Journal of Natural Sciences
June 2019, Vol. 7, No. 1, pp. 10-15
ISSN 2334-2943 (Print) 2334-2951 (Online)

Published by American Research Institute for Policy Development
DOI: 10.15640/jns.v7n1a2
URL: https://doi.org/10.15640/jns.v7n1a2

Allelophatic and Antioxidant Activity of Eight Amazon Species from De Tamshiyacu Tahuayo Reserve

Ursula Monteiro[1], Blanca Diaz[2], Robinson Saldaña[2], Víctor Sotero[1], Dora García[2]

Abstract

In this study were evaluated the antioxidant activity (AAO), allelopathic (ALL) and bioactive substances present in leaves of eight plant species from the Tamshiyacu-Tahuayo Community (Loreto-Peru). The AAO evaluation was performed by the free radical DPPH capture method, using dry and pulverized leaves, macerated in methanol for fifteen days, solutions of 0.01- 5.0 mg/mL and 0.1 mM DPPH were prepared, reading was performed in the UV/vis spectrometer at 517 nm, for the ALL was performed by the Petri plates method, using methanolics extracts of fresh leaves with concentrations of 0.1- 50.0 mg/mL against the growth of pregerminated seeds of *Latuca sativa* , the reading was made by measuring the hypocotyl and radicle of the seedling. In the results of species with high AAO were *Virola sebifera*, *Caryocar glabrum* and *Tapirira guianensis*, which present a percentage of inhibition superior to 50% in concentrations of 5.0 mg/mL. The species with high ALL are *Xylopia benthami*, *Malouetia naias* and *Virola surinamensis*, reaching an EC_{50} for the radicle at concentrations lower than 1.0 - 50.0 mg/L. The concentration of phenolic compounds for these species varied from 115217,18-321815,14 mg/kg, flavonoids from 1030,79-1441,16 mg/kg and proanthocyanidins from 0.65-1,77 mg / 100g. Total alkaloids range from 0,0 - 68,2 m/g evaluation, presenting differences between them.

Key words: allelopathy, antoxidants, Amazonian plants.

1. Introduction

Living beings use oxygen for the generation of energy and free radicals, which is incompatible with life unless there are defense mechanisms against these species, this defense is done through antioxidants (Garcia et al., 2001). Antioxidants are compounds that inhibit or retard the oxidation of other molecules by preventing the initiation and/or propagation of free radical chain reactions. They are divided into two categories, mainly synthetic and natural. The treatment with antioxidants is an alternative to reduce diseases related to oxidative stress, making use of natural plants with antioxidant effect. There are several methods to measure the *in vitro* antioxidant capacity of a species or substance, such as the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical stability method (Morales, 2013).

Plants originate secondary metabolites that intervene in complex interactions between organisms, favoring the process of germination and cell division. These substances provide benefits or damages to other plants and also to animals, and are called allelochemicals, whose direct or indirect detrimental or beneficial effect of the action of these released compounds is called allelopathy (Ahn & Chung, 2005). Not all compounds released by plants are inhibitors; some show stimulating effects depending on their concentration. Therefore, the concept of "allelopathy" as a crop management tool can be one of the practical application alternatives in agroecosystems, towards an approach to sustainable agriculture (Lorenzo & Gonzalez, 2010). In the present study the *in vitro* allelopathic activity of plant species was determined by the plate method against *Latuca sativa* growth. Phenols and alkaloids are secondary metabolites present in plants that consider them to cause antioxidant effects to the former and allelopathic to both.

[1] Foundation for the Sustainable Development of the Lower Jungle of Peru-FUNDESAB-PERU. Samanez Ocampo 576-Iquitos-Perú.
[2] Faculty of Chemical Engineering. National University of Peruvian Amazon. Freyre 632. Iquitos-Peru

Phenols are characterized by the presence of one or more phenolic rings called polyphenols, some are indispensable for plant physiological functions, others participate in defense functions in situations of stress and various stimuli (water, light, etc.) (Quiñones, 2012). It is considered that phenolic compounds are the main antioxidant substances, since they can stabilize free radicals, among them the researchers consider flavonoids as the most interesting, and they are found in different species and in high concentration in the apple, broccoli, cherries, grapes, lemons, oranges, grapefruits among others (Martinez - Flórez et al., 2002).

