PHARMACEUTICAL SCIENCES

Bioassay Screening of Amazonian Plants

RICARDO O. GUERRERO, Ph D*; SHARON M. RIVERA, B Sc†; SULAY RIVERA B Sc‡; LILLY ANN SUEIRO, B Sc**

Objective. **The purpose of this study was to evaluate several biological activities of thirty plant extracts collected in the North West Amazon (Ecuador). Some of these plants are being used for their reputed medicinal properties by the natives of this region.**

Methods. **Five in vitro bioassays were used to screen the plant material. 1. The brine shrimp lethality examination (BSLT) in microplate is a general test that seems capable of detecting a broad spectrum of bioactivity present in crude plant extracts. 2. Free radical scavenging properties were studied in a colorimetric assay using 2,2-diphenyl-1-picrylhydrazyl (DPPH). 3. The β-glucosidase inhibition test is thought to be a method for the evaluation of anti-AIDS, anti-diabetic or anti-obesity compounds. 4. The xanthine oxidase inhibition assay is used to identify potential anti-gout agents. 5. The antibacterial activity that is being used to isolate and identify antibiotic drugs.**

Results. **In the BSLT, we found that *Piscidia carthagenensis* demonstrated very good activity with a LC_{50}: 21.81 µg/mL. It is considered that plant extracts with low LC_{50} values may contain metabolites with cytotoxic, antifungal, insecticidal or pesticide activities. In the antioxidant activity bioassay, several plant extracts were confirmed to have excellent free radical scavenging properties. *Rhus juglandifolia* and *Clusia venusta* leaves exhibited an ED_{50}: 3.12 µg/mL and 3.61 µg/mL, respectively. *Piper reticulatum* (84%), *Inga heteroptera* (77%), *Clusia venusta* (70.9%), and *Rhus juglandifolia* (70.5%) showed fairly good inhibition activity for β-glucosidase. On the other hand, none of the plant extracts was capable of inhibiting xanthine oxidase. Finally, the Gram-positive microorganisms *Staphylococcus aureus* and *Corynebacterium diphteriae* were found to be sensitive to the majority of the plant extracts, whereas the Gram-negative bacteria *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, and *Salmonella typhi* were proved to be resistant toward the plant extracts.**

Conclusions. **It is important to continue investigating our plant kingdom, especially the world tropical reserves as an alternative for finding new or better drugs. It should be essential to follow-up this type of investigation to isolate and elucidate the active principles of the bio-positive plants.**

Key words: Amazonian plants, Ecuador, Brine shrimp, Anti-oxidant activity, β-glucosidase inhibition, Xanthine oxidase inhibition, Anti-bacterial activity.

Ecuador is a small South American country that occupies 0.2% of the planet's area and yet it is blessed with a large biodiversity. It has been estimated that around 25.000 species of plants exist in this country. This number represents approximately 10% of all the plants that occur in the world (1). The eastern portion of Ecuador is called the Amazon Region and it is usually referred to as the "Oriente". It comprises everything east of the Ecuadorian Andes, representing almost half of the territory. This region contains thousands of plant species, many of which might be able to supply bioactive compounds to mankind. Plants already provide us with some 25% of our medicines and they could produce more. In all probability, 95% of all plants remain to be evaluated for their biological utility, two thirds of which are tropical (2). Several ethnic groups inhabit the "Oriente". Among them, the Jíbaros constitute the main Indian group and they number around 27,000 people. Other groups scattered throughout this region include the Kofán, Quichua, Siona-Secoya, Shuar, and Waorani (3).

There are few accounts concerning the medical use of the plants by the Indian groups of the "Oriente". An early description was made by Jacinto Martín, a priest who lived with the Indians for several years. He described 61 plant

From the *School of Pharmacy, Medical Sciences Campus, University of Puerto Rico, San Juan, Puerto Rico, the †Program of Allied Health Studies, College of Natural Sciences, University of Puerto Rico, Rio Piedras Campus, the ‡Department of Chemistry, College of Natural Sciences, University of Puerto Rico, Rio Piedras Campus, and the **School of Family Ecology and Nutrition, College of Education, University of Puerto Rico, Rio Piedras, Puerto Rico.

