. Muell.	SPJ 7061	>100	-	-	
	Ptilotus obovatus (Gaudich.) F. Muell.	SPJ 7056	-	-	-	>100
Asparagaceae						
	Asparagus asparagoides (L.) Druce	SPJ 7009	-	-	-	**2.7**
	Acanthocarpus preisii Lehm.	SPJ 7117	-	-	-	38.2
	Dichopogon capillipes (Endl.) Britta	SPJ 7051				>100
	Thysanotus patersonii R. Br.	SPJ 7076	-	-	-	41.6
Asteraceae						
	Arctotheca calendula (L.) Levyns	SPJ 7022	-	-	-	**0.32**
	Erymophyllum ramosum ssp. *involucratum* (F. Muell.) P. G. Wilson	SPJ 7060	-	-	-	>100
	Rhodanthe chlorocephala ssp. *roseum* (Hook.) P. G. Wilson	SPJ 7050	-	-	-	>100
Chenopodiaceae						
	Halosarcia halocnemoides (Nees) PG. Wilson	SPJ 7104		-	-	>100
Cyperaceae						
	Gahnia trifida Labill.	SPJ 7115	-	-	-	>100
Dilleniaceae						
	Hibbertia acerosa (DC.) Benth.	SPJ 7110				>100
	Hibbertia hypericoides (DC.) Benth.	SPJ 7005	**9.1**	-	-	>100
	Hibbertia subvaginata (Steud.) F. Muell.	SPJ 6993	-	-	-	>100
Ericaceae						
	Conostephium magnum Cranfield	SPJ 7036	>100	-	-	>100
	Leucopogon capitellatus DC.	SPJ 7141	-	-	-	36.7
	Leucopogon parviflorus (Andr.) Lindl.	SPJ 7014	39.9			80.1
	Leucopogon sprengelioides Sond.	SPJ 6986	-	-	-	**12.00**
	Leucopogon verticillatus R. Br.	SPJ 7164	50.9	-	-	40.5
Euphorbiaceae						
	Monotaxis bracteata Nees.	SPJ 7083			-	>100
Fabaceae						
	Bossiaea eriocarpa Benth.	SPJ 6979	-	-	-	39.80
	Bossiaea eriocarpa Benth.	SPJ 6995	-	-	-	37.80
	Bossiaea ornata (Lindl.) Benth.	SPJ 7143	-	-	-	78.7

Table 1. KB data for Western Australian Plants screened during 1982–1985 at Purdue University

Family	Species	Collector's #	root	w-stem	twig-leaf	pl or px
	Daviesia podophylla Crisp.	SPJ 6994	**0.0076**	-	-	**0.0975**
	Gastrolobium capitatum (Benth.) G. Chandler & Crisp	SPJ 7017	**8.3**			67.4
	Gastrolobium polystachyum Meisn.	SPJ 7026	39.4	-	-	>100
	Gastrolobium oxylobioides Benth.	SPJ 7047	>100			>100
	Gastrolobium retusum Lindl.	SPJ 6973	-	-	-	54.1
	Gastrolobium villosum Benth.	SPJ 7169	-	-	-	>100
	Jacksonia hakeoides Meisn.	SPJ 7086	-	48.5		-
	Hardenbergia comptoniana (Andr.) Benth.	SPJ 6007	**6.7**	-	-	44.7
	Kennedia coccinea Vent	SPJ 6969		-	-	~100
	Kennedia coccinea Vent	SPJ 7142	-	-	-	24.2
	Kennedia prostrata R. Br.	SPJ 6971	-	-	-	**3.76**
	Kennedia prostrata R. Br.	SPJ 7018	-	-	-	29.5
	Mirbelia trichocalyx Domin.	SPJ 7111				65.9
Geraniaceae						
	Pelargonium capitatum (L.) L'Herit.	SPJ 7006	-	-	-	**4.4**
Goodeniaceae						
	Dampiera altissima Benth .	SPJ 7046	-	-	-	>100
	Dampiera lavandulacea Lindl.	SPJ 7100	-	-	-	54.1
	Dampiera linearis R. Br.	SPJ 7174	-	-	-	59.7
	Dampiera oligophylla Benth	SPJ 7043	-	-	-	>100
	Dampiera spicigera Benth.	SPJ 7094	-	-	-	31.2
	Lechenaultia biloba Lindl.	SPJ 7032	-	-	-	**19.1**
	Scaevola porocarya F. Muell.	SPJ 7110	-	-	-	26.0
	Scaevola spinescens R. Br.	SPJ 7059	35.2	-	44.9	_
Haemodoraceae						
	Blancoa canescens Lindl.	SPJ 6992	-	-	-	22.60
	Conostylis psyllium Endl.	SPJ 6977	-	-	-	37.80
	Conostylis setigera R.Br.	SPJ 7016	-	-	-	83.9
	Conostylis setosa Lindl.	SPJ 7145	-	-	-	24.2
	Conostylis stylidioides F.Muell.	SPJ 7044	-	-	-	>100
	Conostylis serrulata R.Br.	SPJ 7172	-	-	-	23.9
Iridaceae						
	Fressia refracta (Jacq.) Klait.	SPJ 7021				28.5
Lamiaceae						
	Hemiandra pungens R. Br.	SPJ 7171	-	-	-	>100
	Hemiphora bartlingii (Lehm.) B.J.Conn & Henwood	SPJ 7042				>100
	Lachnostachys eriobotrya (F. Muell.) Druce	SPJ 7038		>100		>100

Table 1. KB data for Western Australian Plants screened during 1982–1985 at Purdue University