The Amazon basin is one of the regions with the greatest biodiversity. The wet and rain forests of the Amazon Basin, jointly with other plant formations (savannas, savannas, dense forests), constitute the set of biomes and more extensive and complex ecosystems in species; reason why it is considered that this region is strategic to maintain the environmental balance and be a biological reserve of global importance. On the other hand, the piedmont Andean regions, as well as some central areas of the Brazilian Amazon, are threatened by exploitation of non-renewable natural resources such as gas and oil. The Ecuadorian Amazon was resembling a chessboard, due to the numerous blocks tankers assigned to it. Faced with this situation, and despite fact that it has increased (almost 30%) over the last decade, protected areas of the region do not are in a position to conserve their biodiversity (Pasquis, 2006).

The objective of this study was to evaluate the antioxidant and allelopatic activity of eight Amazonian vegetable species from the Tamshiyacu Tahuayo communal reserve located in the Loreto Region (Peru).

2. Material and Methods

Material

The plant species studied were *Sapium* sp, *Virola sebifera*, *Xylopia benthamii*, Caryocar glabrum, *Guatteria hyposericea*, *Tapirira guianensis*, *Virola surinamensis* and *Malouetia naias*, found in the Tamshiyacu-Tahuayo Community Reserve. The exsicatas of the species were deposited in the Herbarium Amazonense of the National University of the Peruvian Amazonia (UNAP)

Methods

- **Determination of antioxidant activity *in vitro*.**

The determination of the antioxidant activity in plant species was performed using the DPPH method (Free Radical 1,1-difinyl-2-picryl-hydrazyl with 99.9% purity), following the methodology of (Sotero & Garcia, 2009). For the evaluations were prepared solutions of 0.01; 0.05; 0.10; 0.25; 0.50 mg/mL, from dry and micro powdered leaves and 0.1 mMol DPPH solution

In a 1.5 mL polystyrene cuvette were added 0. 025 mL of extract and 0.975 mL of the free radical. In another cuvette 1mL of DPPH (0.1 mM) was added, the spectrophotometric equipment Uv/vis was read at a wavelength of 517 nm for 5 minutes over an interval of 30 seconds. The readings were done in triplicate for each standard solution. Inhibition of DPPH radical sequestration by increasing solutions of the extracts was determined by the following expression.

Inhibition of DPPH ,$\% = \left[\frac{Ac-Am}{Ac}\right] x100$

Where A_c and A_m, are the absorbance of control (DPPH) and of the sample plus DPPH, respectively.

It was determined by plot intersection of the different concentrations versus percent inhibition and the concentration was taken at 50%.

- **Determination of allelopathic activity *in vitro*.**

Plant materials were washed with distilled water and lixiviated with methanol at the proportion of 5 g in 100 mL of methanol for 15 days, this product is filtered and the product utilized for the previous allelophatic evaluation. With the methanolic extract were realized the bioassays to evaluate their effect on germinated seeds of *Lattuca sativa*. It was impregnated at filter paper (Whatman Nº 1 and 2.7 cm of diameter) and collocated in Petri dishes with 100 µl of the solution to study at concentrations of 10; 3; 1; 0.3 y 0.1 mg/mL. after the evaporation of methanol, they were added with 700 µl of distilled water and there was placed five pre germinated seeds of *L. sativa* by 52 hrs (20ºC in dark).

Every experiment was realized by triplicate and for the blank; after of this was measurement the length of the roots of every seed and compared with the blank seeds. For the bioassay of the fractions it was similar but only was utilizing 100 µl of everyone (Sotero et al, 2016). The 50% Effective Concentration (EC_{50}), was determined by plot intersection of the different concentrations versus percent inhibition and the concentration was taken at 50%. The EC_{50} was determined on the radicle of the seedlings.

• Determination of total phenols and alkaloids.

The phenolic compounds were determined according to the methodology of (Valls, 2000). The extracts were made with three volumes of 25 mL of absolute ethanol, acidified with 1% formic acid, 0.5 grams of dried and pulverized sample was extracted, the extract formed was put to dryness in a stove at 40 °C, until volatilized the ethanol. The dried residue was dissolved in 50% methanol solution, acidified with 1% formic acid and brought to a volume of 10 mL, stored at -20 ° C until analysis.

Anthocyanins and Total Flavonoids.

