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Evaluation of Colombian Rainforest Plants for their DNA Interaction and Cytotoxic Activities

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Abstract : Seventy five plant extracts from four botanical families were assessed for their cytotoxic activities through the DNA interaction and the brine shrimp lethality tests. The best results in the DNA interaction assay were shown by the dichloromethane extracts of the species *Solanum brevifolium* (41%, Solanaceae) and *Clibadium funkize* (31%, Asteraceae), as well as the methanol extracts of both species (*S. Brevifolium*, 28% and *C. funkize* 31%). With regards to the brine shrimp lethality assay the best results were showed by the methanol extracts of *Mabea montana* (LC_{50} = 4.0 mg/L, Euphorbiaceae) and *Solanum ochranthum* (LC_{50} = 1.0 mg/L, Solanaceae); as well as the dichloromethane ones of the species from the Rubiaceae family named *Ladenbergia macrocarpa* (LC_{50} = 4.0 mg/L) and *Rubiacea* sp (LC_{50} = 1.0 mg/L).
Keywords: *Artemia salina*, bioactivity, bioprospection, Brine shrimp lethality assay, DNA-inhibition.

Introduction

It is considered that oxidative damage to cell occurs through several different processes, such as damage to DNA polymerases, prevention of apoptosis, induction of lipid peroxidation, formation of endogenous DNA adducts, among others[1]. Any chemical entity that contributes to neutralize by some specific mechanism the reactive species could preserve DNA integrity and consequently reduce some of the oxidative processes above mentioned and this will be of paramount importance in the search of novel bioactive molecules. Among the plants with good chemopreventive constituents are *Morinda citrifolia*[2], *Hemsleya amabilis*[3], *Aloe arborescens* Miller, *A. Ferox* Miller, *A. barbadensis* Miller, *A. saponaria* Haw, among others[4].

On the other hand, the brine shrimp lethality assay (BSLA) is an indicator used to determine cytotoxicity and insecticidal properties of compounds and plant extracts and it is very useful as a preliminary assessment of bioactivity. It has been used broadly because it is considered efficient, rapid, inexpensive

and requiring small quantities of samples[5]. It is well known that BSLA result has a good correlation with rodent and human acute oral toxicity data[6].

For thousands of years man has depended on natural resources as a wealth of bioactive compounds for treatment of many illnesses that has affected mankind[7]. However, factors as climatic change, deforestation, overpopulation, among others, are disturbing plants diversity in natural reserves and tropical jungles, and many of them will be lost forever. As a consequence, the screening of plants for their bioactive constituents is of transcendental importance, prior the extinction of many plant species worldwide in the near future[8]. Conscious of that, the aim of this work was to assess the biological activity throw the DNA interaction and the brine shrimp lethality assays (BSLA) of 75 plant extracts belonging to four botanical families from Colombian natural reserve.

Material and Methods

Plant materials

The aerial part of 25 plant species belonging to the botanical families Asteraceae, Euphorbiaceae, Rubiaceae and Solanaceae, were collected at The Regional Natural Park Ucumarí (RNPU, Pereira-Colombia). All these plants were authenticated by Dr. F.J. Roldán and voucher specimens were prepared and deposited at The Universidad de Antioquia Herbarium (Medellín, Colombia) and are listed in Table 1.

Extraction of plant material

Each collected plant sample was oven dried at 50 ºC with forced air by 72 h. Then, the dry materials were ground to a fine powder and aliquots of 300 g were extracted successively with *n*-hexane, dichloromethane and methanol (thrice, with portions of 900 mL each time). In each case the solvents were pulled out and separately concentrated in a rotary evaporator at 45 ºC at reduced pressure to dryness and stored at -10 ºC for further work up[9].