Family	Species	Collector's #	root	w-stem	twig-leaf	pl or px
Loganiaceae						
	Logania spermacocea F. Muell.	SPJ 6981	-	-	-	**10.80**
Loranthaceae						
	Amyema preissii (Miq.) Tiegh.	SPJ 7091	--	-	-	21.5
Mollugiaceae						
	Macarthuria australis Endl.	SPJ 6976	-	-	-	>100
Phyllanthaceae						
	Phyllanthus calycinus Labill.	SPJ 7120	-	-	-	**0.22**
	Phyllanthus calycinus Labill.	SPJ 7144	-	-	-	**5.52**
Myrtaceae						
	Astartea cf. fascicularis (Labill.) DC. [*Baeckia* sp. FloraBase id]	SPJ 7077	-	-	-	57.4
	Calytrix glutinosa Lindl.	SPJ 7168	-	-	-	22.2
	Calythropsis aurea G. A. Gardner [= *Calytrix ecalycata* Craven]	SPJ 7041	30.4			>100
	Chamelaucium uncinatum Schauer	SPJ 7011	95.8		39.4	
	Conothamnus trinervis Lindl.	SPJ 7023				>100
	Eremaea fimbriata Lindl.	SPJ 6983	-	-	-	32.80
	Eremaea asterocarpa Hnatiuk ssp. *asterocarpa*	SPJ 7033				58.4
	Hypocalymma angustifolium Endl.	SPJ 7035	71.7	-	-	>100
	Hypocalymma robustum Endl.	SPJ 7019	26.7			>100
	Hypocalymma xanthopetalum F. Muell.	SPJ 6990	-	-	-	>100
	Leptospermum erubescens Schauer	SPJ 6974	**18.3**	74.2	29.7	-
	Leptospermum laevigatum (Gaertn.) F. Muell.	SPJ 7010	>100		>100	-
	Malleostemon sp.	SPJ 7087		-	-	>100
	Melaleuca microphylla SM.	SPJ 7090	28.3			-
	Melaleuca nematophylla L. A. Craven	SPJ 7078		47.7		
	Melaleuca uncinata R. Br.	SPJ 7053	>100	>100	>100	-
	Scholtzia parviflora F. *Muell.*	SPJ 7088			51.1	-
	Scholtzia sp.	SPJ 7040				>100
	Scholtzia sp.	SPJ 7084	28.5	-	36.1	-
	Verticordia acerosa Lindl.	SPJ 7166				26.0
Polypodiaceae						
	Pteridium esculentum (G. Foster) Cockayne	SPJ-7001				25.1
Proteaceae						
	Banksia armata (R.Br.) A.R.Mast & K.R. Thiele var. *armata*	SPJ 7027	>100			>100

Table 1. KB data for Western Australian Plants screened during 1982–1985 at Purdue University

Family	Species	Collector's #	root	w-stem	twig-leaf	pl or px
	Banksia grandis Willd.	SPJ 7149	>100			
	Banksia menziesii R. Br.	SPJ 7020			**3.1** (fasciation)	
	Banksia sessilis var. cygnorum (Gand.) A.R. Mast & K.R. Thiele	SPJ-7002	>100	>26.9	>100-	
	Conospermum crassinervium J. W. Green	SPJ 6997	-	-	-	>100
	Conospermum huegelii Endl.	SPJ 7167	-	-	-	>100
	Conospermum triplinervium R. Br.	SPJ 7108			35.1	-
	Banksia squarrosa (R.Br.) A.R.Mast & A..R. Thiele ssp. *squarrosa*.	SPJ 7170	66.8	>100		
	Grevillea amplexans Benth. ssp. *amplexans*	SPJ-7098	-	-	-	>100
	Grevillea leucopteris Meisn.	SPJ 7039	>100	>100	>100	-
	Grevillea muelleri Benth.	SPJ 7030	65.5	-	-	
	Grevillea pinaster Meisn.	SPJ 7045	>100	>100	>100	
	Grevillea petrophiloides Meisn.	SPJ 7052	>100	>100	>100	-
	Grevillea preissii* Meisn. ssp. *preissii	SPJ 7013	**12.6**	-	-	>100
	Grevillea thyrsoides Meisn.	SPJ 7029	29.1	-	-	31.3
	Hakea conchifolia Hook.	SPJ 7025	32.3	-	-	24.1
	Hakea undulata R. Br.	SPJ 6968	>100	-	-	29.55
	Hakea undulata R. Br.	SPJ 7159			53.8	-
	Hakea varia R. Br.	SPJ 7173	>100	72.4		
	Isopogon asper R. Br.	SPJ 6988	-	-	-	**17.60**
	Isopogon linearis Meisn.	SPJ 6987	**5.34**	-	-	**1.88**
	Isopogon divergens R. Br.	SPJ 7048	>100	-	-	>100
	Lambertia mulitflora Lindl.	SPJ 6984	69.9	>100	**15.4**	-
	Lambertia cf. *multiflora* Lindl.	SPJ 7160	>100	-	-	>100
	Petrophile brevifolia Lindl.	SPJ 7024	-	-	-	>100
	Petrophile linearis R. Br.	SPJ 7034	-	-	-	37.8
	Petrophile shuttleworthiana Meisn,	SPJ 7028	31.2	-	-	61.4
	Petrophile striata R. Br.	SPJ 6985	>100	-	-	**0.36**
	Xylomelum angustifolium Kipp.	SPJ 7109			>100	
Restionaceae						
	Ecdeiocolea monostachya F. Muell.	SPJ 7096	-	-	-	23.8
	Hypolaena exsulca R. Br.	SPJ 6970	-	-	-	79.40
	Lepidobolus quadratus B. G. Briggs & L.A.S. Johnson **(male)**	SPJ 6991	-	-	-	**1.22**
	***Lepidobolus quadratus* (female)**	SPJ 6991	-	-	-	**0.0359**
	Desmocladus virgatus (Benth.) B.G.Briggs & L.A.S.Johnson	SPJ 6989	-	-	-	28.30
	Lyginia sp.	SPJ 6996A	-	-	-	>100
Rhamnaceae						
	Cryptandra arbutiflora Fenzl. *borealis* Rye	SPJ 7054				>100
	Spyridium globulosum Benth.	SPJ-7003	-	>100	69.2	
Rubiaceae						
	Opercularia vaginata Juss.	SPJ 7055	-	-	-	>100