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Address correspondence to: Dr. Ricardo O. Guerrero, Department of Pharmaceutical Sciences, School of Pharmacy, University of Puerto Rico, P.O. Box 365067, San Juan, PR 00936-5067, (787) 758 2525 x 5418; fax (787) 767 2796, Email: rguerrero@rcm.upr.edu

species and their medical utility. Some of these plants have applicability as remedies for ailments such as alopecia and dental caries. More important are those plants which stimulate fertility or suppress the menstruation temporarily (4). Iglesias (1985) reported that 178 plants having medicinal properties are being used by the Quichuas in the area of the upper Napo River (5). Another study (6) in 1988 describes 600 plant species used by five ethnic groups (Kofanes, Quichuas, Sionas-Secoyas, Shuar and Huaorani). The scientific information on the biological activity and bioactive constituents of these plants is insufficient or non-existent.

The most comprehensive report of the plants used by the natives of the North West Amazon comes from the book of Schultes and Raffauf (3). This extensive work describes ethnobotanically 1516 species in 145 families and 594 genera. As the authors indicate, at least 50% of the plants have had little or no investigation of their chemical and pharmacological properties.

In our study, thirty medicinal plant extracts collected in the upper Napo River area were investigated with five *in vitro* bioassays evaluating general and specific biological actions as described below. It was hypothesized that some of the plants would provide positive results, which would help to explain some of their folkloric uses.

Thirty plant species (23 families) were identified and collected in the eastern region of Ecuador (Napo province), by Dr. Victor Hugo Villacrés and Mr. Valdano Tafur in 1995 (Table I). The voucher specimens have been deposited at the "Instituto de Ciencias Naturales, Facultad de Ciencias Químicas, Universidad Central del Ecuador" herbarium. Approximately, 100-200 Gm. dry weight of every specimen collected was submitted to the Laboratory of Natural Products, School of Pharmacy, University of Puerto Rico. Extracts (ethanol, 95%) were prepared and subjected to five *in vitro* bioassays.

Table 1. Amazonian Plants

Scientific Name	Common Name	Family	Part of Plant	Reported Medicinal Properties
Anethum graveolens	Eneldo	*Apiaceae*	Leaves	Carminative,diuretic, soporific, galactogogue (21)
Artocarpus altilis	Fruta de pan	*Moraceae*	Leaves, Stems	Treatment of high blood pressure (22)
Bauhinia picta	--	*Leguminosae*	Fruits	--
Bauhinia picta	--	*Leguminosae*	Leaves, Stems	--
Blechum brownei	Yerba de papagayo	*Acantaceae*	Leaves, Stems	Taken to relieve colds (21)
Brunfelsia grandifolia	Chiric sanango	*Solanaceae*	Leaves	Treatment of arthritis and rheumatism (22)
Campelia zanonia	Quilon quilon	*Commelinaceae*	Leaves	--
Cedrela odorata	Cedro	*Meliaceae*	Leaves, Stems	--
Clusia venusta	--	*Clusiaceae*	Stems	--
Cola acuminata	Kola	*Sterculiaceae*	Leaves, Stems	Used as stimulant (16)
Cordyline terminalis	Marpindo	*Agavaceae*	Leaves, Stems	Cultivated ornamental (22)
Costus scaber	Caña agria	*Costaceae*	Leaves, Stems Fruits	Used for liver disease (22)
Croton lechleri	Sangre de drago	*Euphorbiaceae*	Bark	Latex used to heal wounds (22)
Dacryodes peruviana	Copal	*Burseraceae*	Resin	Latex is mixed with other "copals" to caulk boats (22)
Gleichenia pubescens	--	*Gleicheniaceae*	Leaves, Stems	--
Grias tessmannii	Sachamango	*Lecythidaceae*	Leaves	--
Gustavia longifolia	Sacha chopé	*Lecythidaceae*	Leaves, Stems	The seed is purgative (22)
Inga heteroptera	Guabo de mono	*Leguminosae*	Leaves, Stems	--
Justicia connata	Lumbrisina	*Acantaceae*	Leaves, Stems	--
Piper reticulatum	Cordoncillo verde	*Piperaceae*	Leaves, Stems	--
Piscidia carthagenensis	Barbasco	*Leguminosae*	Leaves, Stems	--
Pollatesta discolor	Yanavara	*Asteraceae*	Leaves, Stems	Wood is used for columns, constructions, etc. (22)
Pothomorphe umbellata	Matico	*Piperaceae*	Leaves, Stems	--
Rhus juglandifolia	Alubillo	*Anacardiaceae*	Leaves	--
Scoparia dulcis	Teatina	*Scrophulariaceae*	Leaves, Stems	Infusion used for bronchitis, cough, diarrhea, etc. (22)
Sida acuta	Pichana	*Malvaceae*	Leaves, Stems	Leaf infusion used as diuretic (22)
Sparanttanthelium glabrum	Chundu huasca	*Hernandiaceae*	Leaves, Stems	Stem decoction for cough, diarrhea, headache, etc. (22)
Trichilia viridis	--	*Meliaceae*	Leaves, Stems	--
Verbena litoralis	Verbena	*Verbenaceae*	Leaves, Stems	Decrease menstrual flow (5)
Zingiber officinale	Jengibre	*Zingiberaceae*	Stems	Macerated rhizomes for arthritis and rheumatism (22)