1 mL of the extract prepared above, anthocyanins at 535 nm and flavonoids at 374 nm were determined by UV/vis spectrophotometry in a 1.5 mL polystyrene cuvette.

Total Phenolics.

The content of total phenol was determined using the Folin index. 40 µl of extract was added with 0.5 mL of the Folin-Ciacalteu reagent and 2 mL of 20% (w/v) sodium carbonate in a centrifuge tube (15 mL), making up to 10 mL. After half an hour, the reading was made by spectrophotometry at 700 nm. The phenolic compounds are determined by the equation of the standard calibration of catechin.

Catechins and Proanthocyanidols

The content of catechin and proanthocyanidols was determined with the vanillin assay by mixing 0.5 mL of extract, 1.25 mL of vanillin in 1% (w/v) methanol and 1.25 mL of 25% (v/v) sulfuric acid in methanol. The blank was prepared simultaneously in the same manner, but replacing the vanillin solution with methanol. After 15 minutes, the reading was made by spectrophotometry at an absorbance of 510 nm. The compounds of catechins and proanthocyanidols are determined by the equation of the standard calibration of catechin.

Total Alkaloids.

The determination of total alkaloids was performed using the methodology of (Shamsa et al., 2008). In whatman paper cartridges, 5.0 g of dry and micro-pulverized sample were weighed, the cartridges sealed and placed in the soxhlet apparatus for extraction with methanol for 16 hours (continuous extraction). The extract obtained was placed in the oven at 45 °C until the methanol was volatilized; the dry residue was dissolved with 2N HCl (approx. 15 mL) and filtered to remove impurities. 1 mL of the extract was washed with 10 mL of chloroform (3 times), the washed solution was adjusted to neutral pH with 0.1 N NaOH, to this solution was added 5 mL of BCG solution and 5 mL of phosphate buffer was added and the complex formed was extracted with 1,2,3 and 4 mL of chloroform by vigorous stirring. The extracts are collected in a 10 mL graduated flask and ground with chloroform. The reading was performed by spectrophotometry at 470 nm, the alkaloids were determined through that of the Atropine calibration curve

3. Results and Discussion

Antioxidant and allelopathic activity evaluation, in the leaves of the plant species under study, determines that if they present activity in different concentrations of both one and the other, where it is possible to distinguish that each species has its own chemical characteristic that uses it as a defense mechanism to environmental situations (predators, pests, molds, free radicals, etc.). According to the chemical substances present in them and the mixture thereof, they can be used for the search of active principles useful for medicine and/or agriculture with subsequent research studies, thus giving these species an additional added value since in many cases are used as building materials and carpentry

Table 1 details the results of comparative antioxidant activity of the plant extracts in their different concentrations, determining the inhibition on the free radical DPPH (0.1 mMol).

Evaluation of the methanolic extracts of the eight plant species studied showed antioxidant activity against the free radical DPPH (0.1 mM) in the concentration of 5.0 mg/mL, highlighting *Caryocar glabrum*, *Virola sebifera* and *Tapirira guianensis*, which presented percentage of antioxidant inhibition of 74.25, 62.75 and 57.40%. The DPPH method tests the antiradical capacity of lipophilic molecules in nonaqueous media. In other researches, this method is used as well as (Castañeda et al. 2008), which evaluated antioxidant capacity in seven peruvian medicinal plants extracts, with concentrations of 1, 50, 100 and 200 μg/mL, presenting high values of free radical scavenging of 100.57 and 110.56% in 100 μg/mL in *Alchornea castaneifolia* and *Calophyllum brasiliense*. The IC_{50} of each species was determined, *Caryocar glabrum* presented a concentration of 2.8 mg/mL, is the minimum concentration in which the plant extract inhibits the free radical DPPH, compared to *Virola sebifera* and *Tapirira guianensis*, which presented 3.5 and 4.1 mg/mL, this could be because they present total phenols and alkaloids, as well as (Orbe & Tuesta, 2013) determined that *Piper lagenaebaccum* Trel presents greater antioxidant activity than the leaves of *Piper tenuistylum*, concerning their IC_{50} valuation.