Table 1. KB data for Western Australian Plants screened during 1982–1985 at Purdue University

Family	Species	Collector's #	root	w-stem	twig-leaf	pl or px
Santalaceae						
	Exocarpos aphyllus R. Br.	SPJ 7058	-	-	29.5	
	Santalum acuminatum (R.Br.) A.DC..	SPJ 7057	-	-	>100	
Scrophulariaceae						
	Eremophila glabra (R. Br.) Ostent. ssp. *albicans* (Bartl.) Chinnock	SPJ 7119	-	-	-	26.0
	Eremophila pterocarpa W. V. Fitzgerald ssp. *pterocarpa* -	SPJ 7074				>100
Solanaceae						
	Anthocercis littorea Labill.	SPJ 7113	55.1	>100	21.1	>100 (fr)
	Solanum simile F. Muell.	SPJ 7008	-	-	-	>100
Stackhousiaceae						
	Stackhousia pubescens A. Rich.	SPJ 6978	-	-	-	**4.57**
Sterculiaceae						
	Thomasia foliosa J. Gray	SPJ 6967	-	-	-	~100
Sylidiaceae						
	Stylidium elongatum Benth.	SPJ 7049				>100
Thymelaeaceae						
	Pimelea rosea R. Br.	SPJ 7112				55.8
Xanthorrhoeaceae						
	Dasypogon sp.	SPJ-6998	-	-	-	>100
	Kingia australis R. Br.	SPJ 7161		22.2	22.2	>100 (lf)
	Xanthorrhoea preissii Endl.	SPJ-6972		38.1		81.8

Abrrevitions. SPJ: Standard USDA ARS abbreviation for collector Richard Spjut. Plant samples were also assigned a "PR" (Plant Record) numbers (Fig 4), but these numbers cannot be traced to voucher specimens. Pl: entire plant. Px: plant excluding root. W-st: woody-stem.

Table 2. Summary of KB and Other Antitumor Active Species for Western Australian Plants					
Active Species (Alphabetical by family/genus/species)	**Plant parts**	**ED_{50}**	**Species in genus**	**NCI Extracts for genus tested/active before 1982**	**Comments: Geographical distribution of genus, related plants screened by the NCI before 1982, pharmacological reports**
Asparagaceae					
Asparagus asparagoides	rt-st-lf-fl	2.7 5	130	59/0	Mostly Africa; *A. asparagoides* native to the Cape Region, invasive elsewhere (Batchelor & Scott 2006). Reported by Wall et al. (1987) under Liliaceae. *A.officinalis* L., extensively cultivated since ancient Greece (Mabberly 1997), this species and *A. racemosus* Willd. screened many times. Mollucidal saponins reported in some species induce apoptosis in human hepatoma cell line HepG2 (Ji et al. 2012); KB insensitive to saponins. Antidiabetic activity in *A. asparaogides* and *A. racemosus* (Hafizur et al. 2012; Somania et al. 2012).
Asteraceae					
Arctotheca calendula	rt-st-lf-fl	0.32	4	3/0	Native to the Cape Region of South Africa, invasive elsewhere.
Olearia muelleri (Sond.) Benth. SPJ-7287	rt-st-lf-fl-fr	5	130	30/1	Mostly Australia, 25 New Guinea, New Zealand. Wall et al.(1987) as *O. calcarea*, voucher reidentified *O. muelleri* by P. G. Wilson, 1982.
Olearia muelleri CSIRO, Oct 1964 collector unknown	tw-lf	≤5			EtOH extract, KB, Jan 1966; recollection requested by Hartwell 13 Apr 1976, request cancelled by Suffness 23 Nov 1977. Jefferies et al. (1974) isolated centauredin, which was reported to have a weak cytotoxic action against KB.
Sonchus hydrophilus Boulos SPJ-7180	rt-st-lf-fl-fr	5	60	46/0	Old World, especially Macaronesia; invasive species *S. asper*, *S. arvensis*, and *S. olearaces* each represented by 6 or more extracts tested. Wall et al. (1987) as *Sonchus* sp.
Celastraceae					
Psammomoya choretroides (F.Muell.) Diels & Loes. SPJ-7230	rt-st-lf-fl	5	4	1/0	Endemic to WA. Wall et al. (1987)
Cyperaceae					
Caustis dioica R. Br. CSIRO, Oct 1965 collector unknown	pl				L1210, July 1967, investigator P. R. Jefferies, University of Western Australia. Active compound(s) unknown. Duke et al. (2013) reported novel prenylated hydroxystilbenes from propolis on a related genus, *Lepidosperma*, occurring on Kangaroo Island. Pterostilbene, xanthorrhoeol, sakuranetin and pinostrobin isolated from propolis in WA by Ghisalberti, Jefferies et al. (1978).

