Forest Plot as a Tool to Demonstrate the Pharmaceutical Potential of Plants in a Tropical Forest of Panama[1,2]

Angela I. Calderon, Cindy K. Angerhofer, John M. Pezzuto, Norman R. Farnsworth, Robin Foster, Richard Condit, Mahabir P. Gupta, and Djaja D. Soejarto

Calderon, Angela I. and Mahabir P. Gupta *(Center for Pharmacognostic Research on Panamanian Flora—CIFLORPAN, Apartado 10767, College of Pharmacy, University of Panama, Republic of Panama),* Robin B. Foster *(Environmental and Conservation Programs, Botany Department, Field Museum, Roosevelt Road at Lake Shore Drive, Chicago, IL 60612, USA, and Smithsonian Tropical Research Institute, Balboa, Republic of Panama),* Richard Condit *(Smithsonian Tropical Research Institute, Apartado 2072, Balboa, Republic of Panama), and* Cindy K. Angerhofer, John M. Pezzuto, Norman R. Farnsworth, and Djaja Doel Soejarto *(Program for Collaborative Research in the Pharmaceutical Sciences, College of Pharmacy, University of Illinois at Chicago, Chicago, IL 60612, USA).* Forest Plot as a Tool to Demonstrate the Pharmaceutical Potential of Plants in a Tropical Forest of Panama. *Economic Botany 54(3):278–294, 2000. Based on literature analysis of 308 angiosperm species inventoried from a 50-hectare forest plot on Barro Colorado Island, Panama, 40 species were selected and 80 samples (two samples for every species; leaf + twig and stembark samples) were collected for investigation of their medicinal/pharmaceutical potential. Extracts of these 80 samples were tested in bioassay systems to assess cancer chemoprevention (eight assays), antiplasmodial, cytotoxicity, and anti-HIV activities. Details of bioassay techniques are described. Of the 40 species, 12 (30%) showed activity in one or more of the 11 bioassay systems used. These active species are* Bombacopsis (= Pachira) quinata, Calophyllum longifolium, Casearia commersoniana, Lozania pittieri, Maclura tinctoria, Mouriri myrtilloides, Olmedia aspera (= Trophis caucana), Pseudobombax septenatum, Spondias radlkoferi, Stylogyne standleyi, Turpinia occidentalis, *and* Vochysia ferruginea. *Because literature data on the chemistry of* Bombacopsis (= Pachira) quinata, Lozania pittieri, Mouriri myrtilloides, Olmedia aspera (= Trophis caucana), Pseudobombax septenatum, *and* Stylogyne standleyi, *are lacking, and similar data on the other six species are deficient, further fractionation and isolation work on these active species potentially may yield novel, biologically active structures. This study demonstrates that a plot-based selection of plant species for evaluation of their potential medicinal/ pharmaceutical value has merit in achieving such a goal, and should be used in a program on plant drug discovery.*

Parcela de Bosque como un Diseño para Demostrar el Potencial Medicinal/Farmaceutico de Plantas de un Bosque Tropical de Panama. *A base de análisis de 308 especies de angiospermas inventariadas de una parcela de 50 hectáreas de un bosque en la Isla de Barro Colorado, Panama, 40 especies fueron seleccionadas y 80 muestras (hojas +ramitas y corteza de tallo, de cada especie) fueron coleccionadas para la investigación hacia su potencial medicinal/farmacéutico. Los extractos de estas 80 muestras fueron suministrados a bioensayos para detectar sus actividades en la prevención de cancer (8 tipos de ensayos), antipalúdica, citotoxicidad, y anti-HIV. Los detalles de los métodos de bioensayos se presentan. De las 40 especies, 12 (30%) demostraron actividad en uno o más de los 11 bioensayos empleados. Estas especies son* Bombacopsis (= Pachira) quinata, Calophyllum longifolium, Casearia commersoniana, Lozania pittieri, Maclura tinctoria, Mouriri myrtilloides, Olmedia aspera (= Trophis caucana), Pseudobombax septenatum, Spondias radlkoferi, Stylogyne standleyi, Turpinia occidentalis, *y* Vochysia ferruginea. *En vista de que* Bombacopsis (= Pachira) quinata, Lozania pittieri, Mouriri myrtilloides, Olmedia aspera (= Trophis caucana), Pseudobombax septenatum, *y* Stylogyne standleyi, *carecen de datos químicos de la literatura, mientras que datos químicos sobre las otras seis especies son deficientes, un trabajo de fraccionamiento y aislamiento sobre estas especies potencialmente nos pueda dar compuestos novedosos, biologicamente activos. Este estudio demuestra que el método de selección de especies de plantas a partir de especies*

inventariadas de una parcela de bosque, para someterlas a pruebas biológicas, tiene sus méritos para empleo más amplio.

In view of the increasing need for investigating plants of the tropical rain forests for their nontimber economic potential, the present research was undertaken. In the present study, the potential economic value of plants from a tropical rain forest of Panama was assessed, focusing on medicinal and pharmaceutical value, by carrying out biological evaluation of samples collected, utilizing a biodiversity-based plant selection approach. Specifically, plants were selected and collected from a large plot that had been already established and inventoried. Although a number of selection approaches (Soejarto 1996) may be utilized in a program to screen plants for biological activities, a plot-based selection approach was utilized based on the following rationale: (1) The results of such an approach may provide semiquantitative results (percent of active species) on the potential pharmaceutical value of plants found within the plot, and hence, in a forest tract; (2) Recollection of active species, even from sterile plants, may be performed using the still-standing and marked plants in the plot as a living reference (Soejarto 1991).

In the present study, the choice of a Panamanian tropical rain forest was based on the following criteria: (a) high plant diversity in these forests (D'Arcy 1987; Gupta 1995); (b) availability of a large forest plot and census data (Foster and Hubbell 1990; Foster, Condit, and Hubbell 1991); (c) availability of botanist collaborators in Panama; (d) familiarity with the country. Proof of a high biodiversity is given by the total number of vascular plant species in this country, estimated between 8000 and 10 000, of which 17.3% are considered endemic (Gupta 1995). Among the nonvascular plants are 350 mosses, plus an unknown number of hepatic and hornwort species (D'Arcy 1987). Thus, Panama

is among the 25 most plant-rich countries in the world (World Conservation Monitoring Centre 1992:84). For the present research, a list of identified angiosperm species as a basis for selection was obtained from the 1990 census data of a 50-hectare forest plot on the Barro Colorado Island (BCI) tropical rain forest (Foster, Condit, and Hubbell 1991). Similar plot-based methodology for the purpose of biological evaluation has been described and used previously (Böhlke 1997; Böhlke et al. 1996; Horgen 1997; Horgen et al. 1997; Soejarto et al. 1996).

Barro Colorado is an island in the Panama Canal Zone, lying midway between the Atlantic and Pacific Oceans. At about 15.6 km^2, BCI is the largest island in Gatun Lake, which was formed between 1911 and 1914 by the damming of the Chagres River to the Canal. BCI was set aside as a biological preserve in 1923 and is currently supervised by the Smithsonian Tropical Research Institute, which operates a modern field station at the Laboratory Clearing, on the northeast shore (Leigh, Rand, and Windsor 1982). The vegetation on Barro Colorado has mature (> 500 years old) semievergreen tropical forest (Foster and Brokaw 1982). There are 1207 species of vascular plants in BCI, including 409 tree and shrub species (Hubbell and Foster 1992). A previous field study by a team of ecologists at the Smithsonian Institution's Tropical Research Station on this island in the early 1980s (Hubbell and Foster 1983) showed that 108 species of trees with diameters of 20 cm and above were found in a 50-hectare plot. A census in 1990 of free-standing woody plants of 1 cm diameter at breast height (dbh) from the same 50-hectare plot yielded 308 species. Such an assortment of plant species was considered an appropriate amount of diversity in searching for compounds with biological activities that could be of potential pharmaceutical value.