Micro-well brine shrimp lethal toxicity (BSLT). BSLT is a general toxicity procedure used as a preliminary stage in the examination of bioactive compounds. Since 1981 this bioassay has been utilized for studies on bioactive substances in plant and marine extracts. The protocol by Solis et al, using serial dilutions in 96-well micro-plates was followed throughout our experiments (7). Samples of the extracts (1 mg/mL) were made up in artificial seawater, adding 50 µL of DMSO before adding the seawater. Aliquots of 100 µl were run in 3 replicates in a 96-microplate. Brine shrimp eggs (*Artemia salina* Leach) were purchased in a pet shop in San Juan, Puerto Rico, and hatched in artificial seawater prepared from salts (Instant Ocean®, Mentor, Ohio 44060, USA) at room temperature. The nauplii were collected after 48-72 hours. A suspension of 10 to 15 nauplii in 100 µL was added to each well of the micro-plate and incubated for 24 hours at room temperature. After this time, the number of dead nauplii was counted using a Bausch & Lomb binocular microscope, (20 X). The total number of brine shrimp was counted after adding 50 µL of methanol to each well and waiting for 30 minutes. Artificial seawater was used as negative control and berberine chloride (Sigma Chemical Co., St Louis, MO, USA) was used as positive control. The probit analysis method described by Finney was used to calculate LC_{50} values and 95% confidence intervals (8). Extracts with $LC_{50} \leq 100$ µg/mL were considered active.

Antioxidant activity. Free radicals are unstable molecules because they contain an unshared electron. To become stable molecules, free radicals seek electrons from other molecules such as DNA, lipids from the cellular membrane, or proteins from body tissues. When these molecules are attacked by free radicals, their molecular structure is altered. These distorted molecules are converted themselves into free radicals that search for, attack and damage neighboring molecules maintaining a chain reaction that creates free radicals and causes cellular damage. It is believed that free radicals contribute to the development of many diseases and to accelerate signs of old age. Antioxidants have the ability to stop this chain reaction by donating electrons to the free radicals.

The method of Joyeux et al, was used in our study (9). A fresh methanolic solution (20 mg/mL) of DPPH (2,2-diphenyl-1-picrylhydrazyl) was prepared. Methanolic solutions of the extracts to be analyzed were also made up at concentrations of 100, 10, and 1 µg/mL.. In the procedure, 1.5 mL of the DPPH solution was mixed with 750 µL of the extract dilutions in triplicate. A blank was prepared, consisting of 1.5 mL of DPPH solution and 750 µL of deionized water. Quercetin (Sigma Chemical Co., Saint Louis, MO, USA) was used as positive control. The mixtures were incubated at 25° C for 5 minutes, and absorbance at 517 nm was measured. The spectrometer was adjusted to 0 with a mixture of methanol-water (2:1). Percentage for the decoloration capacity (free radical scavenging activity) was obtained employing the formula:

% of decoloration potential = 1 – [Absorbance of the extract/Absorbance of blank] x 100

Due to the coloration of the extracts it was necessary to include a negative control that contained methanol instead of the DPPH solution. In these cases, the absorbance value of the negative control was subtracted from the absorbance values of the DPPH solution containing the extract.