Phytochemical screening

The phytochemical screening of plant extracts was performed by using thin layer chromatography (TLC) on Silica Gel 60 F_{254} sheets (Merck, Darmsted Germany) following the procedure described by Wagner and Bladt[10]. In brief, the systems chloroform–ethyl acetate–methanol (2:2:1) was used for elution of methanol extracts while *n*-hexane–ethyl acetate (7:3) were used for elution of dichloromethane and *n*-hexane extracts, respectively; after elution phytocompounds were visualized through the use of the following chromogenic agents: Dragendorff, anisaldehyde-sulfuric acid, vanillin (1%) in sulfuric acid-ethanol, ferric chloride (1%) and aluminum trichloride (2%) in ethanol in order to search for: alkaloids, sterols, terpens, saponins, phenols, tannins and flavonoids, respectively. All determinations were done in triplicate and standards for the respective natural product assayed were used.

DNA interaction assay

In this assay, methanol HPLC grade was purchased from Mallinckrodt (Phillipsburg, NJ, USA), and HPLC-grade water obtained from a Barnstead E-pure system (Barnstead Thermolyne, Dubuque, IA, USA) was used. In addition, herring sperm DNA and vincristine sulfate were purchased from Sigma-Aldrich (St. Louis, MO, USA). A Hewlett Packard HP-1100 liquid chromatograph (Palo Alto, CA, USA) equipped with a diode-array detector (DAD), a 20 µL Rheodine manual injector, a Hypersil ODS column (4.6 µm, 250 mm, 5 mm; Agilent Technologies, Santa Clara, CA, USA) and the software HP Chemstation A. 06.01 was used.

This assay was performed by following the procedure proposed by Pezzuto[11]. In brief, 50 µL of the Herring sperm DNA solution (100 mg/L) was mixed with 50 µL of water. After homogenization, 20 µL of this solution were injected into the HPLC instrument, the elution solvent system H_2O-MeOH (80:20 v/v) was used at a flow rate of 1 mL/min, and the absorbance registered at 250 nm. The procedure was performed in triplicate to obtain DNA absorbance ($A_{DNA\ Absorbance}$). Then, 50 µL of DNA (100 mg/L) solution were mixed thoroughly with 50 µL of each plant extract solution (250 mg/L) to be analyzed, and from this 20 µL were injected into the HPLC instrument. The elution was performed with the same system and flow of the previous steps. The procedure was repeated thrice with the same plant extract to determine sample absorbance (A_{Sample}). The above procedure was repeated, but using 50 µL of vincristine sulfate (100 mg/L) to get the percentage of DNA interaction for the positive control. The DNA interaction percentage (DNA Int %) was determined by applying the following equation:

$$\text{DNA Int \%} = [(A_{DNA\ Absorbance} - A_{Sample})/A_{DNA\ Absorbance}] \times 100$$

Brine shrimp lethality assay

The cytotoxicity of the investigated extracts was determined by using nauplii of the brine shrimp (*Artemia salina*, Leach) through the lethality assay according to the methodology described by McLaughlin and Roger[12]. The brine Shrimp eggs, were purchased locally and hatched in artificial sea water (NaCl 3.8%) at room temperature. After 48 h, the nauplli were collected.

The methanol plant extracts were dissolved in small amount of artificial sea water and those from the dichloromethane were allowed to evaporate for 16 h and after that both extracts were made up to 10 mL with artificial sea water in each vial, followed with homogenization. Then, 10 *Artemia* nauplli were transferred to each vial. Each plant extract was tested at 1000, 100, and 10 mg/L and evaluated by triplicate. All tests were performed in a temperature controlled chamber at 28ºC, during 24 h, under a continuous light regime. In all experiments gallic acid (100 mg/L) and the same solvent used to dissolve the original extract were used as positive and negative controls, respectively. After 24 h, the number of survivors was counted and mortality percentage (M %) for each concentration was calculated by applying the following equation:

Mortality % = No of Dead nauplli x 100/Initial No of live nauplli

The medium lethal concentration (LC_{50}, mg/L) was determined for each plant extract by interpolation in the graph from mortality percentage versus the concentration (mg/L) and through a linear regression analysis by applying the program Microsoft Excel.