To match this biodiversity-based approach in the selection of plants to study, a broad spectrum of biological activities was chosen for screening 80 plant extracts. The rationale for the selection of the bioassays was to find new leads for dis-

covering medicinal agents for diseases that have shown high demographic incidence around the world, but for which no appropiate drugs are available for current therapies. The details of the biological assays selected are discussed under the Materials and Methods section.

MATERIALS AND METHODS

LITERATURE SEARCH AND PRIORITIZATION OF PLANTS FOR COLLECTION

Literature data from previous biological studies on every plant species in the 1990 census of the 50-hectare plot of the tropical rain forest on the Barro Colorado Island were queried from the NAPRALERT database (which summarizes the world literature on medicinal uses, chemistry, and bioactivity of plants; Loub et al. 1985). Based on the amount of data available for a particular species and on data from previous studies of different species of the same genus, certain species were excluded and others were classified into various priorities for collection. To avoid missing crucial information that had been published in the literature, scientific synonyms for each species were verified, and each synonym was also queried from the NAPRALERT database. Subsequently, a list of plants was created according to three priorities depending on the amount of information available for each species. These were: (a) plants that appear never to have been studied chemically and/or pharmacologically (91 species, Table 1); (b) those that have been studied chemically and/or pharmacologically, but need further studies (24 species, Table 2); and (c) those that have been extensively studied both chemically and pharmacologically, to the extent that no additional studies appear to be warranted (four species, Table 3).

Of the 308 species of trees with a diameter of 1 cm at breast height (dbh), which represent 183 genera from the 50-hectare forest plot, 119 (38%) species were found to be of interest for biological evaluation, based on available ethnomedical, chemical, and pharmacological data. The criteria used for exclusion of the remaining species were the amount of pharmacological and chemical data, and the time and budget considerations for plant collection and biological testing.

PLANT COLLECTION STRATEGY

The list of 91 plants of priority one (Table 1) was sent to the Smithsonian Tropical Research

TABLE 1. NINETY-ONE FIRST–PRIORITY SPECIES SELECTED FOR FURTHER STUDY FROM A POOL OF 303 FREE-STANDING WOODY SPECIES FOUND IN 1 HECTARE FOREST PLOT IN BARRO COLORADO ISLAND.

Species[a] (family)
Adelia triloba[b] (Euphorbiaceae)
Allophylus psilospermus[c] (Sapindaceae)
Amaioua corymbosa[b] (Rubiaceae)
Anaxagorea panamensis[c] (Annonaceae)
Bertiera guianensis[c] (Rubiaceae)
Calophyllum longifolium[c] (Guttiferae)
Capparis frondosa[c] (Capparidaceae)
Casearia commersoniana[c] (Flacourtiaceae)
Cassipourea elliptica[c] (Rhizophoraceae)
Cavanillesia platanifolia[b] (Bombacaceae)
Chimarrhis parviflora[b] (Rubiaceae)
Chrysochlamys eclipes[c] (Guttiferae)
Clidemia septuplinervia[c] (Melastomataceae)
Conostegia bracteata[b] (Melastomataceae)
Conostegia cinnamomea[b] (Melastomataceae)
Cordia lasiocalyx[c] (Boraginaceae)
Coussarea curvigemmia[c] (Rubiaceae)
Cyphomandra hartwegii[d] (Solanaceae)
Dendropanax stenodontus (= *arboreus*)[c] (Araliaceae)
Desmopsis panamensis[b] (Annonaceae)
Diospyros artanthifolia[c] (Ebenaceae)
Drypetes standleyi[c] (Euphorbiaceae)
Eugenia nesiotica[c] (Myrtaceae)
Faramea talamancarum[c] (Rubiaceae)
Garcinia intermedia[c] [Syn.: *Rheedia edulis*] (Guttiferae)
Guapira (= *Pisonia*) *standleyana*[c] (Nyctaginaceae)
Guatteria dumetorum[c] (Annonaceae)
Gustavia superba[c] (Lecythidaceae)
Hampea appendiculata[c] (Malvaceae)
Heisteria concinna[c] (Olacaceae)
Koanophyllon wetmorei[c] (Compositae)
Lacmellea panamensis[c] (Apocynaceae)
Lafoensia punicifolia[c] (Lythraceae)
Leandra dichotoma[c] (Melastomataceae)
Licania platypus[d] (Chrysobalanaceae)
Lindackeria laurina[c] (Flacourtiaceae)
Malpighia romeroana[c] (Malpighiaceae)
Marila laxiflora[c] (Guttiferae)
Miconia hondurensis[c] (Melastomataceae)
Miconia impetiolaris[c] (Melastomataceae)
Mouriri myrtilloides[b] (Melastomataceae)
Myrospermum frutescens[c] (Leguminosae)
Nectandra cissiflora[c] (Lauraceae)
Neea amplifolia[c] (Nyctaginaceae)
Ochroma pyramidale[c] (Bombacaceae)
Ocotea oblonga[c] (Lauraceae)
Olmedia aspera (= *Trophis caucana*)[b] (Moraceae)
Pavonia (= *Lopimia*) *dasypetala*[c] (Malvaceae)
Pentagonia macrophylla[c] (Rubiaceae)

TABLE 1. CONTINUED.

Species[a] (family)
Perebea xanthochyma[c] (Moraceae)
Picramnia latifolia[c] (Simaroubaceae)
Piper perlasense[c] (Piperaceae)
Pithecellobium (= *Abarema*) *macradenium*[c] (Leguminosae)
Platymiscium pinnatum[c] (Leguminosae)
Platypodium elegans[b] (Leguminosae)
Pochota (= *Bombacopsis*; *Pachira*) *quinata*[b] (Bombacaceae)
Pochota (= *Bombacopsis*; *Pachira*) *sessilis*[b] (Bombacaceae)
Poulsenia armata[b] (Moraceae)
Pourouma bicolor[c] (Moraceae)
Prioria copaifera[b] (Leguminosae)
Protium panamense[c] (Burseraceae)
Pseudobombax septenatum[b] (Bombacaceae)
Quararibea asterolepis[c] (Bombacaceae)
Quiina schippii[c] (Quiinaceae)
Randia armata[c] (Rubiaceae)
Rinorea sylvatica[c] (Violaceae)
Schefflera morototoni[c] (Araliaceae)
Sloanea terniflora[c] (Elaeocarpaceae)
Sorocea affinis[c] (Moraceae)
Spachea membranacea[b] (Malpighiaceae)
Spondias radlkoferi[c] (Anacardiaceae)
Stylogyne standleyi[b] (Myrsinaceae)
Tachigali versicolor[c] (Leguminosae)
Talisia princeps[c] (Sapindaceae)
Ternstroemia tepezapote[c] (Theaceae)
Tetrathylacium johansenii[b] (Flacourtiaceae)
Tocoyena pittieri[c] (Rubiaceae)
Trattinnickia aspera (Burseraceae)
Trichanthera gigantea[d] (Acanthaceae)
Trophis racemosa[d] (Moraceae)
Turpinia occidentalis[c] (Staphyleaceae)
Unonopsis pittieri[c] (Annonaceae)
Vismia billbergiana[c] (Guttiferae)
Vochysia ferruginea[c] (Vochysiaceae)
Xylopia macrantha[c] (Annonaceae)
Xylosma oligandrum[c] (Flacourtiaceae)

[a] Nomenclature follows the Flora of Panama, checklist and index. (Vol 2, 1987).
[b] Genus is not in NAPRALERT.
[c] Species is not in NAPRALERT, but genus is.
[d] Species is in NAPRALERT, with ethnomedical data only.

