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Evaluation of the Antioxidant Activity of 31 Amazonian Vegetable Species of Tamshiyacu Loreto-Peru

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Abstract

The aim of this study was evaluate the antioxidant capacity of the extracts of the leaves of 31 vegetable species collected at the vicinity of the town of Tamshiyacu, in the department of Loreto, Peru. The percentage of inhibition of free radicals of 2,2-diphenyl-1-picrylhydrazyl (DPPH) was calculated. From the concentration of the best species with high activity, which were three, it was realized the analysis of total concentration of phenolic compounds and alkaloids was analyzed by UV/Vis spectrophotometry. Besides the metanolics extracts were submitted to fractionation in chromatographic column and the fractions with similar molecules, grouped by thin layer chromatography. The final fractions were analyzed by a Gas Chromatograph coupled to mass spectrometry (CG-MS), to identify the molecules present in them and causing this activity. According to the results, the species that showed the best activity at concentrations lower than 5.0 mg/ml were Virola sebifera, Caryocar glabrum and Tapirira guianensis. The concentration of total phenolic compounds was 18580.9, 15180.7 and 11568.7 mg/100g for V. sebifra, C. glabrum and T. guiannensis, and total alkaloids were 36.6, 0.0 and 74.0 mg/100g for these same species. The main secondary metabolites are 3,5-di-tert-butyl-4-hydroxyanisole and normolatedol and caryophyllene, in V. sebifera, diisooctyl dicarboxylate.1,2-benzene and 3, 5-bis (1,1-dimetyl etill-phenol in C. glabrum and diisoctylphthalate, α-panasinseno, and vitamin E in T. guianensis.

Keywords: antioxidants, Amazonian plants, phenolics, CG-MS.

1. Introduction

Certain Amazonian plant species among its many utilities that ethnobotany mentions, is its use as medicinal plants that have been used by the inhabitants of this region since time immemorial and, thanks to the studies of various researchers, it has become known and valued for a proper use of them Duke & Vasquez (1994). The town of Tamshiyacu is the capital of the district of Sargento Lores in the Loreto Region and its primary forests belong for the most part to the Tamshiyacu - Tahuayo Communal Regional Conservation Area of Peru (ACR CTT). Located in zone 18 of the UTM projection system, between coordinates 680 075 E, 9 528 176 N and 768 162 E, 9 444 073 N. It has an area of four hundred twenty thousand and eighty hectares with two thousand five hundred square meters (420,080, 25 ha), Gobierno Regional de Loreto, (2011); Shoobridge, et al., (2004). The antioxidant activity is considered as the sequestration of free radicals, which are found in excess, in the human organism they are considered to cause various pathologies that include aging, cancer, atherosclerosis and other diseases in humans, Ibarra et al., (2011). The main organic molecules provided by the plant species that manage to sequester these free radicals are mainly phenolic compounds, some alkaloids and vitamins A, E and C, Coronado, et al., (2015). The objective of the present study was to perform the evaluation of the antioxidant activity of the foliar samples of 31 plant species of the Amazonian forest of the Tamshiyacu locality and to select the three best with high activity,

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2. Materials and Methods

2.1. Materials

Collection of plant material: Leaves of 31 plant species were collected in the vicinity of the town of Tamshiyacu and are shown in Table 1. The exsicates were deposited in the Herrerense herbarium of the Research Institute of the Peruvian Amazon (IIAP).

Evaluation of antioxidant activity. with the samples of the dried and powdered leaves, the methanolics extracts were prepared with some concentrations of 0.25; 0.1; 0.05 and 0.01 mg/mL. To determine the antioxidant activity, we used the UV/Vis spectrophotometer equipment, Specturlamb brand 22 pc. (KERLAB). In a 1.5 mL polystyrene cuvette, 25 µL of the methanolic extract and 975 µL of 0.1 mmol DPPH solution were added, then the absorbance was measured at a wavelength of 517 ηm, the readings were performed for 5 minutes with 30 second intervals, all reactions were performed in triplicate. The inhibition of DPPH radical sequestration by increasing solutions of the extracts was determined by the following expression, Sotero et al., (2011).

% Inhibition DPPH = [(Ac – Am)/Ac] 100

Where: Ac, is the absorbance of the control (0.1 mmol of DPPH), and Am, is the absorbance of the sample (increasing solutions of the extracts) in a time n.

