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Journal of Ethnopharmacology 66 (1999) 159–165

Journal of ETHNO-PHARMACOLOGY

Ethnobotany in the search for vasoactive herbal medicines

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Received 13 July 1998; received in revised form 12 October 1998; accepted 30 October 1998

Abstract

Plant samples derived from ethno-directed and random collections were screened to determine their effect on pre-contracted rat aortic tissue. Of the 31 ethno-directed species, four were found to be potent relaxants of vascular smooth muscle. The vasoactive species were *Chamguava gentlei*, *Alseis yucatanensis*, *Licaria peckii* and *Nectandra salicifolia*. None of the 32 randomly collected samples produced a relaxation response. These data support the hypothesis that ethno-directed collection is a more efficient means of drug discovery than random plant screens.

Keywords: Ethno-directed; Smooth muscle; Belize; Ethnopharmacology; Rat aorta; Random screen

1. Introduction

In the search for bioactive compounds from plants, one strategy that has been identified as effective is known as 'ethno-directed' screening (Balick, 1994; Adsersen and Adsersen, 1997). This involves working with traditional healers, people who provide health care services to people in their communities. These healers can then identify plants used in folk medicine and the collectors can sample the material as directed by these ethnomedical leads. In some disease systems, the ethno-directed approach has been shown to be a more effective way of identifying leads. For example, working with plants used in folk medicine to treat malaria or fevers in the Amazon region, Carvalho and Kettli (1991) were able to show an activity rate of 18% ($n = 22$) as compared to 0.7% activity derived from plants collected randomly ($n = 273$). Spjut and Perdue (1976) were able to show greater frequencies (19.9%) of primary activity in antineoplastic screens using plants from four families known to treat cancer in traditional medicine (Fabaceae, Liliaceae, Rubiaceae, and Rutaceae)

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0378-8741/99/$ - see front matter
PII: S0378-8741(98)00225-6

versus a background average of 10.4% in a National Cancer Institute survey that analyzed 20 525 species during 1960–1980. Additionally, their analysis of this program revealed that plants used as vermifuges yielded a 29.3% activity rate, fish poisons a 38.6% activity rate and arrow/dart poisons a 52.2% activity rate. In a more recent study, Balick (1990) did not show a significant difference for ethno-directed versus random collections in human cancer cell lines analyzed as part of the subsequent phase of the National Cancer Institute program. Collections of medicinal plants for the most part did not include those used by local people to treat cancer. Lewis and Elvin-Lewis (1995) reported a 71.4% activity rate ($n = 14$) with anti-infective plants used by traditional healers to treat virus 'considered ancestrally related to HIV', versus an overall average of 8.5% activity ($n = 16\,886$) in the NCI primary anti-HIV screens.

The focus of the present study is to identify plants species that are effective in treating cardiovascular disorders such as hypertension, stroke and angina. We compare ethno-directed collection methods to random collections in the search for herbal medicines that affect vascular smooth muscle. The neotropical forests contain much unstudied biodiversity, and show of great potential as a source of bioactive lead compounds (Duke and Vasquez, 1994; Schultes and Raffauf, 1990). It has been estimated that 25% of the medicines in use today in western society have their origins in herbal traditions (Farnsworth 1988).

In this study, 90 samples derived from 63 different plant species were collected by one lab and sent in a blind manner to a second lab for determination of vasoactive response. The samples were divided into two groups; samples from plants that were identified by a traditional healer as having general or specific medicinal use and plants collected by random sampling. In the laboratory, vasoactive samples were defined as those that produced a $> 50\%$ relaxation of rat aortic rings precontracted with norepinephrine (NE). The purpose of this study is to determine whether an ethno-directed approach is a more efficient means of identifying biologically active plants than random plant screens.

2. Materials and methods

2.1. Plant sample collection and extraction

The plant samples were collected and identified by Dr Rosita Arvigo and Dr Michael Balick. Dr Arvigo is a natural medicine specialist cataloging and preserving natural medicines used in the Maya culture at IX Chel farm in San Ignatio, Cayo, Belize; she provided the ethno-directed sampling. Dr Balick and the staff of the New York Botanical Garden provided the taxonomic identification of the plant samples.