Institute (STRI) for review, to determine the ease of their collection in adequate quantities for biological testing (300–500 g dry weight per sample). Forty species were determined to be relatively easy to collect in testing quantities. From a biodiversity point-of-view, these 40 species are taxonomically diverse, consisting of 39

TABLE 2. SECOND PRIORITY SPECIES FROM A POOL OF 303 FREE-STANDING WOODY SPECIES FOUND IN 1 HECTARE FOREST PLOT IN BARRO COLORADO ISLAND.

Species[a] (family)
Aegiphila panamensis[b] (Verbenaceae)
Alibertia edulis[c] (Rubiaceae)
Alseis blackiana[b] (Rubiaceae)
Annona hayesii[c] (Annonaceae)
Annona spraguei[c] (Annonaceae)
Apeiba tibourbou[b] (Tiliaceae)
Brosimum alicastrum[e] (Moraceae)
Casearia guianensis[b] (Flacourtiaceae)
Cespedesia macrophylla[b] (Ochnaceae)
Cupania rufescens[b] (Sapindaceae)
Guarea guidonia[d] (Meliaceae)
Hamelia axillaris[b] (Rubiaceae)
Hasseltia floribunda[c] (Flacourtiaceae)
Herrania purpurea[c] (Sterculiaceae)
Lacistema aggregatum[b] (Flacourtiaceae)
Ormosia amazonica[c] (Leguminosae)
Ouratea lucens[b] (Ochnaceae)
Phoebe (= *Cinnamomum*) *cinnamomifolia*[c] (Lauraceae)
Posoqueria latifolia[c] (Rubiaceae)
Schizolobium parahybum[c] (Leguminosae)
Stemmadenia grandifolia[e] (Apocynaceae)
Triplaris cumingiana[b] (Polygonaceae)
Zanthoxylum belizense (= *ekmanii*)[c] (Rutaceae)
Zuelania guidonia[e] (Flacourtiaceae)

[a] Nomenclature follows the Flora of Panama.
[b] Species is in NAPRALERT, with either biological or chemical data.
[c] Species is in NAPRALERT, chemical data only.
[d] Species is in NAPRALERT, with biological and ethno data.
[e] Species is in NAPRALERT, chemical and ethnomedical data.

TABLE 3. THIRD PRIORITY SPECIES FROM A POOL OF 303 FREE-STANDING WOODY SPECIES FOUND IN 1 HECTARE FOREST PLOT IN BARRO COLORADO ISLAND.

Species[a] (family)
Aphelandra sinclairiana[b] (Acanthaceae)
Jacaranda copaia[c] (Bignoniaceae)
Swartzia simplex[c] (Leguminosae)
Symphonia globulifera[b] (Guttiferae)

[a] Nomenclature follows the Flora of Panama.
[b] Species is in NAPRALERT, with biological, chemical and ethnomedical data.
[c] Species is in NAPRALERT, with both biological and chemical data, but without ethnomedical data.

TABLE 4. PLANTS COLLECTED IN PANAMA IN 1995, SELECTED FROM NINETY-ONE FIRST-PRIORITY SPECIES, BASED ON LOGISTICAL AND SPECIES ABUNDANCE CONSIDERATIONS.[a]

Species (Family) (voucher specimen)	Place, collectors, and date of collection
Adelia triloba (Muell.-Arg.) Hemsl. Euphorbiaceae FLORPAN #2105	District of Panama, Soberania Park, 100 m from the entrance of the Plantation trail, 9°04′ N. lat. and 79°39′ W. long. S. Aguilar and A. Castillo 05/26/95
Allophylus psilospermus Raldk. Sapindaceae FLORPAN #2120	District of Panama, Gamboa, 5 km from entrance of Oleoducto trail, 9°08′ N. lat. and 79°44′ W. long. S. Aguilar and A. Castillo 06/02/95
Amaioua corymbosa HBK. Rubiaceae FLORPAN #2117	District of Panama, Pelado hill (Gamboa) 9°07′ N. lat. and 79°43′ W. long. S. Aguilar and A. Castillo 06/01/95
Annona spraguei Saff. Annonaceae FLORPAN #2091	District of Panama, Plantation trail, Soberania National Park, approximately 9°04′ N. lat. and 79°39′ W. long. S. Aguilar, A. Hernandez and A. Calderon 05/17/95
Bombacopsis (= *Pachira*) *quinata* (Jacq.) Dugand Bombacaceae FLORPAN #2115	District of Panama, Soberania National Park, 100 m of the entrance of the Plantation trail, 9°04′ N. lat. and 79°39′ W. long. S. Aguilar and A. Castillo 06/01/95
Bombacopsis (= *Pachira*) *sessilis* (Benth.) Pitt. Bombacaceae FLORPAN #2103	District of Panama, between Paraiso and summit, 9°03′ N. lat. and 79°38′ W. long. S. Aguilar and A. Castillo 05/26/95
Calophyllum longifolium Willd. Guttiferae FLORPAN #2107	District of Panama, 300 m from the entrance of Cruces trail, 9°07′ N. lat. and 79°38′ W. long. S. Aguilar and A. Castillo 05/29/95
Casearia commersoniana Camb. Flacourtiaceae FLORPAN #2124	District of Chorrera, beside the fence of the Barro Colorado monument, 9°06′ N. lat. and 79°52′ W. long. S. Aguilar and A. Castillo 06/09/95
Cavanillesia platanifolia HBK. Bombacaceae FLORPAN #2110	District of Arraijan, Cocoli, 8°58′ N. lat. and 79°37′ W. long. S. Aguilar and A. Castillo 05/31/95
Coussarea curvigemmia Dwyer Rubiaceae FLORPAN #2093	District of Arraijan, Cocoli, 8°58′ N. lat. and 79°37′ W. long. S. Aguilar, A. Hernandez and A. Calderon 05/22/95
Desmopsis panamensis (Rob.) Saff. Annonaceae FLORPAN #2122	District of Chorrera, beside the fence of the Barro Colorado monument, 9°06′ N. lat. and 79°52′ W. long. S. Aguilar and A. Castillo 06/05/95
Gustavia superba (HBK.) Berg. Lecythidaceae FLORPAN #2101	District of Panama, Soberania National Park, 9°04′ N. lat. and 79°39′ W. long. S. Aguilar and A. Castillo 05/26/95
Heisteria concinna Standl. Olacaceae FLORPAN #2095	District of Arraijan, Cocoli 8°58′ N. lat. and 79°37′ W. long. S. Aguilar and A. Castillo 05/23/95
Lacmellea panamensis (Woods.) Markgr. Apocynaceae FLORPAN #2121	District of Chorrera, beside fence of the Barro Colorado monument, 9°06′ N. lat. and 79°52′ W. long. S. Aguilar and A. Castillo 06/05/95
Lindackeria laurina Presl Flacourtiaceae FLORPAN #2100	District of Panama, Gamboa, entrance to Oleoducto trail, 9°05′ N. lat. and 79°39′ W. long. S. Aguilar and A. Castillo 05/25/95
Lozania pittieri (Blake) L.B. Sm. Flacourtiaceae FLORPAN #2128	District of Capira, Campana hill beside trail to former forest guard house, 8°41′ N. lat. and 79°56′ W. long. S. Aguilar and A. Castillo 06/12/96

TABLE 4.   CONTINUED.