2.2. Methods

Phenolic compounds: For the extraction of phenolic compounds, the technique of Valls et al., (2000) is as followed: 0.5 g of the sample is weighed and extracted successively with 3 volumes of 25 ml of ethanol acidulated with 1% formic acid. The extract is concentrated in a rotary evaporator at 40 °C. The dry residue is redissolved in a 50% methanol solution acidified with a formic acid solution, and brought to a volume of 10 mL. This is stored for subsequent analyzes.

Table 1. List of 31 plant species collected in the vicinity of the town of Tamshiyacu, Loreto-Peru.

Registry Collector	Order/families	Species	Georeference	
			UTM	
1 PAA	Malpighiales/ Euphorbiaceae	Sapium sp.	18M 0714538	9555251
2 PAA	Magnoliales/ Myristicaceae	Virola. sebifera	18M 0714538	9555249
3 PAA	Magnoliales/ Annonaceae	Oxandra sp.	18M 0714501	9555241
4 PAA	Magnoliales/ Myristicaceae	Virola. sebifera	18M 0714495	9557062
5 PAA	Magnoliales/ Myristicaceae	Iryanthera cf. laevis	18M 0714497	9555242
6 PAA	Magnoliales/ Annonaceae	Cymbopetalum cf.longipes	18M 0714451	9555237
7 PAA	Rosales/ Moraceae	Ficus cf. americana	18M 0704508	9560836
8 PAA	Fabales/ Fabaceae	Parkia cf. multijuga	18M 0704522	9560848
9 PAA	Rosales/ Moraceae	Brosimum parinaroides.	18M 0704985	9561001
10 PAA	Gentianales/ Apocynaceae	Couma macrocarpa.	18M 0705215	9561180
11 PAA	Magnoliales/ Annonaceae	Xylopia cf. benthamii	18M 0705289	9561177
12 PAA	Malpighiales/ Caryocaraceae	Caryocar . glabrum	18M 0705951	9558757
13 PAA	Magnoliales/ Annonaceae	Guatteria cf. hyposericea	18M 0705964	9558755
14 PAA	Sapindales/ Anacardiaceae	Tapirira guianensis	18M 0705988	9558712
15 PAA	Magnoliales/ Myristicaceae	Virola cf. surinamensis	18M 0706268	9559258
16 PAA	Gentianales/ Apocynaceae	Llacmelleasp.	18M 0711608	9557071
17 PAA	Magnoliales/ Annonaceae	Guatteria cf. flabellata	18M 0711617	9557063
18 PAA	Fabales/ Fabaceae	Dialium cf. guianense	18M 0711612	9557072
19 PAA	Rosales/ Moraceae	Helicostylis cf. tomentosa	18M 0711610	9557062
20 PAA	Rosales/ Moraceae	Helicostylis cf. turbinata	18M 0711623	9557049
21 PAA	Santalales/ Olacaceae	Mnquartia guianensis	18M 0711855	9556913
22 PAA	Laurales / Siparunaceae	Siparuna cf. sessiliflora	18M 0711854	9556909
23 PAA	Rosales/ Moraceae	Ficus sp.	18M 0711842	9556901
24 PAA	Alismatales/ Araceae	Dracontium cf. amazonense	18M 0711830	9556904
25 PAA	Malpighiales/ Clusiaceae	Vismia cf. macrophylla	18M 0711852	9556899
26 PAA	Caryophyllales/ Nyctaginaceae	Neea cf. divaricata	18M 0711864	9556887
27 PAA	Fabales/ Fabaceae	Zygia cf. macribridei	18M 0711868	9556888
28 PAA	Fabales/ Fabaceae	Diaium sp.	18M 0706244	9561454
29 PAA	Gentianales/ Apocynaceae	Malouetia cf. naias	18M 0706538	9561642
30 PAA	Magnoliales/ Annonaceae	Unonopsis cf. sitipitata	18M 0706764	9561927
31 PAA	Ranunculales/ Menispermaceae	Curarea cf. toxicofera	18M 0706669	9561534

Anthocyanins and total flavonoids: The determination of anthocyanins and total flavonoids is performed by UV/Vis spectrophotometry in 1mL of the extract prepared for the phenolic compounds by reading the absorbance at 535 ηm and 374 ηm respectively, after dilution of the samples. To perform the calculations, the molar extinction coefficient of malvidin-3-glucoside is used: 29500 L/mol cm.