Table 2. Summary of KB and Other Antitumor Active Species for Western Australian Plants					
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H. polystachya Benth. CSIRO, Oct 1965 collector unknown	st-lf				Aq-EtOH extract, WM, Sep 1967, recollection requested by Jonathan Hartwell. 12 Sep 1974. No recollections made. Active agents unknown.
Ericaceae					
Andersonia parvifolia R. Br. SPJ-7321	rt-st-lf-fl	5	22	0/0	Australia, 12-13 in WA (Lemson 1996); Wall et al. (1987)
Leucopogon sprengelioides	st-lf-fl	12.0	130	7/0	Mainly Australia, 71 in WA.
Fabaceae					
Acacia pulchella R. Br. CSIRO, July 1965, collector unknown	pl		1000	622/25	Aq-EtOH WM, Nov 1967. Recollection requested by Hartwell, 12 Sep 1974. Activity in genus—mostly aqueous extracts in assays sensitive to tannins—employed early in the 1960's; later, tannins extracted before testing, then a single solvent extraction employed, then WM dropped from screen. Estimate number of species is for Australia. Number extracts screened includes species now classified in other genera. Genus listed as GESOC (Genera Extensively Screened Or Collected) in Spjut (1985).
Daviesia podophylla	rt st-lf-fl	0.0076 0.0975	120	18/0	Australia, especially southwestern WA
Gastrolobium capitatum	rt	8.3	35	5/0	Genus endemic to Australia; CSIRO listed one other species screened for antitumor activity with negative results.
Gastrolobium parviflorum (Benth.) Crisp SPJ-7346	rt	10			Wall et al. (1987) as *Oxylobium parviflorum*
Hardenbergia comptoniana	rt	6.7	3	3/0	Australian genus, closely related to *Kennedia*
Hovea elliptica (Sm.) DC. SPJ-7364	rt				3-Cell Cancer Assay (RTI, unpubl. rpt., Oberlies, (pers. comm.).
Kennedia prostrata	st-lf-fl	3.76	16	4/0	Australia, New Guinea, 11 WA; endophyte *Streptomyces* spp. in *K. nigricans* Lindl. produce munumbicins, compounds that have antibiotic and antifungal activities (Castillo et al. 2002). KB screening discovered maytansine (in *Maytenus*, Celastraceae) and colubrinol (in *Colubrina*, Rhamnaceae). Maytansinoids found in bacteria, *Actinosynnema*, generally not synthesized by flowering plants (Cassady et al. 2004); microbial associations suggested for variation in antitumor activity in the moss *Claopodium crispifolium* (Spjut et al. 1988),

					which may even result from synergistic effect from both maytansinoids and *Nostoc* compounds (e.g. cryptophycins, Al-awar & Shih 2005), but only ansamitocin P-3 reported in the moss (Suwanborirux et al. 1990; Yu & Floss 2005).
Geraniaceae					
Pelargonium capitatum	rt-st-lf-fl	4.4	280	59/0	Mostly Africa. Active species native to coastal southern Africa. Contains essential oils with reported antimicrobial and antifungal activities (Guerreni et al. 2011).
Goodeniaceae					
Lechenaultia biloba	rt-st-lf-fl	19.1	26	0/0	Australia, 20 WA.
Haemodoraceae					
Anigosanthus humilis ssp. humilus Lindl. SPJ-6975 or 7092	rt-st-lf-fl	5	11	0/0	Endemic to WA. Wall et al. (1987) without collector or extract numbers.
Anigosanthus manglesii D. Don SPJ-8095 or 7190B	rt-st-lf-fl	10			Wall et al. (1987) without collector or extract numbers.
Hemerocallidaceae					
Dianella revoluta R. Br. CSIRO, Oct 1965 collector unknown	pl		35-45	15/1	Africa, Pacific Islands, Australia; Aq/EtOH, WM, Nov 1967; recollection requested 12 Aug 1964. WM sensitive to saponins in Liliales s.l.
Iridaceae					
Moraea flaccida (Sweet) Steud. SPJ-7184	bu-st-lf-fl	0.5	195	13/1	Southern Africa, includes *Homeria.* Wall et al. (1987) as *H. miniata*, reidentified by "tss," 28 Sep 1999.
Loganiaceae					
Logania spermacocea	rt-st-lf-fl (bud)	10.8	25	2/0	22 Australia, 1 New Caledonia, 1 New Zealand
Myrtaceae					
Calythropsis aurea (Myrtaceae)	rt	NCI 60 cell line		1/0	Differential cytotoxicity in NCI 60-cell line, activity similar to known tubulin-interactive compounds; new active chalcones, calythropsin and dihydrocalythropsin isolated (Beutler et al. 1993). Included in *Calytrix* (Craven 1990).
Eucalyptus calophylla R. Br. C CSIRO, Sep 1965, collector unknown	sb		>500	404/35	Largely Australia. Species active in WM, KB and/or P-388, collected in Australia, Hawaii, California, Africa, Brazil, India. Essential oils common (Nagpal et al. 2010; Padovan et al. 2013).
Leptospermum erubescens	rt	18.3	79	26/2	SE Asia; 25 Australia; *L. laevigatum* (Sol. ex Gaertn.) F. Muell., Aq/EtOH of seed from Africa active in WM, Apr 1968; *L. scoparium* J.R. Forst. & G. Forst., ws-sb, 95% EtOH extract active in KB from Lanai, HI. Essential oils in *L.*