Plant samples were prepared for testing by decoction. Twenty milliliters of boiling de-ionized water was poured over 2 g of ground, dried material in a 50 ml conical tube and allowed to boil for 15 min. Solid material was removed by filtration. The dry weight of the extracts were typically between 10 and 15 mg/ml.

2.2. Tissue preparation

Measurements of the contraction of aortic smooth muscle were performed according to accepted protocols (Rapoport 1987). Adult male Sprague–Dawley rats (175–250 g) were euthanized by 100% CO_2 inhalation followed by decapitation and the thoracic aorta isolated. The aorta was cleaned of fatty deposits and connective tissue and cut into four rings, each 5 mm in length. The rings were denuded of endothelium by inserting forceps into the lumen of the vessel and manually rotating it. The aortic rings were attached to an isometric force transducer, placed in a 15 ml temperature controlled tissue bath, and bathed in oxygenated Krebs–Ringer bicarbonate solution (KRB, 118.5 mM NaCl, 4.7 mM KCl, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 2.5 mM $CaCl_2$, 24.9 mM $NaHCO_3$, 10 mM glucose, and 30 μM EDTA) at 37°C. The KRB solution was continually gassed with 95% O_2/5% CO_2.

2.3. Contraction measurements

Tissues were mounted with 3.0 g resting tension and allowed to equilibrate until the tension was maintained. Tissues were then tested by contrac-

Table 1
Ethno-directed samples[a]

Dalbergia glabra (RA924)		*Ocimum campechianum* (RA941)	
T	−1.6 ± 0.7%	L	1.6 ± 1.6%
Trichilia havanensis (RA925)		*Koanophyllon* alb. (RA942)	
L	−2.2 ± 0.6%	L	3.2 ± 2.6%
B	0.0 ± 2.0%	*Simarouba glauca* (RA943)	
Phytolacca rivinoides (RA926)		B	−0.3 ± /0.3%
L&T	−19.2 ± 6.1%	F	−3.9 ± 4.6%
R	−2.8 ± 1.5%	***A. yucatanensis**** (RA944)	
Bursera simaruba (RA927)		**L**	**103.6 ± 12.0%**
B	−2.6 ± 1.3%	**B**	**105.3 ± 3.2%**
L	−0.9 ± 0.5%	*Lantana camara* (RA945)	
Vitex gaumeri (RA928 & RA936)		L	1.2 ± 0.8%
B	0.2 ± 2.0%	*Annona* cf. *Reticulata* (RA947)	
L	−1.7 ± 1.9%	L	−9.3 ± 7.2%
Cupania belizensis (RA930)		*Phyllanthus liebmannianus* (RA948)	
L	1.0 ± 0.7%	L	−10.4 ± 3.4%
Hamelia patens (RA931)		*Lonchocarpus hondondurensis* (RA949)	
L	0.1 ± 4.2%	L	−0.7 ± 1.3%
C. gentlei* **(RA932)**		R	0.9 ± 1.2%
L	**81.7 ± 15.6%**	B	−0.4 ± 0.4%
B	**70.5 ± 5.2%**	*Acacia cornigera* (RA951)	
Guazuma ulmifolia (RA933)		L&T	−2.7 ± 0.4%
L	−1.5 ± 0.5%	R	−0.9 ± 0.6%
B	−1.2 ± 0.5%	B	−1.9 ± 0.7%
Stachytarpheta cayen. (RA934)		*Casearia sylvastris* (RA952a)	
L&S	5.7 ± 3.6%	L	−4.2 ± 1.6%
Lygodium venustrum (RA935)		*Prophyllum punctatum* (RA9953)	
L&T	−0.4 ± 0.4%	L	−1.4 ± 0.5%
Lacistem aggregatum (RA937)		*Trichilia pallida* (RA954a)	
L	−0.8 ± 0.8%	L	−0.5 ± 0.7%
B	−0.8 ± 0.2%	***L. peckii**** (RA955a)	
Cecropia sp. (RA938)		**B**	**114.1 ± 11.1%**
L	−3.1 ± 1.3%	*Bursera simaruba* (RA956a)	
Byrsonima crassifolia (RA939)		L	−2.5 ± 0.7%
B	−1.4 ± 1.3%	***N. salicifolia**** (RA957)	
Acacia dolichostachys (RA940)		**L**	**68.3 ± 5.0%**
L	−0.2 ± 3.3%	*Eugenia* sp. (RA958)	
R	−2.2 ± 1.4%	L	13.2 ± 17.6%
B	0.3 ± 0.7%		