***ß*-Glucosidase inhibition.** It is known that castanospermine and 1-deoxynojirimycin hydrochloride block HIV-induced syncytium formation and also interfere with HIV infectivity *in vitro* (10). Castanospermine (1,6,7,8 tetrahydroxyoctahydroindolizine) is an alkaloid isolated from the seeds of the tree *Castanospermun australe* and is known to be a potent *ß*-glucosidase inhibitor (11). It seemed reasonable therefore to use the *ß*-glucosidase inhibition procedure to recognize extracts that have castanospermine or 1-deoxynojirimycin-like effects.

The enzyme *ß*-glucosidase (almond emulsin), the substrate p-nitrophenyl-*ß*-D-glucopyranoside and the inhibitor 1-deoxynojirimycin HCl, were purchased from Sigma Chemical Co., Saint Louis, MO, USA. The assay was followed from the literature[12] with the modification that a positive control (1-deoxynojirimycin HCl) was used in our experiments. The procedure involves the hydrolysis of p-nitrophenyl-*ß*-D-glucopyranoside by *ß*-glucosidase (almond emulsin). The reaction mixture contained in a final volume of 1.9 ml the following components: 25 mM sodium acetate buffer, pH 5.0 (0.2 ml); 400 µg p-nitrophenyl-*ß*-D-glucopyranoside (0.2 ml); plant extract 200 µg; and enough enzyme to give an optical density change from 1.0 to 2.0 at 410 nm during an incubation period of 30 min. at 37°C. The plant extract (2 mg) was dissolved in 10 µl of DMSO and 990 µl of water and was filtered through 0.8 µm Millex®-PF filter units. After an incubation period of 10 min., the enzyme was added to initiate the reaction. This reaction was allowed to proceed for another 30 min. and was stopped by adding 1.3 ml of 0.4 M glycine buffer, pH 10.3. The liberated p-nitrophenol was measured at 410 nm in a Beckman DU-6 spectrometer. The assay was performed in triplicate. Controls in which the enzyme was added following the addition of the glycine buffer were utilized to eliminate the interference of the color of the extract. The activity of the enzyme *ß*-glucosidase was expressed as the percentage inhibition and calculated by:

1 - (absorbance of the enzyme with the plant extract/ absorbance of the enzyme without the extract) x 100.

Xanthine-oxidase (XO) inhibition. Allopurinol, a xanthine oxidase inhibitor is used in the treatment of gout. The search for new and useful drugs in treating human gout should include the investigation of plant resources.

The enzyme, xanthine, and allopurinol were purchased from Sigma Chemical Co., Saint Louis, MO, USA. Allopurinol was used as a positive control. The inhibition of XO was measured according to the procedure of Noro et al (13). Solutions of plant extracts were prepared by dissolving 2 mg of the extract in 1 ml of a 5% DMSO in water solution. This was added to 2.9 ml of 1.15 M phosphate buffer (pH = 7.5) and 0.1 ml of enzyme solution. After pre-incubation of the mixture at 25° for 15 min. the reaction was started by adding 2 ml of substrate solution and was stopped after 30 min. by adding 1 ml of 1N HCl. The absorbance was measured at 290 nm. A blank was prepared in the same way, with the enzyme being added to the assay mixture after the HCl. XO inhibitory activity was expressed as the percentage inhibition of XO and calculated by $1 - [A_{extract}/A_{no\ extract}] \times 100$, where A is the absorbance of the samples.

Antibacterial assay. The agar diffusion method (14) was used to investigate the susceptibility of bacteria toward the plant extracts. The Gram-positive bacteria used as test microorganisms were *Staphylococcus aureus* (ATCC No. 25923) and *Corynebacterium diphteriae* (ATCC No. 13812). The Gram-negative bacteria used were *Escherichia coli* (ATCC No. 25922), *Pseudomonas aeruginosa* (ATCC No. 27853), *Proteus vulgaris* (Quality Control Sample), and *Salmonella typhi* (Medical Center Sample) were the testing microorganisms. The plant extracts in known concentrations (100 µg and 500 µg) in appropriate solvent were added to sterile small filter papers (6 mm diameter), and placed over the agar plates inoculated with the microorganism. The plates were incubated for a period of 24 hours at 37°C and analyzed for growth inhibition. The diameters of the zone of inhibition were measured to the nearest mm. Inhibition ≥ 7 mm indicated antibacterial activity; 6 mm or less confirmed resistance of the microorganism toward the plant extracts. Ampicillin (Sigma Chemical Co., Saint Louis, MO, USA) at 10 mg/mL served as a standard antimicrobial agent.