Species (Family) (voucher specimen)	Place, collectors, and date of collection
Luehea seemannii Tr. et Pl. Tiliaceae FLORPAN #2109	District of Arraijan, Cocoli 8°58′ N. lat. and 79°37′ W. long. S. Aguilar and A. Castillo 05/31/95
Maclura tinctoira (L.) D. Don ex Steud. Moraceae FLORPAN #2113	District of Arraijan, Cocoli 8°58′ N. lat. and 79°37′ W. long. S. Aguilar and A. Castillo 05/31/95
Miconia impetiolaris (Sw.) D. Don Melastomataceae FLORPAN #2090	District of Panama, Plantation trail, Soberania National Park, approximately 9°04′ N. lat. and 79°39′ W. long. S. Aguilar, A. Hernandez and A. Calderon 05/17/95
Mouriri myrtilloides (Sw.) Poir. Melastomataceae FLORPAN #2126	District of Chorrera, beside fence of the Barro Colorado Monu- ment, 9°06′ N. lat. and 79°52′ W. long. S. Aguilar and A. Castillo 06/09/95
Ochroma pyramidale (Cav. ex Lam.) Ur- ban Bombacaceae FLORPAN #2112	District of Arraijan, Cocoli 8°58′ N. lat. and 79°37′ W. long. S. Aguilar and A. Castillo 05/31/95
Olmedia aspera (= *Trophis caucana*) R. et P. Moraceae FLORPAN #2106	District of Panama, entrance to the Cruces trail 9°06′ N. lat. and 79°37′ W. long. S. Aguilar and A. Castillo 05/29/95
Pentagonia macrophylla Benth. Rubiaceae FLORPAN #2097	District of Panama, Gamboa, 1 km from Oleducto trail, 9°05′ N. lat. and 79°39′ W. long. S. Aguilar and A. Castillo 05/25/95
Perebea xanthochyma Karst. Moraceae FLORPAN #2125	District of the Chorrera, beside fence of the Barro Colorado monument, 9°06′ N. lat. and 79°52′ W. long. S. Aguilar and A. Castillo 06/09/95
Picramnia latifolia Tul. Simaroubaceae FLORPAN #2114	District of Arraijan, Cocoli, 8°58′ N. lat. and 79°37′ W. long. S. Aguilar and A. Castillo 05/31/95
Poulsenia armata (Miq.) Standl. Moraceae FLORPAN #2098	District of Panama, Gamboa, 1 km from the Oleducto trail, 9°05′ N. lat. and 79°39′ W. long. S. Aguilar and A. Castillo 05/25/95
Prioria copaifera Griseb. Leguminosae FLORPAN #2127	District of the Chorrera, beside fence of the Barro Colorado Monument, 9°06′ N. lat. and 79°52′ W. long. S. Aguilar and A. Castillo 06/09/95
Protium panamense (Rose) I. M. Johnst. Burseraceae FLORPAN #2099	District of Panama, Gamboa, 1 km from the Oleducto trail, 9°05′ N. lat. and 79°39′ W. long. S. Aguilar and A. Castillo 05/25/95
Pseudobombax septenatum (Jacq.) Dugand Bombacaceae FLORPAN #2102	District of Panama, between Paraiso and summit of 9°03′ N. lat. and 79°38′ W. long. S. Aguilar and A. Castillo 05/26/95
Garcinia (= *Rheedia*) *edulis* (Seem.) Pl. et Tr. Guttiferae FLORPAN #2123	District of Chorrera, beside fence of the Barro Colorado monu- ment, 9°06′ N. lat. and 79°52′ W. long. S. Aguilar and A. Castillo 06/09/95
Rinorea sylvatica (Seem.) Kuntze Violaceae FLORPAN #2108	District of Panama, the Cruces trail, 9°06′ N. lat. and 79°37′ W. long. S. Aguilar and A. Castillo 05/29/95
Schefflera morototoni (Aubl.) Maguire, Stey. et Frod. Araliaceae FLORPAN #2094	District of Arraijan, Cocoli, 8°58′ N. lat. and 79°37′ W. long. S. Aguilar, A. Hernandez and A. Calderon 05/22/95

TABLE 4. CONTINUED.

Species (Family) (voucher specimen)	Place, collectors, and date of collection
Sorocea affinis Hemsl. Moraceae FLORPAN #2096	District of Arraijan, Cocoli, 8°58' N. lat. and 79°37' W. long. S. Aguilar, A. Hernandez and A. Calderon 05/23/95
Spondias radlkoferi J.D. Sm. Anacardiaceae FLORPAN #2111	District of Arraijan, Cocoli, 8°58' N. lat. and 79°37' W. long. S. Aguilar and A. Castillo 05/31/95
Stylogyne standleyi Lund. Myrsinaceae FLORPAN #2116	District of Panama, Soberania National Park, 100 m of the entrance of the Plantation trail, 9°04' N. lat. and 79°39' W. long. S. Aguilar and A. Castillo 06/01/95
Tachigali versicolor Standl. et L.O. Wms. Leguinosae FLORPAN #2118	District of Panama, Pelado hill (Gamboa) 9°07' N. lat. and 79°43' W. long. S. Aguilar and A. Castillo 06/01/95
Tetrathylacium johansenii Standl. Flacourtiaceae FLORPAN #2092	District of Arraijan, Cocoli, 8°58' N. lat. and 79°37' W. long. S. Aguilar, A. Hernandez and A. Calderon 05/22/95
Turpinia occidentalis (Sw.) G. Don Staphyleaceae FLORPAN #2129	District of Capira, Campana hill beside the trail to the former forest guard house. 8°41' N. lat. and 79°56' W. long. S. Aguilar and A. Castillo 06/12/95
Vismia billbergiana (Buerl.) Guttiferae FLORPAN #2119	District of Panama, Pelado hill (Gamboa), 9°07' N. lat. and 79°43' W. long. S. Aguilar and A. Castillo 06/01/95
Vochysia ferruginea Mart. Vochysiaceae FLORPAN #2104	District of Panama, highway to Gamboa, 9°03' N. lat. and 79°38' W. long. S. Aguilar and A. Castillo 05/26/95

ª Two samples were collected from each species: LP = leaf + twig sample, and SB = stembark sample.

genera in 22 families (Table 5). After a collection budget was established, collection work and identification of the plants collected were undertaken by staff botanists of STRI.

PLANT COLLECTION

Samples of the 40 species were collected in an area outside of the plot within a 50-mile radius (to reduce the probability of chemical variability) on Barro Colorado Island (Table 4). This sampling method was used because Barro Colorado Island is a Nature Monument, and collection in an experimental plot was not allowed. This restriction attempts to strengthen conservation efforts in that experimental area, in the extraction of plant resources. For each species, two different samples were collected, namely, a leaf + twig sample (1 sample) and a stembark sample (1 sample); each sample was labeled using the collector's collection number and the part of the plant collected. Samples were dried in a herbarium dryer at 30–50°C with electric fans for air circulation. This measure was intended to prevent sample molding and deterioration, due to the high humidity in the Panamanian tropical rain forest. For every tree species collected, four sets of voucher herbarium specimens, fully labeled, were prepared to document the plant samples. One set of duplicate specimens remains at STRI (SCZ), one set was sent to the herbarium of the Field Museum in Chicago (F), one set was kept at the CIFLORPAN Herbarium in Panama City, and one set was sent to the National Herbarium of the Smithsonian Institution (US), Washington, DC.