					scoparium reported to have antifungal and antibiotic activities (Song et al. 2013).
Melaleuca pentagona Labill. SPJ-7323	rt	10	280	89/9	Largely Australia, 5 Indo-Malaysia; *M. quinquenervia* (Cav.) S.F. Blake, invasive, sb-$ChCl_3$ and Lf-EtOH extracts from HI active in KB in 1977; *M. stypheliodes* Sm. native to E Australia, cultivated elsewhere, $ChCl_3$ extract of st-lf-fr from, Kauai active in KB,1977, EtOH and MeOH extracts of sb-st-lf active in KB; $ChCl_3$ extract of *M. viridiflora* Sol.ex Gaertn.tw, native to E Australia, active in KB, Sep 1979. Among 15 plant extracts of Myrtaceae screened for antitumor activity, *Melaleuca leucodendron* L. showed "≥90% toxicity in HepG2 cytotoxicity and ≥3 fold increase in apoptotic background signal at 100 ppm." (El Manawaty et al. 2013). Essential oils common in Myrtaceae (Padovan et al. 2013). Tea tree oil and "terpinen-4-ol from *Melaleuca alternifolia* (Maiden & Betche) Cheel reported to significantly affect the in vitro viability of 2 murine tumour cell lines; AE17 and B16 in a dose- and time- dependent manner as assessed by the MTT assay" (Greay et al. 2010).
Verticordia chrysantha Endl. SPJ-7322	rt-st-lf-fl	0.5	105	2/0	Wall et al. (1987) as *V*. cf. *grandiflora*
Phyllanthaceae					
Phyllanthus calycinus	rt-st-lf-fl st-lf-fl	0.22, 5.52	600	191/10	Tropical/subtropical. The 191 extracts screened represent 42 species, 5 of which were active: *P. acuminatus* Vahl, rt P-388, Costa Rica, Dec 1973, *P. beillei* Hutch. lf Kenya, KB, Nov 1969, *P. neogranatensis* Muell. Arg. tw KB Colombia Nov 1976, *P. reticulatus* Poir. st-lf-fl, India, Aug 1975, *Phyllanthus* sp.lf KB, Colombia, Dec1976 (Saufferer USDA Mem. 1981). Phyllanthoside of interest for development as anticancer drug (Petit et al. 1984) based on activity in B-16 Melanoma and human breast cancer, phase I clinical trials in UK. Englerin A isolated from *P. engleri* Pax (Ratnayake et al. 2009), an eastern Africa species; patent issued Apr 2013 for potential development in treating renal cancers.
Proteaceae					
Banksia armata (R.Br.) A.R.Mast & K.R.Thiele var. *armata* SPJ-6957	st-lf-fl	5	76	20/0	Australia, 1 extends to New Guinea, 60 endemic to WA. Wall et al. (1987) as *Dryandra armata*, without collection or extraction numbers; species listed in Table 1 under another collection number, SPJ-7027, divided into 2 samples, both >100; however, KB data could be from the same sample. Vouchers for both collection numbers in PERTH identified as *B. armata* var. *armata.*

Banksia lemanniana Meisn. SPJ-7337	rt ws-sb fr	10 1 1			Wall et al.(1987) as *Banksia laevigata*
Banksia menziesii	fasciation	3.1			Fasciation on *Banksia* an abnormal outgrowth of a densely entangled redwood like fibrous stems, appearing like a parasitic plant but peculiar growth thought to be due to endophyte.
Grevillea cagiana McGill. SPJ-7252	rt	5	362	62/1	357 Australia, 1 New Guniea, 1 New Caledonia, 1 Sulawesi; rt and ws-sb active in 3-Cell Cancer Assay (RTI unpubl. data, Oberlies pers. comm). Aqueous extract of *G. stricta* R. Br. from Queensland active (Aug 1967) in WM, probably tannin. Robustacides isolated from twigs-leaves of a *Grevillea* cultivar reported to have anitmalarial activity (Ovenden et al. 2011),
Grevillea excelsior Diels SPJ-7244	rt	5			Wall et al. (1987)
Grevillea pilosa A.S.George subsp. *pilosa* SPJ-7359	rt ws-sb tw-lf-fl	10 10 10			Wall et al. (1987)
Grevillea thelmanniana	rt	12.6			Also reported active by RTI, unpubl. report Oct 1983, without KB data.
Hakea costata Meisn. SPJ-6915	rt	5	149	27/0	Australia. Wall et al. (1987)
Isopogon asper	rt-st-lf-fl	17.60	53	2/0	Australia, 47 in WA.
Isopogon linearis	rt st-lf-fl	5.34 1.88			
Isopogon scabriusculus Meisn. subsp. *stenophyllus* Foreman	rt	5			Wall et al. (1987)
Lambertia multiflora	tw-lf-fl	15.4	10	2/0	9 WA, 1 E Australia.
Persoonia saundersiana Meisn., SPJ-7265	rt ws-sb		98	7/0	Australia, 15 WA. Samples previously identified *P.* aff. *diadena* F. Muell., tw-lf not active (Cragg correspondence, 15 Aug 1985, no data),
Petrophile striata	st-lf-fl	0.357	42	0/0	Australia.
Xylomelum angustifolium Kipp SPJ-7109	fr	4	6	2/0	Australia. Wall et al. (1987)
Restionaceae					
Lepidobolus quadratus	rt-st-lf-fl Male Female	 1.22 0.0559	6	0/0	Southern Australia. First reported biological activity for Restionaceae..
Rhamnaceae					

Pomaderris brevifolia N.G.Walsh, SPJ-7340	rt-ws-sb	10	70	14/0	Australia, 5 spp. New Zealand; Wall et al. (1987) as *P. ovaria*.
Scrophulariaceae					
Eremophila sp. SPJ-7070	rt	No data	215	25/0	Australia. Wall et al. (unpubl rpt, 1 Oct 1983; herbarium label, voucher?). Collected near Shark Bay. Decoction of leaves in many species used by Aborigines for colds, aches, as a laxative, purgative, or for diarrhea (Richmond 1993).
Solanaceae					
Solanum ellipticum R. Br. SPJ-7068	rt	No data	1700	764/33	Cosmopolitan. Wall et al. (unpubl rpt, 1 Oct 1983). Alkaloids isolated from other species active in KB, SA, WM: Solamarine, Solapalmatenine, Solpalmatine (Hartwell 1976).
Stackhousiaceae					
Stackhousia pubescens	rt-st-lf-fl	4.57	14	2/0	Australia, Micronesia