[a] The effect of extracts from ethno-directed samples on NE induced contractions. Results are presented as percent relaxation/SEM (negative numbers represent contraction). Relaxations greater than 100% represent loss of tone below baseline. Vasorelaxant species are in bold and asterisked. Voucher specimen numbers are provided in parentheses. (T, twig; L, leaf; B, bark; S, stem; R, root).

tion with 0.3 μM norepinephrine (NE) followed by 10 μM carbachol at the peak of contraction (rings properly denuded of endothelium should give no response to carbachol, Furchgott and Zawadzki, 1980). This procedure was repeated to insure the stability of the smooth muscle and the removal of the endothelium. Rings that perform poorly in test contractions (<1.0 g of active tension) or showed a relaxation to carbachol were discarded.

2.4. Plant extract testing

Extracts from the plant samples were tested for their effect on NE-induced contraction. The aortic rings were contracted with 0.3 μM NE; sufficient

Table 2
Random samples[a]

Melampodium costaricense (JW1553)		*Senna reticulata* (RA720)	
P	$-2.9 \pm 1.4\%$	L, IF, FR	$2.7 \pm 1.2\%$
Melanthera nivea (JW1554)		*Sapindus saponaria* (RA960)	
P	$-1.9 \pm 0.9\%$	L & FR	$-1.8 \pm 1.0\%$
Chamaesyce lasiocarpa (JW1555)		*Myroxylon balsamum* (AR988)	
P	$-1.0 \pm 2.2\%$	L & FR	$-0.1 \pm 1.7\%$
Croton xalapensis (JW1556)		*Tabernaemontana alba* (AT1081)	
T, L, IF	$-1.5 \pm 1.6\%$	L & IF	$4.4 \pm 2.5\%$
Solanum erianthum (JW1557)		*Laetia thamnia* (AT1144)	
T, L, IF	$2.1 \pm 4.8\%$	L & FR	$4.4 \pm 2.3\%$
Cleome viscosa (JW1558)		*Erythrina folkersii* (AT1146)	
T, L, FR	$5.7 \pm 2.7\%$	T, L, FR	$0.0 \pm 1.8\%$
R	$-0.1 \pm 0.1\%$	*Aspidosperma cruetum* (AR1156)	
Ficus insipida (JW1559)		L & T	$6.6 \pm 4.1\%$
R	$0.8 \pm 0.8\%$	*Zygia peckii* (AT1326)	
B	$-0.1 \pm 0.7\%$	L & FR	$-12.1 \pm 5.2\%$
T&L	$-5.2 \pm 2.7\%$	*Montrichardia aborescens* (AT1364)	
Citharexlum hexangulare (JW1560)		L & T	$-2.6 \pm 0.9\%$
R	$-0.4 \pm 1.3\%$	*Amphitecna latifolia* (AT1378)	
B	$-1.0 \pm 1.0\%$	L	$-0.5 \pm 0.7\%$
T, L, FR	$-2.2 \pm 1.0\%$	*Attaleya cohune* (MB2064)	
Ficus obtusifolia (JW1561)		L	$0.4 \pm 1.7\%$
R	$0.8 \pm 1.4\%$	*Gaussia maya* (MB3249)	
B	$-0.6 \pm 0.7\%$	L	$-4.7 \pm 1.2\%$
T, L, FR	$-3.7 \pm 1.4\%$	*Coutoubea spicata* (MB3483)	
Blomia prisca (JW1562)		L & T	$0.9 \pm 1.6\%$
L	$-1.3 \pm 2.0\%$	*Pouteria sapota* (MB3753)	
B	$2.2 \pm 1.6\%$	L & FR	$-7.2 \pm 5.2\%$
Cieba pentandra (JW1563)		*Hampea stipitata* (GO53)	
R	$4.6 \pm 3.2\%$	L & FR	$-6.4 \pm 3.4\%$
B	$-0.7 \pm 0.5\%$	*Sapranthus campechianus* (WA103)	
T & L	$-2.6 \pm 1.3\%$	T, L, FR	$-0.6 \pm 0.9\%$
Guarea glabra (JW1564)	1	*Matopium brownei* (WA110)	
R	$1.2 \pm 1.7\%$	L & FR	$-7.8 \pm 5.1\%$
B	$0.9 \pm 2.0\%$	*Manihot aesculifolia* (WA134)	
T, L, FR	$0.9 \pm 0.7\%$	L & FR	$-3.4 \pm 1.8\%$
Cysdista potosina (WA2327)		*Zanthoxylum kellermanii* (WA2329)	
L & FR	$0.1 \pm 1.1\%$	L & FR	$-9.1 \pm 3.4\%$