Results and Discussion

DNA interaction

The results in the DNA interaction are listed in Table 1. In general, the highest answers in this assay were shown by members of the Solanaceae family followed by those of the Asteraceae one. The best DNA-interaction were showed by the dichloromethane extracts of *Solanum brevifolium* (41%, Solanaceae), *Clibadium funkize* (31%, Asteraceae) and *Solanum ovalifolium* (28%, Solanaceae). In addition, the methanol extracts from *C. funkize* (31%), and those from the Solanaceae family *S. brevifolium* (28%) and *Solanum deflexiflorum* (25%) displayed moderated DNA-interactions. These results suggested that some plant extract constituents induce extract-DNA adducts formation and they may have highly toxic phytochemicals with potent carcinogenic and mutagenic activity[1].

The great DNA interaction displayed by members of the Solanaceae family in this bioassay could be attributed to the presence of steroidal alkaloids detected by the phytochemical screening, which due to their amphipilic nature, spatial conformation and redox reactivity can in some way interact with DNA[13]. For instance, dihydrosolacongestidine showed DNA topoisomerase II activity in the yeast *Saccharomyces cerevisiae* mutant assay[14]. There are also reports showing reduction of tumor cell proliferation, induction of apoptosis, inhibition of DNA synthesis or cell cycle arrest in a variety of cancer cell lines by this type of secondary metabolites[3].

The DNA interaction displayed by *Clibadium funkize* (31%, Asteraceae) in this work correlates very well with the data reported for the methanol extracts of members of the Asteraceae family named *Schistocarpha sinforensi* Cuatrec., *Aspilia quinquenervis* S.F. Blake and *Verbesina nudipes* S.F. Blake that showed strong DNA inhibitory activity[15]; in addition, *Vernonia triflosculosa* Kunth (Asteraceae) showed good inhibition against HaCat cells through a DNA binding assay[16].

Table 1. Results from the cytotoxicity, percentage of DNA inhibition and phytochemical screening of plant extracts from Colombia flora.

Family	Plant species / Voucher	Extracts[1]	Cyto toxicity activity CL_{50} (mg/L)	Percentage of DNA inhibition[2]	Phytochemical compound[3]				
					I	II	III	IV	V
	Mikania leiostachya Benth / FJR 3924	H	>1000	NE	-	-	+	-	+
		DC	>1000	15	-	-	+	+++	++
		MT	52	24	++	+	+	+	-
	Calea angosturana Hieron / FJR 3915	H	30	NE	-	-	+	-	-
		DC	>1000	2	-	+	++	+	++
		MT	77	5	++	++	+	++	-
	Clibadium funkize / FJR 3909	H	>1000	NE	-	-	+	+	-
		DC	899	31	+	+	+++	-	++