Table 1. Percentage of antioxidant inhibition of eight plant extracts

.Specie	Concentrations, mg/mL						IC_{50}, mg/mL
	0.01	0.05	0.10	0.25	0.50	5.00	
	Inhibition percent						
Sapium sp.	16.04	11.88	10.78	11.98	7.03	40.56	ND
Virola sebifera.	4.97	5.74	6.68	18.53	27.04	62.75	3.5
Xylopia benthamii.	-3.21	-1.59	0.43	-0.51	9.46	43.70	ND
Caryocar glabrum.	3.96	6.96	8.21	18.47	24.62	74.25	2.8
Guatteria hyposericea.	2.80	3.17	3.51	5.55	10.37	44.42	ND
Tapirira guianensis.	6.30	6.72	10.33	14.15	19.16	57.40	4.1
Virola surinamensis.	6.57	8.52	7.44	13.06	16.80	46.19	ND
Malouetia naias.	13.18	3.66	2.09	4.11	4.08	30.48	ND

In Table 2, the percentage of growth and of inhibition of the plant extracts in their different concentrations is distinguished, giving an inhibitory effect on the radicle of the lettuce seeds.

The allelopathic evaluation was determined by the percentage of inhibition and the EC_{50} of the plant extracts against the growth of the radicle of the *lettuce sativa* seedling, all presented allelophatic value in their concentration of 50.0 mg/mL, *Xilopia benthamii* presented high allelopathic value with, *Malouetia naias* with 90.14, 92.12, 91.83%, and *Virola surinamensis* with 62.67, 76.73 and 91.94% in the concentrations of 3.0, 10.0 and 10.0, respectively. 50.0 mg/mL. *Xylopia benthamii* presented a minimum inhibitory concentration of 0.5 mg/mL, compared to *Virola surinamensis* and *Malouetia naias* with 1.1 and 1.2 mg/mL, concentrations that inhibit the growth of the radicle in the seedling. (Yaisys B. 2006) describes that the allelopathic effects partially or totally deteriorate the germination and growth of the plants, they can also be of positive or negative character, direct or indirect, according to the concentration of the substances

Table 2. Percentage of allelopathic inhibition of plant extracts on the radicle of the *Latuca sativa* seeds.

. Specie	Concentrations, mg/mL						IC_{50}, mg/mL
	0.1	0.3	1.0	3.0	10.0	50.0	
	Inhibition percent						
Sapium sp.	3.17	19.88	31.56	51.72	59.41	87.53	ND
Virola sebifera.	-11.16	7.94	11.39	45.62	66.57		
Xylopia benthamii.	36.63	38.01	61.52	66.59	91.01	93.09	ND
Caryocar glabrum.	-0.01	-0.47	12.20	32.71	84.56	88.48	2.8
Guatteria hyposericea.	17.04	26.72	40.78	56.68	85.02	92.16	ND
Tapirira guianensis.	13.58	25.10	40.32	53.22	67.97	87.56	4.1
Virola surinamensis.	32.71	35.71	44.46	62.67	76.73	91.94	ND
Malouetia naias.	-21.95	-2.24	29.58	90.14	92.12	91.83	ND

Tables 3 and 4, details the concentrations of phenolic compounds and total alkaloids in mg/100 g which were determined from each plant species under study.

Plants synthesized and accumulated in their organs, secondary metabolites, involved in complex interactions between living organisms, phenols and total alkaloids of eight plant species were evaluated, with respect to the content of anthocyanins presented high values *Caryocar glabrum*, *Virola sebifera*, *Virola surinamensis* and Xylopia bentahamii, with 939,5, 713,8, 641,9 and 641,6 mg/kg, in flavonoids *Caryocar glabrum*, *Guatteria hyposericea* and *Tapirira guianensis* presented values of 143.89, 1441,8 and 1441,1 mg/kg. With respect to phenolics *Virola surinamensis*, *Xylopia enthamii* and *Malouetia naias* presented values of 321815,1, 255929,6 and 232987,4 mg/kg, in catechins and proanthocyanidins *Caryocar glabrum*, *Virola sebifera* and *Malouetia naias* presented values of 1,77, 1,58 and 1,28 mg/kg