Catechin and Proanthocyanidins: It is done by the vanillin test. 0.5 ml of the extract is mixed with 1.25 ml of vanillin in 1% methanol (w/v) and with 1.25 ml of 25% sulfuric acid (v/v) in methanol. The white is prepared simultaneously in the same way, but replacing the vanillin solution with methanol. It is left to rest for 15 minutes and then the absorbance reading is made at 510 ηm.

Total phenolic compounds: Measurement of the Folin index is carried out, for which 40 µl of the extract prepared for phenolics are treated, with 0.5 ml of Folin-Ciocalteau reagent and 2mL of 20% sodium carbonate (w/v), and they are taken to 10 ml. After half an hour, the absorbance reading is carried out at 765 ηm. To establish the calibration, catechin standards of concentrations between 0 - 100 mg/L are used.

Alkaloids: The method indicated by Shamsa et al., (2008), is used, 5 g dry pulverized sample is weighed and extracted with methanol in soxhlet equipment for 12 continuous hours. The extract was filtered and the methanol was separated in a rotavapor at 45 ºC, redissolved with 2N HCl and then filtered, 1 mL of this solution was transferred to a decanting pear and washed three times with 10 mL of chloroform. The pH of this solution is neutralized with 0.1 N NaOH, 5mL of BCG and 5mL of phosphate buffer are added, the mixture is shaken and the complex formed is extracted with 1, 2, 3, and 4mL of chloroform with stirring. The extracts are collected in a 10mL vial and then enriched to volume with chloroform to this solution is proceeded to perform the absorbance reading at 470 ηm. The data are quantified with a standard curve of atropine.

Chromatographic fractionation and identification of polar molecules by gas chromatography coupled to mass spectrophotometry (CG-Ms). The chromatographic fractionation of methanolic extracts, in open column with silica gel No. 100, of the three species with high antioxidant activity was performed, and identification of the molecules by thin-layer chromatography, grouping the fractions with similar molecules, then they were subjected to Gas chromatography equipment with mass spectrometry, using Agilent Technologies 7890ª ® with an Agilent 122-5532 DB 5MS ® capillary column of 30 m, with internal diameter of 0.25 mm. The initial temperature of the oven was 100 °C/03 min, followed by a ramp of 20 °C/3 min up to 300 °C/19 min; the maximum temperature of the oven was 325 °C. A Split injection was used and the helium gas flow was 2 mL/min. The fragments for the analyses were recorded with the parameters for a scan of 50 to 500 m/z, Sotero et al., (2016).

3. Results

Table 2 shows the antioxidant activity of the leaves of the 31 plant samples studied, ordered by families, where all the evaluations of the antioxidant activity of this species are summarized, it is observed that the three species that exceed 50 % inhibition of antioxidant activity at a concentration lower than 5.0 mg/ml are, Virola sebifera, Caryocar glabrum and Tapirira guianensis.

Tables 5, 6 and 7 show the molecules identified by the GC-Ms of the extracts of the species Virola sabifera, Caryocar glabrum and Tapirira guianensis, respectively.

Table 2. Percentages of Inhibition of the methanolics extracts of the leaves of the 31 plant species under study at different concentrations, using the DPPH method.

Family	Species	Concentration, mg/mL					
		5,0 mg/ml	**0,5 mg/ml**	**0,25 mg/ml**	**0,1 mg/ml**	**0,05 mg/ml**	**0,01 mg/ml**
Annonaceae	Oxandra sp.	32,59	10,01	12,11	18,35	18,37	17,67
Annonaceae	Guatteria hyposericea	44,42	10,37	5,55	3,51	3,17	2,80
Annonaceae	Cymbopetalum.longipes	26,19	0,05	0	0	0	0
Annonaceae	Xylopia benthamii	43,70	9,46	0,51	0,43	0	0
Annonaceae	Guatteria flabellata	45,56	26,74	7,10	3,68	2,40	1,51
Annonaceae	Unonopsis sitipitata	3,15	0	0	0	0	0
Apocynaceae	Couma macrocarpa.	19,48	7,18	4,10	3,26	0,55	1,78
Apocynaceae	Llacmelleasp.	42,11	17,09	10,75	10,90	10,90	10,50
Apocynaceae	Malouetia. naias	27,84	9,52	13,27	21,55	0	0