Results

The results are presented in Tables 2 and 3.

Table 2. Biological activities of amazonian plants

Species	BSLT CL_{50} (µg/mL)	Antioxidant activity ED_{50} (µg/mL)	ß-Glucosidase Inhibition percentage	Xanthine Oxidase Inhibition percentage
A. graveolens	*	$34.85_{(48.8\text{-}25.7)**}$	12.37	‡
A. altilis	*	$11.01_{(14.8\text{-}9.2)}$	16.81	‡
B. picta (Fruits)	*	--	48.20	‡
B. picta (Leaves-stems)	*	$9.14_{(25.1\text{-}14.6)}$	59.83	‡
B. brownei	*	$70.84_{(126.5\text{-}45.9)}$	46.03	‡
B. grandifolia	*	$150.39_{(301.7\text{-}96.5)}$	--	‡
C. zanonia	*	--	17.86	‡
C. odorata	*	$5.10_{(6.8\text{-}3.8)}$	34.14	‡
C. venusta	*	$3.61_{(5.9\text{-}2.5)}$	70.93	‡
C. acuminata	*	$4.27_{(5.7\text{-}3.1)}$	65.24	‡
C. terminalis	*	$17.94_{(23.8\text{-}13.7)}$	31.73	‡
C. scaber	*	$27.10_{(34.9\text{-}21.1)}$	24.67	‡
C. lechleri	*	$120.56_{(351.4\text{-}62.2)}$	45.95	‡
D. peruviana	*	--	20.20	‡
G. pubescens	643.5	$16.73_{(22.4\text{-}12.6)}$	22.28	‡
G. tessmannii	*	$50.77_{(85.9\text{-}33.6)}$	2.36	‡
G. longifolia	*	$17.32_{(22.8\text{-}13.2)}$	48.29	‡
I. heteroptera	*	$4.38_{(6.01\text{-}3.10)}$	--	‡
J. connata	*	$18.15_{(27.6\text{-}12.4)}$	9.08	‡
P. reticulatum	*	--	84.34	‡
P. carthagenensis	$21.8_{(30.8\text{-}13.7)**}$	--	12.82	‡
P. discolor	*	$14.45_{(19.88\text{-}106.43)}$	49.20	‡
P. umbellata	*	--	0	‡
R. juglandifolia	*	$3.12_{(4.01\text{-}2.39)}$	70.58	‡
S. dulcis	*	$102.52_{(424.30\text{-}46.97)}$	38.55	‡
S. acuta	*	$13.75_{(18.55\text{-}10.23)}$	--	‡
S. glabrum	*	$30.88_{(46.47\text{-}21.58)}$	--	‡
T. viridis	*	$6.51_{(9.05\text{-}4.62)}$	49.13	‡
V. litoralis	*	$7.86_{(10.63\text{-}5.77)}$	25.84	‡
Z. officinale	*	$10.25_{(14.09\text{-}7.44)}$	--	‡

* > 1,000
** 95% confidence intervals
‡ No activity

Table 3. Antibacterial Activity of Amazonian Plants Against *Staphylococcus aureus* and *Corynebacterium diphteriae*

Scientific name	Extract concentration (µg)	*S. aureus* Inhibition zone (mm)	*C. diphteriae* Inhibition zone (mm)
A. graveolens	100	8	21
	500	--	26
A. altilis	100	11	8
	500	13	--
B. picta (Fruits)	100	16	18
	500	--	23
B. picta (Leaves-Stems)	100	13	23
	500	15	25
B. brownei		NP	NP
B. grandifolia	100	9	--
	500	13	9
C. zanonia		NP	NP
C. odorata	100	8	12
	500	11	17
C. venusta	100	11	24
	500	14	28
C. acuminata	100	9	12
	500	11	18
C. terminalis	100	--	13
	500	12	18
C. scaber	100	9	12
	500	11	20
C. lechleri		NP	NP
D. peruviana	100	R	R
	500		
G. pubescens		NP	NP
G. tessmannii		NP	NP
G. longifolia	100	--	7
	500	13	13
I. heteroptera	100	10	15
	500	11	--
J. connata		NP	NP
P. reticulatum	100	--	--
	500	9	--
P. carthagenensis	100	--	13
	500	9	18
P. discolor	100	--	16
	500	13	23
P. umbellata	100	13	11
	500	15	21
R. juglandifolia	100	11	15
	500	12	19
S. dulcis		NP	NP
S. acuta		NP	NP
S. glabrum	100	R	R
	500		
T. viridis	100	8	12
	500	13	20
V. litoralis		NP	NP
Z. officinale	100	R	R
	500		
Ampicillin	10 µg/ml	11	47