1. Species names in bold from Table 1.
2. Plant part abbreviations: bu—bulb, lf—leaf, fl—flower, fr—fruit, pl—plant (entire plant with roots), rt—root, sb—stem-bark, st—stem (herbaceous), ws-sb—woody-stem with stem-bark (usually applied when bark is not practical to collect) .
3. Collectors: SPJ—Richard Spjut et al. (Chuck Edson, L. Lacy, Robert Phillips, Graeme White).
4. Tumor abbreviations from Abbott et al. (1967): KB—human cancer cells of the nasopharynx (cell culture); L1210—Lymphoid leukemia (mouse); SA—Sarcoma 180 (albino mouse); WM—Walker 256 carcinosarcoma intramuscular (rat).
5. Species numbers in genera from Mabberley (1997), FloraBase (2013) and other literature cited.
6. Number of extracts tested and active for genera from NCI (1980). Other active species from CPAM (1977, 1982). Hartwell communications from notes in USDA ARS Active Book—Australia (e.g., Fig. 3).

screening in previous studies (Barclay & Perdue 1976; Perdue 1976; Spjut 2010). A review by Barclay and Perdue (1976) of 20,525 species screened by the NCI reported 4.1% active extracts and a cumulative yield of 10.4% active species based on screening in KB and often three other bioassays (Abbott et al. (1967, e.g., Fig. 4); in 1969, the P-388 was substituted for the L1210, and the other assays that were sensitive to tannins and saponins were dropped from the prescreen (Hartwell 1976; Perdue 1982). Perdue (1976) in a review of antitumor activity according to p1ant parts for 1,041 species collected in the Amazon of Peru, showed that while the incidence of antitumor activity in KB and/or P-388 was highest in stem-bark, it was still < 5%; however, higher frequencies of antitumor activity were found for stem-bark and other parts collected by Perdue (1976) in East Africa when sorted by vegetation types (Spjut 2010). But again these results included the P-388 actives in addition to the KB actives with results still below 10%, compared to 14–17% active plant parts for the WA samples reported in this paper (Table 1).

Even more significant is the 10% active extracts at ≤10 µg/ml (Table 1, 18 active/181 tested). Abbott et al. (1967) indicated activity in the KB screen at ≤10 µg/ml should yield 1–2% active extracts. Thus, the incidence of KB activity at 10% among the WA plant extracts (Table 1)—which is five-fold more than expected (Abbott et al. 1967)—is phenomenal. It should be noted that although the number of samples of WA plants screened at RTI is not known; the RTI reported more active species than the Purdue University screening. The RTI may have screened extracts from all 413 samples that remain incompletely accounted for, whereas it is known that Purdue University screened 53% of their share of the 340 WA samples. Nevertheless, it would seem that the KB actives reported by the RTI corroborate the phenomenal KB results obtained from Purdue University.

The higher incidence of KB activity in WA plants is attributed to a number of factors: (1) The Mediterranean WA flora consisting predominantly of shrubs compared to the diversity of annuals in western North America that are generally not collectable in 1-2 kg (dried); (2) collecting in different WA floristic provinces to obtain as much diversity as possible; (3) emphasis on obtaining root, bark and fruit samples; (4) not over-collecting in large genera such as *Acacia* and *Eucalyptus*; (5) not having to deal with previously collected species referred to as "SLOP" (Species Low On Priority, Spjut 1985) since I had already determined that the WA flora would yield many species new to the NCI screen, and (6) that unpublished studies indicated antitumor activity in Mediterranean shrublands would be greater than what has been generally found in wet tropical and temperate forests (Spjut 2010).

A limited search on pharmacology and chemistry of the antitumor WA active plants (PubMed, Google) revealed little or no information on their active compounds as evident from the absence of such data under comments in Table 2; therefore, many novel antitumor active compounds await discovery upon further screening of their recollections.

One example is the 15 KB active species in eight genera of Proteaceae (Table 2). Reports on their biologically active compounds appear limited to other related species or genera in Proteaceae. Phenolic gylocosides referred to as robustacides, were isolated from twigs-leaves of a cultivar of *Grevillea* sp. (Ovenden et al. 2011), and resorcinolic lipids, which are also associated with endophytes, occur in a number of Proteaceae genera and also in genera of other families (Kozubek & Tyman 1999). But they are probably not the compounds responsible for the KB activity in the sample of *Banksia menziesii* fasciation, even though a *Streptomyces* endophyte was found to produce antibiotic compounds, named Kakadumycins, isolated from *Grevillea pteridifolia* Knight (Castilleo et al. 2003). Anti-HIV screening of WA plant samples by the NCI discovered a potent, novel HIV-inhibitory naphthoquinone trimer from the root of *Conospermum* cf. *incurvum* Lindl. (*Spjut & Edson 7139*), named conocurvone (Decosterd et al. 1993).

The Myrtaceae—rich in essential oils (monoterpenes, sesquiterpenes; Stefanello et al. 2011; Padovan 2013)—is an example of where KB activity was less frequent; only one of 30 samples

represented by 20 species, was active, root of *Leptospermum erubescens* (Table 1, 18.3). This is not surprising since the KB assay does not react to essential oils, especially monoterpenes (Hartwell 1976), but sesquiterpene lactones, which are common in Asteraceae, have shown antitumor activity in KB and P388 Leukemia (Hartwell 1976), while it may be noted that they do not degrade upon drying of samples (Stuessy 2013). Myrtaceae oils, on the other hand, are often extracted from fresh material, 50% of the studies noted by Padovan et al. (2013). Moreover, tea tree (*Melaleuca alternifolia*) oil and terpinen-4-ol can "significantly affect the in vitro viability of 2 murine tumour cell lines, AE17 and B16 in a dose-and time-dependent manner as assessed by the MTT assay" (Greay et al. 2010). Additionally, novel chalcones, which are open chain flavonoids, were discovered in dried root samples of *Calythropsis aurea* that exhibited a differentiated pattern of cytoxicity in the NCI 60-cell line (Beutler et al. 1993), and several chalcones are being used for treatment of viral disorders, cardiovascular diseases, parasitic infections, pain, gastritis, and stomach cancer (Batovska & Todorova 2010). Although *Calythropsis* has been included in *Calytrix* (Craven 1990), it can be distinguished from *Calytrix* by the absence of a calyx and presence of 4- rather than 5-merous flowers (Craven 1987; Keighery 2004). KB results in both *Calythropsis* root (30.4) and *Calytrix glutinosa* whole plant (22.2) were marginal (Table 1). Thus, one may expect *L. erubescens* root to contain novel antitumor active compounds.