[a] The effect of extracts from randomly collected samples on NE induced contractions. Results are presented as percent relaxation/SEM (negative numbers represent contraction). Voucher specimen numbers are provided in parentheses (T, twig; L, leaf; B, bark; S, stem; R, root; P, plant; IF, inflorescence; FR, fruit).

time was allowed for the tonic phase of the contraction to achieve equilibrium. A 300 μl aliquot of the extract was added to each of the baths and sufficient time was allowed for equilibration of the tissue. The bath was then washed out and the tissues were allowed to recover. When the tissues returned to the original resting tension, they were contracted with 0.3 μM NE, to determine if they reach a level of tension comparable to before exposure to the extract. If the tissues had recovered properly, the baths are washed out, the tissues contracted with 0.3 μM NE, and then exposed to the another extract. Usually, three to four extracts were tested per aorta. Each plant extract was tested in at least six tissues from at least three different aortas.

3. Results

The 90 plant samples tested in this study were collected from 63 different species. Some samples represent different parts of the same species (e.g. bark and leaf of the same plant). Of the 63 species tested, 31 species (46 samples) were chosen by ethno-directed methods and 32 species (44 samples) were random plant samplings. The ability each of the 90 samples to relax NE contracted aortic rings was determined and can be seen in Tables 1 and 2.

These results are summarized in Table 3. None of the randomly selected species showed significant vasorelaxing ability. Of the ethnopharmacological collections, six samples from four different species were found to be potent relaxants of vascular smooth muscle. The vasoactive species were *Chamguava gentlei (*leaf and bark), *Alseis yucatanensis* (leaf and bark), *Licaria peckii* (bark), and *Nectandra salicifolia* (leaf). Physiograph traces showing representative relaxations produced by extracts from these species can be seen in Fig. 1.

4. Discussion and conclusions

The data in Table 2 show that ethno-directed methods of drug discovery were more efficient than random sampling in this study. Of the 31 ethno-directed plants, four species (12.9% of those tested) were effective in relaxing the tissues. None of the samples in a similar-sized group of randomly collected plants produced relaxation. This shows that ethno-directed methods were more efficient in identifying vasoactive plants in this study.

Table 3
Summary of the results in Tables 1 and 2[a]

	Number	Relaxations	% of total
Ethnobotanical samples			
Species	31	4	12.9
Samples	46	6	13.0
Random samples			
Species	32	0	0.0
Samples	44	0	0.0

[a] The number of vasoactive species identified by ethno-directed means was significantly greater than by random sampling (P for χ^2 distribution <0.05).

Although the ethno-directed pharmacy was more effective than random sampling, one might expect a higher percentage of the ethno-directed samples to be vasoactive. There are a number of possible explanations for this. First, the ethno-directed pharmacy includes all plants employed by the practitioner, irrespective of the intended use of the plant. Many plants were screened that are not used for conditions that would suggest a vascular component. For example, plants used for rashes, external parasites, etc. may not be expected (*a priori*) to produce vascular relaxation. These plants were included in the study to cast the widest possible net to find bioactive molecules. In fact, one species that is used as external antifungal agent, *N. salicifolia*, was identified as vasoactive and may have been excluded if stricter criteria for acceptance into the ethno-directed group were used.

Secondly, substances that produce a decrease in blood pressure in vivo may do so without directly affecting the vascular smooth muscle. Drugs may produce their effects through altering levels of a circulating metabolite, altering renal physiology, or by a central mechanism (Gilman et al. 1990). Plant extracts that produce their effects through these types of mechanisms would not be selected in the screening process.