NP: Not performed
R: Resistant

Discussion

In the micro plate brine shrimp lethality test, only one plant showed a LC_{50} value under 100 µg/mL: *Piscidia carthagenensis* (LC_{50}: 21.81 µg/mL), suggesting the presence of bioactive compounds, which to our knowledge, have not been yet identified. A literature search on NAPRALERT database (October, 2002) did not find any scientific report on this plant (16). Interestingly, PubMed database specified in one study that extracts of a related *Piscidia* plant, *P. piscipula* exhibited antifungal activity (17). *Piscidia carthagenensis* will need to be studied more thoroughly to isolate and characterize the active constituents. The BSLT is considered to be a convenient method to monitor the separation of these bioactive components. A correlation has been established between plant extracts with low LC_{50} values and cytotoxic, antifungal, insecticidal or pesticide activities (15).

The DPPH method for the assessment of antioxidant activity has been evaluated as rapid, simple, independent of sample polarity, and very convenient for the quick screening of plant extracts[18]. Our results revealed that most of the Amazonian plant extracts presented excellent activity. In fact, eight of the thirty extracts (26.66%) showed ED_{50}s below 10 µg/mL. For comparison, our positive control, quercetin had an ED_{50} :1.61 µg/mL. The most active extracts with free radical scavenging activity were: *Rhus juglandifolia* (ED_{50} :3.12 µg/mL), *Clusia venusta* (ED_{50} :3.61 µg/mL), and *Cola acuminata* (ED_{50} :4.26 µg/mL). There were no scientific reports for *R. juglandifolia* and *C. venusta* in the NAPRALERT and PubMed databases (16,17), October 2002. As for *C. acuminata*, NAPRALERT cites caffeine, triterpenes and flavonoids isolated from different parts of the plant. The extract has also shown smooth muscle relaxant and antispasmodic activities (16). Other plant extracts: *Inga heteroptera, Cedrella odorata, Trichillia viridis, Bauhinia picta,* and *Verbena littoralis* also presented good antioxidant activity. According to the examined databases

(NAPRALERT and PubMed), to this date some of these plants (*I. heteroptera, B. picta,* and *T. viridis)* have not been investigated for their active constituents and biological activities.

The *ß*-glucosidase inactivation is thought to have medicinal relevance for the search of anti-AIDS and diabetes/antiobesity agents (19). The in vitro bioassay offered several moderately good results. Four extracts (13.33%) exhibited inhibition values superior to 70%. Our positive control, 1-deoxynojirimycin HCl displayed 80.19% of inhibition at 9.5 x 10^{-9} N. The predominantly active extracts were *Piper reticulatum* (84%), *Inga heteroptera* (77%), *Clusia venusta* (70.9%), and *Rhus juglandifolia* (70.5%). Curiously, the last three extracts showed very good activity as free radical scavengers. As for *P. reticulatum*, the scientific literature reveals that an acetone extract of the aerial parts collected in Trinidad yielded two 6-substituted 5,6-dihydropyran 2-ones 1 and 2 and dihydrowisanidine (20). As indicated above, no information on bioactivities or chemical compounds has been found on *I. heteroptera, C. venusta,* and *R. juglandifolia* to date.

The xanthine oxidase inhibition bioassay proved to be unsuccessful in the screening of the Amazonian plants. While our positive control, Allopurinol exhibited inhibition percentages ranging from 75 to 100, the plant extracts presented no inhibition at all.