Asteraceae		MT	>1000	31	-	-	++	++	+
	Pentacalía americana (Kunth) Cuatrec. / FJR 3916	H	>1000	NE	-	-	-	-	-
		DC	>1000	19	-	-	++	+++	+++
		MT	192	13	++	+++	+	-	-
	Vernonia canescens Kunth / FJR 3908	H	150	NE	-	-	+	+	+
		DC	60	0	-	-	+++	-	+++
		MT	171	1	+	+	++	++	+
Euphorbiaceae	*Acalypha* sp / FJR 3914	H	>1000	NE	-	-	++	+	-
		DC	>1000	19	+	-	+++	++	++
		MT	25	22	+	-	-	-	+
	Tetrorchidium andinum Müll. Arg. / FJR 3927	H	348	NE	-	-	+	+	-
		DC	150	2	-	++	++	+++	++
		MT	129	4	-	+	+	+	+
	Acalypha diversifolia Jaq / FJR 3917	H	>1000	NE	-	-	++	++	-
		DC	>1000	15	+	+	++	++	+++
		MT	114	18	+	+	-	-	++
	Acalypha platyphylla Müll. Arg. / FJR 3910	H	608	NE	-	-	++	++	-
		DC	>1000	1	+	++	+++	+	+++
		MT	41	7	++	+	-	-	+
	Alchornea coelophylla Pax & K. Hoffm. / FJR 3906	H	>1000	NE	-	-	++	++	-
		DC	252	0	++	-	+++	+++	++
		MT	114	3	+++	+	++	++	+
	Euphorbia sp L. / FJR 3918	H	>1000	NE	-	-	-	+	-
		DC	>1000	11	-	-	+	+	++
		MT	103	22	-	+	-	-	+
	Hyeronima macrocarpa Müll. Arg. / FJR 3200	H	>1000	NE	-	-	+	+	-
		DC	>1000	0	+	+	+	+++	+++
		MT	225	14	++	++	+++	+++	++
	Hyeronima antioquiensis Cuatrec. / FJR 3905	H	>1000	NE	-	-	++	++	+
		DC	>1000	5	-	-	+++	++	+
		MT	98	13	+	+	+	++	++
	Mabea montana Müll. Arg. / FJR 3912	H	>1000	NE	-	-	+	++	-
		DC	>1000	0	-	-	++	-	++
		MT	4	13	++	++	+	+	+
Rubiaceae	*Cinchona pubescens* Vahl / FJR 3161	H	155	NE	-	-	+	++	-
		DC	20	1	-	-	++	+++	-
		MT	79	11	-	-	-	+	+++
	Ladenbergia macrocarpa (Vahl) Klotzch / FJR 3903	H	178	NE	-	-	+	++	-
		DC	4	11	+	-	+++	+++	+++
		MT	57	0	+	+	-	+	+++
	Palicourea acetosoides Wernham / FJR 3904	H	530	NE	-	-	++	++	+
		DC	459	0	+	++	++	-	++
		MT	632	14	-	-	+	++	++
	Palicourea sp Aubl. / FJR 3907	H	>1000	NE	-	-	++	++	+
		DC	>1000	12	-	+	++	+++	++
		MT	146	3	-	-		+	+
	Psychothria sp L. / FJR 3911	H	>1000	NE	-	-	+	++	-
		DC	>1000	0	-	-	++	+	+
		MT	662	5	-	-	+++	+++	++
	Rubiaceae sp Juss. / FJR 3913	H	662	NE	-	-	+	++	+
		DC	1	0	-	+	++	+++	++
		MT	112	9	++	+	++	+++	++
	Solanum brevifoluim	H	217	NE	-	-	++	++	-

Solanaceae	Dunal / FJR 3923	DC	125	41	-	++	+	+++	+++
		MT	19	28	-	-	+	+	++
	Solanum ochranthum Dunal / FJR 3922	H	166	NE	-	-	+	+	-
		DC	531	16	+	++	++	+	++
		MT	1	18	+	+	+	+	+++
	Solanum leucocarpon Dunal / FJR 3717	H	>1000	NE	-	-	++	+	-
		DC	>1000	16	-	-	+++	+	++
		MT	91	22	-	-	-	-	+++
	Solanum ovalifolium Dunal / FJR 3714	H	>1000	NE	-	-	+	+	+
		DC	>1000	28	+	+	+	++	+++
		MT	256	14	++	++	+	-	+
	Solanum deflexiflorum Bitter / FJR 3718	H	64	NE	-	-	++	+	-
		DC	>1000	23	-	-	+++	-	+++
		MT	80	25	-	++	+	+	+++
Positive control		Gallic acid	1		Tannic acid	Quer-cetin	Lanosterol, Stigmasterol	Diosgenin, Hecogenin	Lycorine, Quinine, Morphine Papaveri-ne
		Vincris-tine		50					

[1] Extracts: H= *n*-hexane, DC= Dichloromethane, MT= Methanol
[2] NE = Not Evaluated
[3] Phytochemical compound: I= Tannins; II= Flavonoids; III= Sterols; IV= Saponins; V= Alkaloids.