Plants used in traditional medicine are often cited as a rational basis for finding new medicinal drugs; however, ethnobotanical relationships to antitumor active plants in this study are not apparent. Many Australian plants reportedly used in Aboriginal medicine occur in the subtropical woodland and forests regions, compared to relatively few reports from the Mediterranean flora (empirical obs.). Of relevance is *Scaevola spinescens* (Goodeniaceae) used to treat boils, rashes, and skin disorders (Cock & Kukkonen 2011) and anecdotal reports on Internet sites that mention use against cancer; root and twig-leaf samples of this species were inactive in KB (Table 1), which is consistent with the plant reported to be nontoxic (Cock & Kukkonen 2011). A review on 11 medicinal species of *Eremophila* (Scrophulariaceae) indicated that only leaves are employed in folk medicine (Richmond 1993); the KB activity was only in root, and in only one sample from ten species that I collected in WA (Table 2, Spjut 1982).

The high incidence of KB activity in root of WA woody plants is undoubtedly related to the perennial root being in contact with the microbial world as opposed to aerial parts of the plant exposed to the animal world. Various microbial-root symbiotic associations exist with most plants (e.g., rhizobial, actinorrhizal, mycorrhizal). Maytansine, discovered from KB screening of *Gymnosporia* (Celastraceae), is an ansamitocin generally produced by actinomycetes commonly found in soil, namely *Actinosynnema pretiosum* Hasegawa et al. 1983, for which there is substantial evidence for its presence in the rhizosphere of *Gymnosporia* spp. and the closely related maytansinoid shrub, *Putterlickia verrucosa* (E. Mey ex Sond.) Szyszyl (Cassady et al. 2004; Wings et al. 2013).

As reported by Kinghorn et al. (2003), many of their discovered novel antitumor active agents were from root of tropical plants. Six examples are: (**1**) stilbenoids 5-[(1E)-2-(4-hydroxyphenyl)ethenyl]-4,7- dimethoxy-3-methyl-2H-1-benzopyran-2-one from *Ekebergia benguelensis* C. DC. (Meliaceae), (**2**) naphthoquinone 2-(1 -hydroxyethyl)naphtho[2,3-b]furan-4,9- quinine from *Ekmanianthe longiflora* (Griseb.) Urb. (Bignoniaceae), (**3**) pervilleines that reverse multidrug resistance, discovered from *Erythroxylum pervillei* Baillon (Erythroxylaceae), (**4**) 13-hydroxy-15-oxozoapatlin from *Parinari curatellifolia* Benth. (Chrysobalanaceae), (**5**) norditerpenoid, (16S)-ent-16,17- dihydroxy-19-nor-kaur-4-en-3-one from *Exostema acuminata* Urb. (Rubiaceae), and (**6**) clerodane-type diterpenoids, intrapetacins and casearvestrins from *Licania intrapetiolaris* Spruce ex Hook. (Chrysobalanaceae). Additionally, Kinghorn et al. (2003) employed the KB assay in fractionation and isolation of the active compounds along with LNCaP (human hormone-dependent prostate), Lu1 (human lung) cells and other cell lines.

Plants collected as whole samples (Table 1, pl), or of just the entire top portion without root (px), also showed a higher incidence of KB activity (13.7%) than twig-leaf samples (4.2%). Perdue et al. (1970), in a review of herbaceous species screened for antitumor activity, found more active species when samples were made of separate plant parts, but there was little difference in activity between root and aerial parts; however, their review did not differentiate between annual and perennial herbs as well as shrubs. The relatively few root samples from herbs collected in California during 1965 in a report by Abbott et al. (1967) were from the annual *Amsinckia tesselata* A. Gray, and perennials *Astragalus douglasii* (Torrey & A. Gray) A. Gray var. *parishii* (A. Gray) M.E. Jones, *Dicentra chrysantha* Walp., *Marah fabaceus* Greene, and *Rumex hymenosepalus* Torr. This is in contrast to examples of common California shrubs from which only the tops of plants were picked: *Acamptopappus sphaerocephalus* A. Gray, *Artemisia californica* Less., *A. tridentata* Nutt., *Eriodictyon crassifolium* Benth, *Eriogonum fasciculatum* Hook & Arn., *Hymenoclea salsola* Torr. & . Gray, *Purshia glandulosa* Curran, *Salazaria mexicana* Torr., *Salvia mellifera* Greene, and others (Abbott et al. 1967).