As for the antibacterial activity bioassay, the Gram-negative bacteria *E. coli, P. aeruginosas, P. vulgaris,* and *S. typhi* were in general resistant to the extracts. The Gram-positive microorganisms *Staphylococcus aureus* and *Corynebacterium diphteriae* resulted to be sensitive to most of the Amazonian plant extracts. As it can be seen in Table III, *Bauhinia picta* extract from the fruits showed the best activity, with a 16 mm inhibition zone at 100 µg/mL against *S. aureus*. At 500 µg/mL, the extracts of *B. picta* (leaves-stems) and *P. umbellata* displayed 15 mm inhibition zones for the same microorganism. In the case of *C. diphteriae*, the extract of *B. picta* (leaves-stems) shows again a superior inhibition zone (23 mm) at 100 µg/mL. The extract of *C. venusta* at 500 µg/mL demonstrated the greatest inhibition zone (28 mm). Our standard, Ampicillin at 10µg/ml produced an 11 mm average inhibition zone for *S. aureus* and 44 mm average inhibition zone for *C. diphteriae*.

Conclusion

This study has identified several plant extracts with antibacterial, antioxidant and *ß*-glucosidase inhibitory activities, despite their general inactivity in the brine shrimp lethality assay. It does not seem to be a correlation between the BSLT and the other biological activities. According to the main medical and plant related databases (NAPRALERT and PubMed), some of these species have not been searched for their bioactive chemical constituents (16,17). It should be useful to continue the investigation of these plants. The problem appears when the countries where these species occur are reluctant to provide large amounts of the plant material for this purpose. It is rationally feared that a possible drug discovery from these plants may leave the source countries without any economical benefit, as this has happened in the past. Therefore, collaborations with academic research universities through agreements of mutual assistance should be encouraged. There is much to gain in the study of our natural resources before the potential danger of extinction becomes imminent.

Resumen

Este estudio fue diseñado para evaluar las actividades biológicas de treinta extractos de plantas coleccionadas en el noroeste amazónico (Ecuador). Algunas de estas plantas son usadas por sus supuestas propiedades medicinales por los nativos de esta región. Para examinar los extractos se usaron cinco bioensayos *in vitro*:

1. El examen de mortalidad de los camarones salinos (*Artemia salina* Leach) en micro-plato (BSLT) es un procedimiento general que parece ser capaz de detectar un espectro amplio de bio-actividades presentes en extractos crudos de plantas.
2. Las propiedades antioxidantes fueron estudiadas en un ensayo colorimétrico usando 2,2-difenilo-1-picrilhidrazilo (DPPH).
3. El examen de inhibición de la *ß*-glucosidasa que se cree es un método para la evaluación de drogas anti-sida, anti-diabéticas o anti-obesidad.
4. El ensayo de inhibición de la xantina oxidasa que podría ayudar en el descubrimiento de agentes anti-gota.
5. La actividad antibacteriana que está siendo usada para aislar e identificar drogas antibióticas.

Como resultados encontramos que en el BSLT, *Piscidia carthagenensis* demostró una actividad óptima con una LC_{50} 21.81 µg/mL. Se considera que los extractos de plantas con valores de LC_{50} bajos pueden contener metabolitos con propiedades citotóxicas, anti-fúngicas, insecticidas o pesticidas. En el bio-ensayo de actividad antioxidante, se confirmó que algunos extractos de plantas mostraban excelentes actividades de destrucción de radicales libres. Las hojas de *Rhus juglandifolia* y *Clusia venusta* exhibieron valores de ED_{50} : 3.12 µg/mL y 3.61 µg/mL, respectivamente. *Piper reticulatum* (84%), *Inga heteroptera* (77%), *Clusia venusta* (70.9%) y *Rhus*

juglandifolia (70.5%) mostraron porcentajes de inhibición de β-glucosidasa bastante elevados. Del otro lado, ninguno de los extractos de plantas fue capaz de inhibir la xantina oxidasa. Finalmente, se encontró que los microorganismos Gram-positivos *Staphylococcus aureus* y *Corynebacterium diphteriae* fueron sensitivos a la mayoría de los extractos de plantas, mientras que las bacterias Gram-negativas *Escherichia coli*, *Pseudomonas aeruginosas*, *Proteus vulgaris* y *Salmonella typhi* ofrecieron resistencia con respecto a los extractos de plantas. Concluimos que es importante continuar la investigación de nuestro reino vegetal, especialmente de nuestras reservas tropicales como una alternativa para encontrar nuevas o mejores drogas. También es necesario continuar este tipo de investigación para aislar e identificar los principios activos de las plantas bio-positivas.

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