CHEMICAL EXTRACTION

The processing and extraction of plant material (consisting of 80 samples, representing 40 species) were carried out in the Research Center for Pharmacognostic Studies on Panamanian Flora (CIFLORPAN) at the University of Panama. Methanol extraction of 20 g of each sample was performed overnight.

TABLE 5. TAXONOMIC DIVERSITY OF PLANT SAMPLES COLLECTED AND INVESTIGATED.

Family	Number of genera	Number of species
Anacardiaceae	1	1
Annonaceae	2	2
Apocynaceae	1	1
Araliaceae	1	1
Bombacaceae	4	5
Burseraceae	1	1
Euphorbiaceae	1	1
Flacourtiaceae	4	4
Guttiferae	3	3
Lecythidaceae	1	1
Leguminosae	2	2
Melastomataceae	2	2
Moraceae	5	5
Myrsinaceae	1	1
Olacaceae	1	1
Rubiaceae	3	3
Sapindaceae	1	1
Simaroubaceae	1	1
Staphyleaceae	1	1
Tiliaceae	1	1
Violaceae	1	1
Vochysiaceae	1	1
TOTAL	22 39	40

SELECTION OF BIOLOGICAL ASSAYS

Bioassays were selected to represent diseases with a high incidence of occurrence in the world. These include cancer chemopreventive, antiplasmodial, cytotoxicity, and anti-HIV reverse transcriptase assays. Bioassays were performed in the Bioassay Research Facility of the PCRPS, College of Pharmacy, University of Illinois at Chicago.

CANCER CHEMOPREVENTIVE ASSAYS

Antimutagenicity Assay. This assay is based on the ability of mutated bacteria (*Salmonella typhimurium* strain TM677) to form colonies in the presence of the purine analog 8-azaguanine (8-AG). The assay was carried out in Petri dishes as described previously (Skopek et al. 1978a,b). A decrease in the mutant fraction due to the presence of a test substance, compared to the mutagen alone, indicates antimutagenic activity.

Induction of Quinone Reductase with Cultured Hepa 1C1C7 Cells. This is a simple system for rapid detection and measurement of the induction of phase II enzymes that detoxify carcinogens, based on the direct assay of the activity of quinone reductase. The induction of quinone reductase was based on the procedure described by Prochaska, Santamaria, and Talaly (1992). In brief, the extracts were evaluated for the induction of quinone reductase in Hepa 1c1c7 cells, and their cytotoxicity was assessed through the determination of protein with crystal violet staining.

Inhibition of TPA-Induced Ornithine Decarboxylase Activity. The aim of this assay is to look for potential plant extracts that could inhibit phorbol ester-induced ornithine decarboxylase (ODC) activity in cell culture. Both ODC enzyme activity and the resulting polyamines are essential for cellular proliferation of normal mammalian cells; however, they are overexpressed in various cancer cells (Auvinen et al. 1992).

The ornithine decarboxylase activity was assayed directly in 24-well plates using mouse epidermal cells, line 308, derived from adult Balb/c mouse skin by measuring the release of $[^{14}C]CO_2$ from L-[1-^{14}C]ornithine as described by Lichti and Gottesman (1982), and the cytotoxicity of the extracts towards the cell line was measured by protein determination.

Antioxidant Activity. This assay detects potential antioxidant activity of plant extracts through their scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radicals independently of any enzymatic activity (Smith et al. 1987).

This assay was performed according to the method carried out by Smith (1982; Smith et al. 1987). The reaction mixtures containing plant extracts and DPPH ethanolic solution in 96-well microtiter plates were incubated at 37°C for 30 min. The absorbance was measured at 515 nm (Blois 1958).

Inhibition of Cyclooxygenase Activity. Oxidants have an important role in the promotion of carcinogenesis (Pezzuto 1995). They activate phospholipase A_2, leading to production of arachidonic acid. Arachidonic acid, in turn, serves as a substrate for cyclooxygenase, whose function is to catalyze the production of prostaglandins. Cyclooxygenase activity is considered to be related to tumor promotion as exemplified by the fact that the inhibition of TPA-induced ODC activity by indomethacin is overcome by concurrent application of PGE_2 (Verma, Ashendew,

and Boutwell 1980). For instance, prostaglandins contribute to the growth and possible spread of tumors in human colon carcinogenesis (Marnett 1992).

Cyclooxygenase activity was determined by monitoring the co-oxidation of a peroxidase co-substrate as a prescreen of samples.The consumption of oxygen was used to confirm the inhibition of cyclooxygenase activity by active samples. The procedure adopted was described by Kulmacz and Lands (1987).

Induction of Differentiation with HL-60 Cells. This assay is used to search for agents that induce terminal differentiation in HL-60 cells. The induction of granulocytes, monocytes, eosinophils, or macrophage-like cells, without toxicity, is a unique response not detected in other assays in this panel (Pezzuto 1995).

The plant extracts were evaluated for induction of HL-60 cell differentiation through the following assays: the reduction of nitroblue tetrazolium (NBT) (indicative of monocytes and granulocytes), which shows the ability to produce superoxide when challenged with phorbol ester. The method was described by Ostrem et al. (1987) and by Sokoloski et al. (1993). The nonspecific/specific esterase assay is indicative of monocytes and macrophage-like cells and monocytes and other cell types, respectively, described by Zhou et al. (1989) and Sokoloski et al. (1993). The inhibition of [³H] thymidine incorporation was intended to evaluate the level of HL-60 cell proliferation, through the determination of the [³H]thymidine incorporation into the DNA (Collins et al. 1978).

Estrogenic and Antiestrogenic Activity with Ishikawa Cells. This test exploits the hormone-dependent characteristic of human breast cancers. This is evaluated by measuring agents that are capable of displacing estrogen, which may demonstrate either antagonistic or agonistic activity (Pezzuto 1995).

This bioassay utilized the Ishikawa human endometrial adenocarcinoma cell line grown in 96-well plates. The estrogenic activity was measured by alkaline phosphatase, which is markedly stimulated by estrogens in these cells, so that the enzyme can be quantified in situ using a chromogenic substrate. Antiestrogenic activity was determined by blockage of the action of estradiol. The protocol followed was described by Pisha and Pezzuto (1997).

Inhibition of DMBA-Induced Lesion Forma-tion in Mammary Organ Culture. The development of preneoplastic alveolar lesions in mammary gland organ culture is an attractive model by which to evaluate the efficacy of potential chemopreventive agents prior to an in vivo carcinogenesis study (Mehta and Moon 1986).

The extracts, at a concentration of 10 μg/mL, were tested in mammary glands from four-week old balbc/c mice in organ culture (McCormick and Moon 1986) with some hormones. The percentage of inhibition of mammary lesions produced by DMBA (7,12-dimethylbenz(a)anthracene) was assessed.