It is interesting to note that two WA samples of *Olearia muelleri* (Asteraceae), one collected and screened during the 1960's, and another collected and screened 20 years later, were both active in KB. Perdue (1982) noted HeLa cell contamination being a problem for some cancer

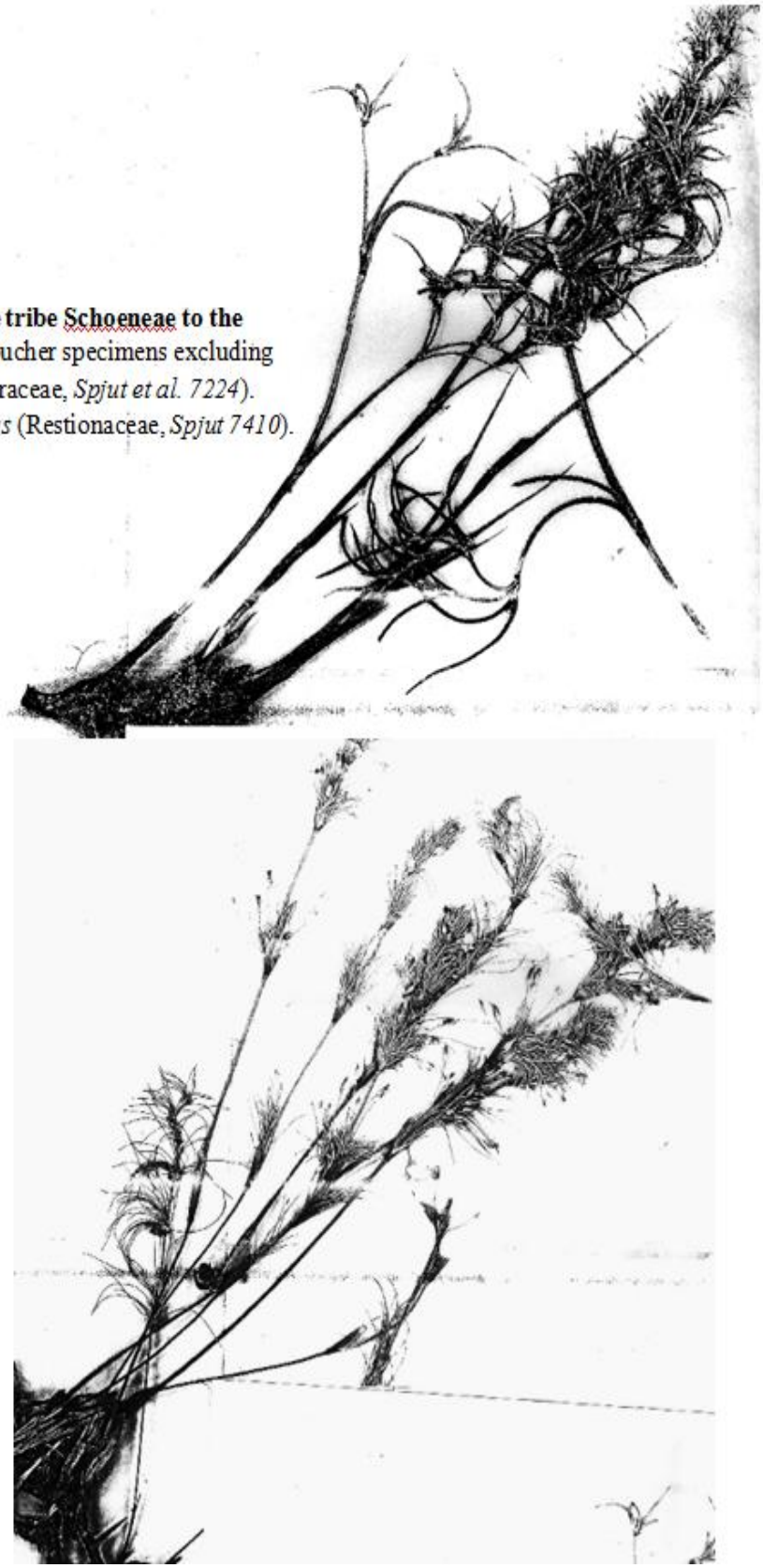

Fig. 6. Similarity of Cyperaceae tribe Schoeneae to the Restionaceae. Photocopies of voucher specimens excluding labels. Top: *Caustis dioica* (Cyperaceae, *Spjut et al. 7224*). Bottom: *Desmocladus fasciculatus* (Restionaceae, *Spjut 7410*).

bioassays; however, as evident from the preceding discussion, the KB results for WA plants are consistent with other chemical and screening data.

A significant lead to new anticancer drug(s) is the KB activity in Restionaceae (*Lepidobolus quadratus*) and the L1210 (LE) activity in a related family Cyperaceae, *Caustis dioica* (Table 1,2). *Lepidobolus quadratus* (Table 1) was strongly cytotoxic (1.22, 0.0559), and several other Restionaceae were marginal (≤30 µg/ml) to KB activity, *Ecdeiocolea monostachya* (23.8) and *Desmocladus virgatus* (28.3). *Caustis dioica* (Cyperaceae, Table 2) belongs to a group of dryland sedges in the Schoeneae tribe that have a Gondwana distribution (Barret 2013). Their similar morphology of flexuous stems, clustered branches arising from dark-brown leaf sheaths and dioecious habit as well as their ecology on dry soils, is much like the Restionaceae (Fig. 6).

The Cyperaceae, Restionaceae, and other related families classified in the order Poales have been negatively perceived for yielding novel antitumor compounds, referred to as "FONI" for Families of No Interest (Barclay & Perdue 1976, Cyperales). However, in view of the fact that only about 1 in 12,000 plant samples showed L1210 activity (Statz & Coon 1976), and that all compounds or closely related compounds from all fourteen L1210 active plants in Hartwell (1976) advanced to clinical trials among a total of 322 active compounds listed—it seems reasonable to conclude that further screening of *Caustis* and related Schoeneae, Restionaceae and also the related Anarthriaceae will lead to discovery of one or more novel, clinically useful anticancer drugs. Indeed, novel prenylated hydroxystilbenes have recently been discovered in propolis associated with *Lepidosperma* (Schoeneae) for which a patent has been filed in regard to the their potential for treating various cancers and "skin aging" (C. Duke et al. 2012).

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