Table 3. Determination of the total phenolics of eight plant species

Species	Antocianins mg /100g	Flavonoids mg /100g	Phenolics mg/100g	Catechins and proanthocyanidins. mg/100g
Sapium sp.	40.812	103.079	11521.718	0.065
Virola sebifera.	71.384	143.898	18580.874	0.158
Xylopia benthamii.	64.161	142.776	25592.969	0.065
Caryocar glabrum.	93.951	144.333	15180.714	0.177
Guatteria hyposericea.	39.651	144.188	13545.343	0.067
Tapirira guianensis.	41.943	144.116	11568.779	0.104
Virola surinamensis.	64.190	143.717	32181.514	0.116
Malouetia naias.	34.082	142.341	23298.743	0.128

Table 4. Determination of total alkaloids of eight plant species

Species	Alkaloids µg/100g	Alkaloids. mg/100g
Sapium sp.	0.1180	0.0039
Virola sebifera.	0.0400	0.0013
Xylopia benthamii.	0.2200	0.0073
Caryocar glabrum.	ND	ND
Guatteria hyposericea.	0.3000	0.0100
Tapirira guianensis.	0.0810	0.0027
Virola surinamensis.	0.0090	0.0003
Malouetia naias.	0.0840	0.0028

4. Acknowledgements

We thank CONCYTEC - INNOVATEPERU for the financial support of this study

5. Conclusion

Results of species with high antioxidant activity differ from those with a high allelopathic activity, although it is observed that the majority compounds are the phenolics when compared to the alkaloids

References

Ahn, J. K., Chung, I. M. (2005). Allelopathic potential of rice hulls on germination and seedling growth of barnyardgrass. *Agronomy Journal,* l. 92: 1162-1167**.**

Castañeda C. B., Ramos LL. E., Ibáñez V. L. (2008)**.** Evaluación de la capacidad antioxidante de siete plantas medicinales peruanas. *Revista Horizonte Médico* 8(1): 56-72.

García B., Vicente G., et al., (2001). Plantas con propiedades antioxidantes. Instituto superior de ciencias básicas y preclínicas "Victoria de Girón". *Revista Cubana*; 20 (3): 231-5.

Lorenzo P. y González. (2010). Alelopatía: una característica ecofisiológica que favorece la capacidad invasora de las especies vegetales. Ecosistemas, *Revista Ciéntifica y Técnica de Ecología y Medio Ambiente. Ecosistemas* 19 (1): 79-91.

Martínez-Flórez, S., J. Gonzáles-Gallego, J.M Culebras & Mª J. Tuñón, (2002). Los flavonoides: propiedades y acciones antioxidantes. *Nutr. Hosp.* V. 17 (6) 271-278

Morales P., Fernández R., et al. (2013). Valoración de la actividad antioxidante de verduras silvestres. VII Congreso Ibérico de Agroingenieria y Ciencias Hortículas, Madrid, Pág. 26-29.

Orbe Saavedra, P., & Tuesta Pinedo, G. (2013). Universidad Nacional de la Amazonia Peruana – Iquitos-Perú. Thesis. 101p.

Pasquis, R. (2006). Conservación de la biodiversidad amazónica: un enfoque regional *Bois et forêts des tropiques*, N° 290 (4): 61-72'

Quiñones M., *et al* (2012). Los polifenoles, compuestos de origen natural con efectos saludables sobre el sistema cardiovascular. *Revista Nutrición Hospitalaria,* 27 (1): 76-89.

Shamsa F., Monsef H., et al. (2008). Spectrophotometric determination of total alkaloids in some iranian medicinal plants. Department of Medicinal Chemistry. Department of Pharmacognosy, Faculty of Pharmacy. Page 17-20.

Sotero V., García D. (2009). Manual de análisis químico de alimentos, un aporte significativo a la investigación, análisis y determinación básico de los alimentos. Primera edición, Iquitos- Perú.

Sotero, V., Suarez, P, Vela, J.E., García de Sotero, D. Fujii, Y: (2016). Allelochemicals of three Amazon plants Identified by GC-Ms. *International Journal of Engineering and Applied Sciences* (IJEAS). 3 (2) 1-7

Yaisys, B., (2006). Revisión bibliográfica: la utilización de la alelopatía y sus efectos en diferentes cultivos agrícolas. *Cultivos tropicales* 27 (3): 5-16.

Valls J., et al. (2000). Importancia de los compuestos fenólicos en la calidad de los vinos tintos de crianza. Unidad de Enología. Dpto. de Bioquímica y Biotecnología, Universidad Rovira y Virgili (Taragona). *Alimentación y tecnología*, 2: 119-124.