ANTIPLASMODIAL ASSAY

This microdilution method was developed for measuring the activity of potential antimalarial drugs against cultured intraerythrocytic asexual forms of the human malaria parasite (Desjardins et al. 1979). The quantitative measurements of the antiplasmodial activity in this assay is based on the inhibition of uptake of a radiolabeled nucleic acid precursor by the parasite during short-term culture in microtiter plates.

The antiplasmodial activity of extracts was assessed with an in vitro radioisotope-incorporation method. Concentrations of both extracts and standards that inhibited parasite-specific incorporation of [³H]hypoxanthine by 50% (IC_{50}) were determined by nonlinear regression analysis. Zero drug controls indicate 100% incorporation (Desjardins et al. 1979).

CYTOTOXICITY ASSAY

This assay is a rapid and sensitive method that was developed for in vitro anticancer screening by measuring the cellular protein content of human epidermoid carcinoma (KB) cultures in 96-well microtiter plates (Likhitwitayawuid et al. 1993; Skehan et al. 1990). The KB cell line was purchased from the American Type Culture Collection (Rockville, MD).

INHIBITION OF HIV-1 REVERSE TRANSCRIPTASE ASSAY

The evaluation for the inhibition of HIV-1 reverse transcriptase is a useful approach to finding new natural products that could be specific inhibitors. Attention is focused on this enzyme because it is specific for the replication and infectivity of retroviruses including HIV. The viral RT has no counterpart in the human cell, consequently, it is unique to the virus.

TABLE 6. SUMMARY OF CHEMOPREVENTIVE ACTIVITIES OF PROMISING PANAMANIAN PLANTS.

Species	Family	Part tested	Cox-1[a] (% inhib)	Anti-ox[b] IC50	QR[c] CD[d]/IC50 = CI[e]	HL-60 cell activity[f] ED50	Anti-mut[g] (% inhib)	MMOCh[b] (% inhib)
Bombacopsis (= Pachira) quinata (Jacq.) Dugand	Bombacaceae	SB		18.9				
Calophyllum longifolium Willd.	Guttiferae	LP				17.6	23	
Casearia commersoniana Camb.	Flacourtiaceae	SB		20.0				
Lozania pittieri (Blake) L.B. Sm.	Flacourtiaceae	SB	79					
Maclura tinctoria (L.) D. Don ex Steud.	Moraceae	LP			3.8/19.1 = 5		29	42.8
Mouriri myrtilloides (Sw.) Poir	Melastomataceae	SB		17.7				42.8
Pseudobombax septenatum (Jacq.) Dugand	Bombacaceae	LP				12.6		
Spondias radlkoferi J.D. Sm.	Anacardiaceae	LP	77	7.14				37.5
Vochysia ferruginea Mart.	Vochysiaceae	LP	72					

IC50 and ED50 values are expressed in μg/mL:
[a] Inhibition of cyclooxygenase using polarographic method.
[b] DPPH free radical scavenging activity.
[c] Induction of quinone reductase.
[d] Conc. of 2-fold of quinone reductase.
[e] Chemopreventive index.
[f] Induction of HL-60 cell differentiation.
[g] Antimutagenic activity.
[h] Inhibition of DMBA-induced lesion formation in mammary organ culture.

This assay was conducted as described by Tan et al. (1991). For screening, all plant extracts initially were tested for enzyme inhibition with homodimer p66 enzyme. Extracts with inhibition of >50% were then tested for the presence of polyphenolic compounds (tannins) utilizing FeCl₃ reagent. Tannins were removed from plant extracts using insoluble polyvinylpyrrolidone (PVP). Then, extracts were tested for enzyme inhibition of the dimer p66/p51. Calculation of IC50 values for active extracts with or without tannins was performed.

RESULTS

Based on the methodology described above, 80 plant samples from 40 different species of flowering plants, distributed in 39 genera and 22 families were selected from the 50-hectare forest plot. Collection of samples (Table 4) was performed during 17 May through 12 June, 1995, each sample in the amount of 300–500 g dry weight. Half (40) of the samples collected consisted of leaf + twig and the other half of stembark. These samples were extracted and the ex-

tracts were sent to the PCRPS in Chicago. A complete list of the voucher herbarium specimens and of the localities of the 40 species is presented in Table 4. For each species, data on the specific name, the family, the type of samples collected, the voucher herbarium specimen number (FLORPAN), their exact collecting localities, the habitat, and the collector's name are given.

EVALUATION OF PANAMANIAN PLANTS IN CANCER CHEMOPREVENTION ASSAYS

Bioassay results are presented in Table 6. Types of bioassays are indicated in the column heading and abbreviated as follows: COX 1 = cyclooxygenase assay with polarigraphic oxygen electrode; ANT-OX = DPPH free radical scavenging activity (antioxidant activity); QR = induction of quinone reductase with cultured Hepa 1c1c7 cells; HL-60 = induction of HL-60 cell differentiation; ANTMUT = antimutagenic activity; MMOC = inhibition of DMBA-induced lesion formation in mammary organ culture.

Inhibition of Cyclooxygenase Activity (COX

1). The 80 extracts were tested for their ability to inhibit cyclooxygenase activity, using the colorimetric method. Fifty-nine extracts were active in the co-substrate assay with IC_{50} values < 25 μg/mL and tested for their ability to inhibit cyclooxygenase activity using the polarographic oxygen electrode method. The extracts active in the oxygen consumption assay at 70 μg/mL were: stembark of *Lozania pittieri* (79% inhibition), leaf + twig of *Spondias radlkoferi* (77% inhibition), and leaf + twig of *Vochysia ferruginea* (72% inhibition) (Table 6).

Antioxidant Activity (ANTI-OX). Several samples exhibited high antioxidant activity with IC_{50} values of less than 20 μg/mL out of 80 samples tested. These samples were: leaf + twig of *Spondias radlkoferi,* active at 7.14 g/mL; stembark of *Mouriri myrtilloides,* at 17.7 μg/mL; and stembark of *Bombacopsis (= Pachira) quinata,* at 18.9 μg/mL (Table 6).

Inhibition of TPA-Induced Ornithine Decarboxylase Activity (ODC). In the TPA-induced ornithine decarboxylase activity assay, the criterion for activity is defined as IC_{50} values of < 4 μg/mL. Consequently, none of the 80 extracts exhibited significant activity (Table 6).

Induction of Quinone Reductase with Cultured Hepa 1C1C7 Cells (QR). From 80 extracts tested, the leaf + twig sample of *Maclura tinctoria* exhibited two-fold induction of quinone reductase activity at 3.8 μg/mL and IC_{50} of 19.1 μg/mL, with a corresponding chemopreventive index of 5 (Table 6).

Antimutagenicity (ANTIMUT). Ten extracts showing activity in antioxidant, cyclooxygenase inhibition, cell differentiation, and quinone reductase induction assays were tested in the antimutagenicity assay. The criterion for activity was given by >25% inhibition of mutagenicity. Eight out of 10 extracts did not show activity, whereas leaf + twig extract of *Calophyllum longifolium* (23%) and leaf + twig extract of *Maclura tinctoria* (29%) showed a weak inhibitory activity (Table 6).

Estrogenic and Antiestrogenic Activity with Ishikawa Cells (ISHIK). None of the extracts showed activity in the displacement of estrogen binding to the estrogen receptor in the Ishikawa cell assay.