Brim shrimp lethality assay

The results on the brine shrimp lethality assay (BSLA) are reported in Table 1. According to Meyer[5] a compound is cytotoxic if it has a LC_{50} < 1000 mg/L and it is none cytotoxic if it has a LC_{50} > 1000 mg/L. Taken in consideration these authors, the best results in the BSLA were given by the methanol extracts of *Solanum ochranthum* Dunal (LC_{50} = 1.0 mg/L, Solanaceae), *Mabea montana* Müll-Arg (LC_{50} = 4.0 mg/L, Euphorbiaceae) and the dichloromethane extracts of the Rubiaceae species *Ladenbergia macrocarpa* (Valh) Klotzch (LC_{50} = 4.0 mg/L), *Rubiacea* sp. Juss. (LC_{50} = 1.0 mg/L) and the hexane one of *Calea angosturiana* Hieron (LC_{50} = 30 mg/L, Asteraceae). According to the phytochemical screening (Table 1) the cytotoxic activities showed by *M. montana* can be attributed to tannins and flavonoids present in the methanolic extracts; in the dichloromethane ones, the species from the Rubiaceae family displayed the presence of sterols and saponins as main secondary metabolites constituents.

In addition, in this work two methanolic extracts from the Euphorbiaceae family belonging to the genus *Acalypha*, named *A. platyphylla* Müll-Arg and *A. Diversifolia* Jacq displayed good cytotoxic activities with a medium lethal concentration values of 41 mg/L and 114 mg/L, respectively; in contrast, in a different study, *Acalypha indica* L. did not display any cytotoxic activity at all in the same bioassay[17]. The good cytotoxic activity showed by the dichloromethane and methanol extracts of *C. pubescens* with LC_{50} values of 20 and 79 mg/L, respectively correlates very well with the activity reported for *Cinchona* spp in the same assay developed by Krishnaraju[18].

On the other hand, the methanolic plant extracts from the species belonging to the genus *Solanum* named *S. ochranthum* (LC_{50} = 1.0 mg/L) and *S. brevifolium* Dunal (LC_{50} = 19 mg/L) displayed good cytotoxic activities. In general, these results are opposite to those showed by members of the same genus but from Indians plants whose LC_{50} values in three of them were higher than 100 mg/L[18].

The result of the cytotoxic activity displayed by members of the Asteraceae family in this work correlates well with those from *Wedelia paludosa* DC. (Asteraceae), since the cytotoxic activity of the dichloromethane extract through the BSLA was 149.6 µg/mL and the secondary metabolites associated to this activity were *ent*-kaurenoic and grandiflorenic acids[19]. In addition, the cytotoxic activities of the Asteraceae extracts correlate very well with those reported by

Niño[9], for the same family where the methanol extracts of *Liabum asclepiadeum* Sch. Bip. and *Munnozia polymnioides* (DC.) H Rob & Brettell also displayed cytotoxic activities with LC_{50} values of 10 and 40 mg/L, respectively.

The plant extracts with positive results in both assays were the methanolic extracts from the Solanaceae family named *Solanum brevifolium* Dunal (LC_{50} = 19 mg/L; 28%) and *Solanum deflexiflorum* (LC_{50} = 80 mg/L; 25%); while, from the Asteraceae family the methanolic plant extracts from *Mikania leiostachya* (LC_{50} = 52 mg/L; 24%) and *Acalypha* sp (LC_{50} = 25 mg/L; 22%) were also active in both bioassays. These extracts contain phenolic and alkaloidal compounds which might be responsible for these effects.

Conclusion

Here, we reported new data related to the cytotoxic activity and DNA interaction of plant extracts from Colombian flora. The results are the bases for new studies concerning the knowledge of the biological activities of wild plants from The Regional Natural Park Ucumarí. From these results can be inferred that the plant extracts assessed are a source of potent cytotoxic compounds as well as agents with antitumor properties. The cytotoxic and DNA-interaction activity displayed by several species in this work warrants further investigation.

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References

1. Fu P.P., Xia Q., Lin G., Chou M.W., Genotoxic pyrrolizidine alkaloids-mechanisms leading to DNA adduct formation and tumorigenicity, Int J Mol Sci., 2002, 3, 948-964.

2. Chan-Blanco Y., Vaillant F., Pérez A.M., Reynes M., Brillovet J-C., Brat P., The noni fruit (*Morinda citrifolia* L.): A review of agricultural research nutritional and therapeutic properties, J Food Compos Anal., 2006, 19, 645-654.