Another possibility is that some plants may produce vasorelaxation by an endothelium dependent mechanism. However, endothelium dependent mechanisms may be less specific than mechanisms directly involving the smooth muscle cells. A recent study showed that many commonly consumed plant foods produced endothelium dependent vasorelaxation in rat aorta (Fitzpatrick et al., 1995). Thirty-four of the 53 extracts tested in this study produced endothelium dependent relaxation in the tissues. This suggests that endothelium dependent relaxations may be common to many plant products. In the present study, the endothelium was removed to focus on plants that directly relaxed smooth muscle and eliminate less specific effects.

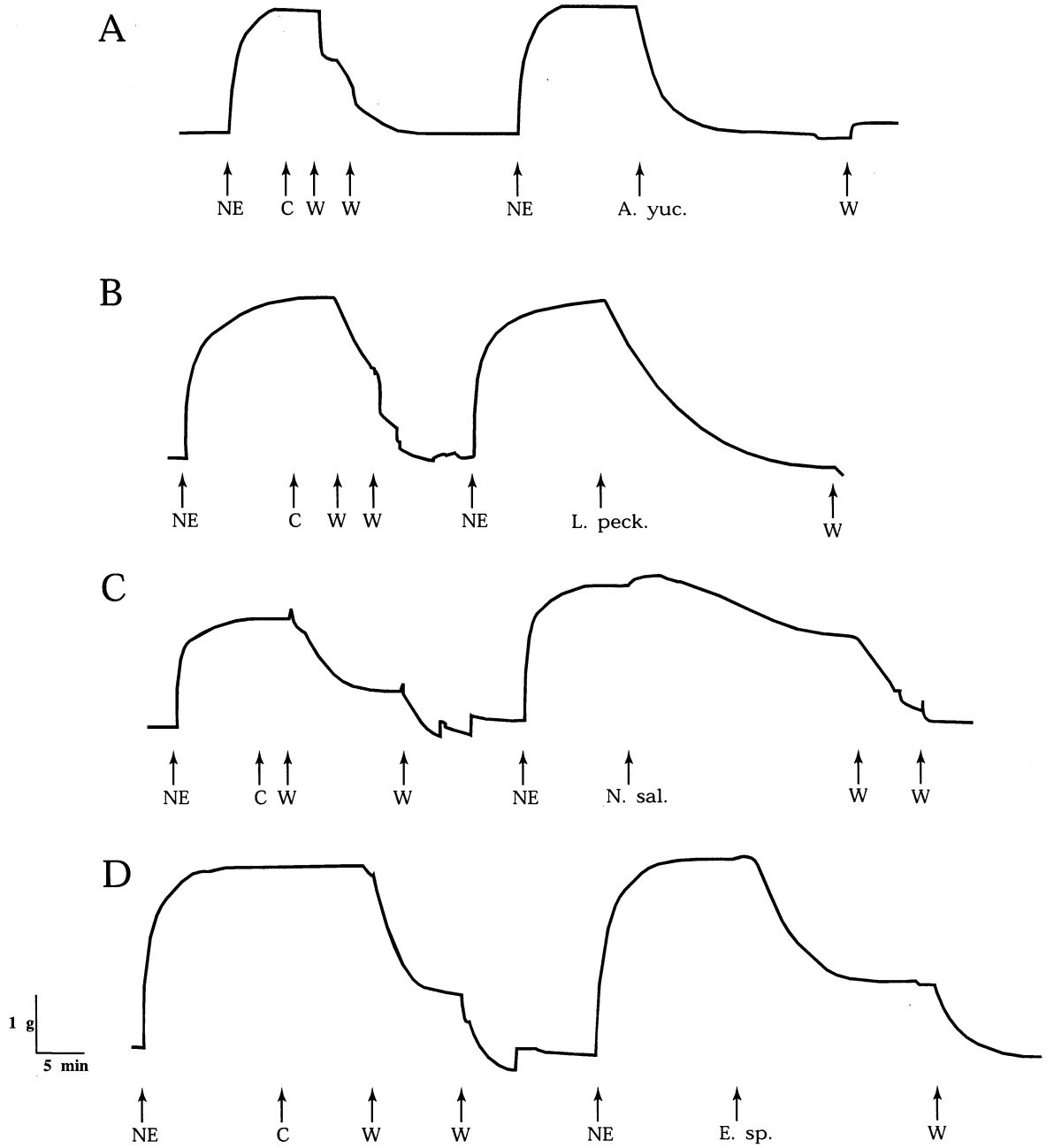

Fig. 1. Representative relaxations produced by each of the vasoactive species. (A) *A. yucatanensis* (bark). (B) *L. peckii* (bark) (C) *N. salicifolia* (leaf). (D) *C. gentlei* (bark). (NE-0.3 μM norepinephrine, C-10 μM carbachol, W-washout).