Induction of HL-60 Cell Differentiation (HL-60). Of 80 extracts tested, initially, two samples showed weak activity in this assay, at a concentration of 20 μg/mL with an incubation period

of four days. The criterion of activity used was a response rate of >40%. When the concentration was doubled (40 μg/mL), leaf + twig of *Calophyllum longifolium* exhibited an ED_{50} value of 17.6 μg/mL and leaf + twig of *Pseudobombax septenatum* exhibited an ED_{50} value of 12.6 μg/mL (Table 6).

Inhibition of DMBA-Induced Lesion Formation in Mammary Organ Culture (MMOC). Nine extracts that were active in at least one of the previous assays were tested in this assay with the objective of finding promising chemopreventive activity. *Maclura tinctoria* (leaf + twig) and *Mouriri myrtilloides* (stembark) exhibited moderate activity, each with inhibition of 42.8% in the DMBA-induced lesions in mammary organ culture, based on a cut-off point for activity of 55% (Table 6).

Based on the results presented above, as summarized in Table 6, and on consideration of literature data, two species, *Maclura tinctoria* (leaf + twig) and *Mouriri myrtilloides* (stembark), may be considered first priority candidates as potential chemopreventive agents, due to the good correlation that exists between the induction of quinone reductase activity, antimutagenic activity, and the inhibition of DMBA-induced lesion formation in mammary organ culture. Furthermore, in the case of *M. myrtilloides,* based on the present study, a relationship has been observed between antioxidant activity and inhibition of DMBA-induced lesion formation in a mammary organ culture.

The second priority comprises two species that have been shown to be active in at least two assays, other than the above. The first species is *Calophyllum longifolium* (leaf + twig), which exhibited significant activity in the HL-60 cell differentiation assay and a weak activity in the antimutagenicity assay, and the second, *Spondias radlkoferi* (leaf + twig), showed antioxidant activity as well as inhibition of cyclooxygenase.

The third priority comprises species that were active in only one of the assays. These include *Bombacopsis (= Pachira) quinata* (stembark) and *Casearia commersoniana* (stembark), each of which was active in the antioxidant activity assay, and *Lozania pittieri* (stembark) and *Vochysia ferruginea* (leaf + twig) were active in the inhibition of cyclooxygenase activity. The last two species, by virtue of their cyclooxygenase

TABLE 7. ANTIPLASMODIAL AND CYTOTOXIC ACTIVITIES OF EXTRACTS OF SELECTED PANAMANIAN PLANTS.

Species (family)	Part tested	KB ED_{50}[b]	Clone D6 IC_{50}[c]		Clone W2 IC_{50}[c]	
Annona spraguei Saff. (Annonaceae)	SB	>20 000	5100		4480	
Calophyllum longifolium Willd. (Guttiferae)	LP	>20 000	2760		2150	
Calophyllum longifolium Willd. (Guttiferae)	SB	>20 000	3840		3600	
Cavanillesia platanifolia HBK. (Bombacaceae)	SB	>20 000	4780		5430	
Lindackeria laurina Presl (Flacourtiaceae)	LP	>20 000	4720		3550	
Lozania pittieri (Blake) L.B. Smith (Flacourtiaceae)	LP	>20 000	4160		5030	
Maclura tinctoria (L.) D. Don ex Steud. (Moraceae)	SB	>20 000	2680		2930	
Olmedia aspera R. et P. (= *Trophis caucana*) (Moraceae)	LP	>20 000	2490		2030	
Turpinia occidentalis (Sw.) G. Don (Staphyleaceae)	SB	>20 000	6350		8600	
Antiplasmodial standard drugs:						
Chloroquine		11 200	3.97	0.01	84.18	0.54
Quinine		7700	10.49	0.97	25.15	3.74
Mefloquine		3000	3.99	0.80	1.2	0.4
Artemisinin		>20 000	4.52	0.56	2.72	0.74

[a] LP = leaf + twig; SB = stembark.
[b] ED_{50} values are expressed in ng/mL.
[c] IC_{50} values are expressed in ng/mL.

inhibition, are possible candidates as a source of anti-inflammatory agents.

EVALUATION OF PANAMANIAN PLANTS IN ANTIPLASMODIAL AND CYTOTOXICITY ASSAYS

Of the 80 plant extracts examined in this study, only three exhibited activity, albeit weak, in the antiplasmodial assay (Table 7), namely, leaf + twig of *Calophyllum longifolium*, with IC_{50} values of 2760 ng/mL for clone D6 and 2150 ng/mL for clone W2; stembark of *Maclura tinctoria*, with IC_{50} values of 2680 ng/mL for clone D6 and 2930 ng/mL for clone W2; and leaf + twig of *Olmedia aspera* (= *Trophis caucana*), with IC_{50} values of 2490 ng/mL for clone D6 and 2030 ng/mL for clone W2. The remaining extracts failed to show in vitro antiplasmodial activity against either of the *Plasmodium falciparum* clones tested. All extracts were evaluated for cytotoxicity with human epidermoid carcinoma (KB) cells, and no cytotoxicity was observed at the test concentration of 20 μg/ml (Table 7), which implies that any plant extracts that exhibited activity in the antiplasmodial bioassay are relatively selective.

EVALUATION OF PANAMANIAN PLANTS FOR INHIBITION OF HIV-1 REVERSE TRANSCRIPTASE ASSAY

All the extracts evaluated in the HIV-1 RT assay initially were tested for enzyme inhibition with the homodimer p66 enzyme. All extracts that exhibited >50% inhibition were then tested for the presence of polyphenolic compounds (tannins), by utilizing $FeCl_3$ reagent. All samples that contained tannins were treated with insoluble polyvinylpyrrolidine (PVP) and tested again. This procedure removed the tannins so the activity of nontannin, inhibitory compounds could be detected. Because tannins and related compounds have been reported as HIV-1 reverse transcriptase inhibitors (Lee et al. 1992), the PVP treatment makes possible the detection of novel anti-HIV compounds, other than the currently known class of compounds (Tan et al. 1991). Samples that showed negative reaction for tannins and those showing positive reaction for tannins, but that retained the HIV-1 RT inhibitory activity after PVP treatment, were subjected to IC_{50} determinations. For nontannin-containing samples, the results obtained showed IC_{50} values of 95 μg/mL for the leaf + twig sample of *Lozania pittieri*, 82 μg/mL for the stembark sample of *Spondias radlkoferi*, and 96 μg/mL for the leaf + twig sample of *Stylogyne standleyi* (Table 8). For the active plant extracts that were positive for the presence of tannins, IC_{50} values were determined with the understanding that the true IC_{50} values would be less than the values observed.

The following tannin-containing species, *Calophyllum longifolium* (leaf + twig), *Lozania pit-*

TABLE 8. HIV-1 REVERSE TRANSCRIPTASE INHIBITORY ACTIVITY OF EXTRACTS OF SELECTED PANAMANIAN PLANTS USING P66-P51 HETERODIMER ENZYME.

Species	Family	Part used[a]	% HIV-1 RT inhibition	IC$_{50}$ (μg/ml)
Calophyllum longifolium Willd.	Guttiferae	LP	61.9	>5.04
Lozania pittieri (Blake) L.B. Sm.	Flacourtiaceae	LP	55.8[b]	95
Lozania pittieri (Blake) L.B. Sm.	Flacourtiaceae	SB	99.8	>76
Pseudobombax septenatum (Jacq.) Dugand	Bombacaceae	LP	83.8	>87
Spondias radlkoferi J.D. Sm.	Anacardiaceae	LP	98.8	>68
Spondias radlkoferi J.D. Sm.	Anacardiaceae	SB	68.1[b]	82
Stylogyne standleyi Lund.	Myrsinaceae	LP	63.2[b]	96
Stylogyne standleyi Lund.	Myrsinaceae	SB	83.9	>92
Turpinia occidentalis (Sw.) G. Don	Staphyleaceae	LP	91.2	>82

[a] LP = leaf + twig; SB = stembark.
[b] Extracts not treated with PVP.
[c] Active extracts have >50% of inhibition.

tieri (stembark), *Pseudobombax septenatum* (leaf + twig), *Spondias radkolferi* (leaf + twig) and *Turpinia occidentalis* (leaf + twig), all exhibited interesting IC$_{50}$ values less than 90 μg/mL (Table 8).