3. Kazi A., Urbizu D.A., Kuhn D.J., Acebo A.L., Jackson E.R., Greenfelder G.P., Kumar N.B., Dou Q.P., A natural musaceas plant extract inhibits proteasome activity and induces apoptosis selectively in human tumor and transformed, but not normal and non-transformed, cells, Int J Mol Med., 2003, 12, 879-887.

4. Kim H.S., Lee B.M., Inhibition of benzo[*a*]pyrene–DNA adduct formation by *Aloe barbadensis* Miller, Carcinogenesis, 1997, 18, 771–776.

5. Meyer B.N., Ferrigni N.R., Putnam J.E., Jacobsen L.B., Nichols D.E., MacLaughlin J.L., Brine shrimp: a convenient general bioassay for active plant constituents, Planta Med, 1982, 45, 31-34.

6. Lagarto P.A., Silva Y.R., Guerra S.I., Iglesias B.L., Comparative studies of the assay of *Artemia salina* L and the estimate of the medium lethal dose (LD_{50} value) in mice, to determine oral acute toxicity of plant extracts, Phytomedicine, 2001, 8, 395-400.

7. Newman D.J., Cragg G.M., Snader K.M., Natural products as sources of new drugs over the period 1981-2002, J Nat Prod., 2003, 66, 1022-1037.

8. Harvey A.L., Natural products in drug discovery, Drug Discov Today., 2008, 13, 894-901.

9. Niño J., Narváez D.M., Mosquera O.M., Correa Y.M., Antibacterial, antifungal and cytotoxic activities of eight Asteraceae and two Rubiaceae plants from colombian biodiversity, Braz J Microbiol., 2006, 37, 566-570.

10. Wagner H., Bladt S., Plant Drug Analysis. A Thin Layer Chromatography, Berlin, Germany: Springer, 1996.

11. Pezzuto J.M., Che Ch-T., McPherson D.D., Zhu J.T., Topeu G., Eedelmeier C.A.J., Cordell G.A., DNA as an affinity probe useful in the detection and isolation of biologically active natural products, J Nat Prod., 1991, 54, 1522-1530.

12. McLaughlin J.L., Roger L.L., The use of biological assays to evaluate botanicals, Drug Inf J., 1998, 32, 513-524.

13. Trouillas P., Corbière C., Liagre B., Duroux J-L., Beneytout J-L., Structure-function relationship for saponin effects on cell cycle arrest and apoptosis in the human 1547 osteosarcoma cell: a molecular modelling approach of natural molecules structurally close to diosgenin, Bioorg Med Chem., 2005, 13, 1141-1149.

14. Niño J., Correa Y.M., Mosquera O.M., Biological activities of steroidal alkaloids isolated from *Solanum leucocarpum,* Pharm Biol., 2009, 47, 255–259.

15. Correa Y.M., Niño J., Mosquera O.M., DNA interaction of plant extracts from colombian flora, Pharm Biol., 2007, 45, 111-115.

16. Kos D., Castro V., Murillo R., Poveda L., Merfort I., Ent-Kaurane glycosides and sesquiterpene lactones of the hirsutinolide type from *Vernonia triflosculosa,* Phytochemistry, 2006, 67, 62-69.

17. Krishnaraju A.V., Rao T.V.N., Sundararaju D., Vanisree M., Tsay H.B., Subbaraju G.V., Assessment of bioactivity of Indian medicinal plants using brine shrimp (*Artemia salina*) lethality assay, Int J Appl Sci Eng., 2005, 3, 125-134.

18. Krishnaraju A.V., Rao T.V.N., Sundararaju D., Vanisree M., Tsay H.B., Subbaraju G.V., Biological screening of medicinal plants collected from eastern Ghats of India using *Artemia salina* (Brine Shrimp Test), Int J Appl Sci Eng., 2006, 4, 115-125.

19. Batista R., Brandão G.C., Braga F.C., Oliveira A.B., Cytotoxicity of *Wedelia paludosa* D.C. extracts and constituents, Rev Bras Farmacogn., 2009, 19, 36-40.

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