In conclusion, this work has shown that the use of ethnobotanical information is a more effective means of drug discovery than random plant screening. In this study four species were identified (*C. gentlei*, *A. yucatanensis*, *L. peckii*, and *N. salicifolia*) that directly relaxed rat aortic smooth muscle. This represent 12.9% of the species screened. None of the randomly selected species produced an effect.

Acknowledgements

The authors are grateful to Leopoldo Romero and James Mesh for assistance in the plant collecting and preparation, and to Jeffrey Augello for his help in preparing the samples for analysis. We would also like to acknowledge Daryl Lamson, Z. Ken White, Todd Terpening, Carmen Costable, Dan Infantino, Joshua Llinas, Marianne Roscoe, and Michele Plumadore for their help in screening the samples. We acknowledge the support of the Forestry Department of Belize and the Conservation Division who facilitated the collection activities in Belize. This work was partially supported by the US Agency for International Development through a grant to The New York Botanical Garden and Ix Chel Tropical Research Foundation. We are grateful to The U.S. National Cancer Institute, The Edward John Noble Foundation, The Rockefeller Foundation, The Metropolitan Life Insurance Foundation, The Overbrook Foundation, The Philecology Trust, The Rex Foundation, The John and Catherine T. MacArthur Foundation, The Nathan Cummings Foundation and the Gildea Foundation for their support of the Belize Ethnobotany Project since 1987, and the State University of New York for support of the pharmacological experiments.

References

Adsersen, A., Adsersen, H., 1997. Plants from Reunion Island with alleged antihypertensive and diuretic effects—an experimental and ethnobotanical evaluation. Journal of Ethnopharmacology 58, 189–206.

Balick, M.J., 1990. Ethnobotany and the identification of therapeutic agents from the rainforest, in bioactive compounds from plants. Ciba Foundation Symposium 154, 22–39.

Balick, M.J., 1994. Ethnobotany, drug development and biodiversity conservation—exploring the linkages. In: Chadwick, D.J., Marsh, J. (Eds.), Ethnobotany and the Search for New Drugs. Wiley, Chichester, NY, pp. 4–18.

Carvalho, L.H., Kettli, A.U., 1991. Antimalarial chemotherapy with natural products and chemically defined molecules, Memorias do Instituto Oswaldo Cruz, 86(II)181–184.

Duke, J.A., Vasquez, R., 1994. Amazonian Ethnobotanical Dictionary. CRC Press, Boca Raton, FL.

Farnsworth, N.R., 1988. Screening plants for new medicines. In: Wilson, E.O. (Ed.), Biodiversity. National Academy, Washington, DC, pp. 83–97.

Fitzpatrick, D.F., Hirschfield, S.L., Ricci, T., Jantzen, P., Coffey, R.G., 1995. Endothelium-dependent vasorlelaxation caused by various plant extracts. Journal of Cardiovascular Pharmacology 26, 90–95.

Furchgott, R.F., Zawadzki, J.V., 1980. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature 288, 373–376.

Gilman, A.G., Rall, T.W., Nies, A.S., Tayler, P., 1990. The Pharmacological Basis of Therapeutics, 8th ed., McGraw-Hill, NY, USA.

Lewis, W.H., Elvin-Lewis, M.P., 1995. Medicinal plants as sources of new therapeutics. Annals of the Missouri Botanical Garden 82, 16–24.

Rapoport, R.M., 1987. Effects of norepinephrine on contraction and hydrolysis of phosphatidylinositides in rat aorta. Journal of Pharmacology and Experimental Therapeutics 242, 188–194.

Schultes, R.E., Raffauf, R.F., 1990. The Healing Forest. Dioscorides Press, Portland, OR.

Spjut, R.W., Perdue, R.E., 1976. Plant folklore: a tool of predicting sources of antitumor activity? Cancer Treatment Report 60, 979–985.