In view of the low IC$_{50}$ value of *Calophyllum longifolium* (Table 8), it may be concluded that this species (leaf + twig) might be more potent than plants that contain no tannins, thus making it a high-priority candidate for bioassay-guided fractionation.

SUMMARY OF BIOLOGICAL ACTIVITIES

The overall occurrence of biological activity in species studied in each of the assays is summarized in Tables 9-A and 9-B. As can be seen from these tables, in eight of the biological systems tested, at least one species showed activity. Absence of activity was observed in the Ishikawa cell assay, TPA-induced ornithine decarboxylase assay, and cytotoxicity assay. Some of the species scored as active were active in more than one test system. When two extracts from one species were active, that species was counted as one. The same criteron was used to score the other three species with lower levels of activity.

The species showing activity in one or more assays were *Calophyllum longifolium, Maclura tinctoria, Vochysia ferruginea, Pseudobombax septenatum, Mouriri myrtilloides, Stylogyne standleyi, Turpinia occidentalis, Bombacopsis* (= *Pachira*) *quinata, Lozania pittieri, Olmedia aspera* (= *Trophis caucana*), *Casearia commer-*

soniana, and *Spondias radlkoferi.* The total number represents 30% of the species studied.

DISCUSSION AND CONCLUSIONS

A biodiversity-based plant selection approach for the biological evaluation of a pool of taxonomically diverse tree species found in a Panamanian forest plot, followed by bioassay of samples of these plants in various bioassay systems, has successfully led to the identification of 12 of 40 species (30%) that showed activity in a panel of 11 biological assays.

Plot-based plant selection has the following advantages. Firstly, a forest plot provides an adequately diverse assortment of species and genera in a well defined area, which, in turn, provides an adequate chemical diversity as basis for biological screening. The area of a forest plot, 0.1–1 hectare, is large enough, yet small enough, to allow the performance of an efficient sampling method on the plant species diversity. A collection of plants from a plot, even if only tree species or woody species are targeted, can provide a wide spectrum of taxonomic diversity that a general collecting survey does not normally provide. Plot sampling can capture efficiently a broader spectrum of chemical diversity represented within a particular tract of forest, as compared to a general collecting survey. Secondly, a plot-based plant selection provides a semi-quantitative result (percent of active species from a well-defined unit of area) on the potential pharmaceutical value of plants found within the plot. An extrapolation of such finding may lead

TABLE 9-A. RESULTS OF BIOLOGICAL EVALUATION OF ANGIOSPERM SPECIES COLLECTED IN PANAMA.

Test results	COX-2[a]	ANT-OX[b]	ODC[c]	QR[d]	ISHIK[e]	ANTMUT[f]
Active	3	3	0	1	0	0
Moderately active	0	19	0	0	0	2
Weakly active	5	2	0	0	0	0
Inactive	32	16	40	39	40	8
Subtotal	40	40	40	40	40	10

[a] Inhibition of cyclooxygenase with polarographic method.
[b] DPPH free radical scavenging activity.
[c] Inhibition of TPA-induced ornithine decarboxylase activity.
[d] Induction quinone reductase.
[e] Estrogenic and antiestrogenic activities with Ishikawa cells.
[f] Antimutagenic activity.

to a sound estimate of the medicinal or pharmaceutical potential of plants from the forest tract.

In a truly random, plot-based approach, using a small plot size, such as 0.1 hectare, where a small number of species is found, one can perform bioassays on plant samples collected from the pool of species found within this plot, even without prior taxonomic determination. When one or more species have been shown to be active in a particular bioassay, marked and numbered trees within the plot would facilitate recollection of the active species.

In a plot of a 1-hectare or larger size, a larger number of species is found (Whitmore 1984:5). To reduce such a large number of species into a more manageable pool for biological evaluation, a preliminary "literature screening" to prioritize species following taxonomic determination of all species found within the plot should be performed using published ethnobotanical and experimental (chemical and biological/pharmacological) data. Such a method was used in our study. We reduced a pool of 308 species found in a 50-hectare plot to a pool of 91 "high priority" species, followed by further selection of species for collection based on (a) ease of field identification, (b) ease of collection, in terms of location, and (c) availability of material, namely, size of plant, to allow the collection of 300–500 grams dry weight. Collection of these 40 species was performed outside the 50-hectare plot because we were not allowed to collect marked and numbered plants/species within the experimental plot. This "terminal" species selection is equivalent to using marked and numbered plants in the plot as a reference for the recollection of active species.

The plant selection approach used in our study could expand the possibility of finding more than 30% of active species in the screening effort, if the number of plant species collected and screened was increased to include all 91 species we considered to be of "high priority."

In our study, the NAPRALERT database indicates that of those species in our assays that showed activity, *Pseudobombax septenatum*, *Mouriri myrtilloides*, *Stylogyne standleyi*, *Bombacopsis* (= *Pachira*) *quinata*, *Lozania pittieri*, and *Olmedia aspera* (= *Trophis caucana*) have

TABLE 9-B. RESULTS OF BIOLOGICAL EVALUATION OF ANGIOSPERM SPECIES COLLECTED IN PANAMA.

Test results	HL-60[a] activity	MMOC[b]	KB cytotoxicity[c]	Antiplasmodial activity	HIV-1 RT inhibition[d]
Active	1	2	0	0	9
Moderately active	1	1	0	3	0
Weakly active	0	0	0	0	0
Inactive	38	6	40	37	31
Subtotal	40	9	40	40	40

[a] Induction of HL-60 cell differentiation.
[b] Inhibition of DMBA-induced lesion formation in mammary organ culture.
[c] Cytotoxicity assay with KB cells.
[d] Inhibition of HIV-1 reverse transcriptase.

no recorded phytochemical literature data. This suggests that these species potentially could provide novel chemical structures if fractionation and isolation work were performed. The discovery of a novel, in vitro, active antimalarial compound, costaricine, a bisbenzylisoquinoline alkaloid, from *Nectandra salicifolia* (Lauraceae) from a Costa Rican forest plot (Böhlke 1997; Böhlke et al. 1996) and of another novel cytotoxic compound, ardisenone, an alkeny phenol, from *Ardisia iwahigensis* (Myrsinaceae) from a forest plot of Palawan (Philippines) (Horgen 1997; Horgen et al. 1997), two species that had not been studied chemically, has demonstrated that plot-based plant selection has improved the chances of finding novel compounds from relatively small, but taxonomically diverse lots of species.

Our research, and others employing plot-based plant selection method provide an effective approach in the selection of plants for biological evaluation. Such an approach may increase the chances of discovering novel, biologically active compounds from plants of the tropical rain forests, of which only a minuscule percentage (estimated at less than 1%; Balick et al. 1996: xi) has been investigated for their medicinal/pharmaceutical potential.

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