Anacardiaceae	Tapirira guianensis	79.41	23.53	20.59	11.76	11.76	8.82
Araceae	Dracontium amazonense	25,82	12,69	7,72	7,11	7,08	8,50
Caryocaraceae	Caryocar glabrum	74,25	29.41	23.53	14.71	11.76	5.88
Clusiaceae	Vismia macrophylla	23,87	10,43	7,27	6,23	5,83	6,32
Euphorbiaceae	Sapium sp.	40,56	7,03	11,98	10,78	11,88	16,04
Fabaceae	Parkia multijuga	6,82	1,40	2,81	1,50	0,85	0,10
Fabaceae	Dialium guianense	11,41	3,17	2,08	0,71	0,26	0
Fabaceae	Zygia macribridei	11,49	0	0,27	0	0	0
Fabaceae	Diaium sp.	30,48	5,55	4,09	2,11	2,18	2,42
Menispermaceae	Curarea toxicofera	22,85	2,91	1,37	0	0	0
Moraceae	Brosimum parinaroides.	17,89	3,92	0,84	1,33	1,46	0,36
Moraceae	Helicostylis turbinata	22,21	12,94	13,78	2,36	2,85	4,39
Moraceae	Ficus americana	9,85	0	0	0	0	0
Moraceae	Helicostylis tomentosa	13,10	1,99	4,71	4,56	4,67	4,22
Moraceae	Ficus sp.	34,48	23,94	24,97	24,72	18,53	19,23
Myristicaceae	Virola. sebifera	81.08	40	32.43	24.32	8.11	5.41
Myristicaceae	Virola sebifera	43,16	12,25	9,96	6,52	0,94	0,12
Myristicaceae	Iryanthera laevis	35,22	6,42	5,87	-2,54	-5,89	-6,06
Myristicaceae	Virola surinamensis	46,19	16,80	13,06	7,44	8,52	6,57
Nyctaginaceae	Neea divaricata	10,33	0	0,98	0	0	0
Olacaceae	Mnquartia guianensis	42,25	8,38	4,20	1,65	1,41	2,00
Siparunaceae	Siparuna sessiliflora	21,32	4,64	2,01	2,03	2,52	2,78

Table 3. Phenolic compounds present in the leaves of three plant species with a high percentage of antioxidant activity.

Species	**Anthocyanins**	**Flavonoids**	**Phenolics**	**Catechins and proanthocyanidins**
	mg /100g	mg /100g	mg/100g	mg/100g
Virola sebifera	71,38	143,90	18580,87	0,16
Caryocar glabrum	93,95	144,33	15180,71	0,18
Tapirira guianensis	41,94	144,12	11568,78	0,10

Table 4. Total Alkaloids present in the leaves of three plant species with a high percentage of antioxidant activity.

Species	**Total, alkaloids** mg/kg	**Total, alkaloids** mg/100g
Virola sebifera	36,03	3,60
Caryocar glabrum	n.d	n.d
Tapirira guianensis	74,00	7,40

Table 5. Molecules found by CG-MS in the methanol fractions of the leaves of the 2PAA specie (Virola sebifera)

	F1-2PAA			
N°	Retention time, min	Molecules	Prob. %	Area
1	9.99	(5a) preganane-3,20a-diol, 14a- [4-methyl-3-oxo- (1-oxa-4-azabuten-1,4- (diyl)] diacetate	63.4	11.5
2	11.67	4-piperidine acetate, 1-acetyl-5-ethyl-2- [3- (2-hydroxyethyl-1H-indol-2-yl] -o-methyl-methyl	66.8	23.2
3	11.35	octadecane, 3-ethyl-5- (2-ethylbutyl)	64.0	23.2
4	14.61	1H-inden-1-one, 2,3-dihydro-5,8-dimethoxy-3-methyl	64.1	12.1
5	16.62	Folic acid	61.8	19.9
	F2- 2PAA			
1	40.16	2'-methylene, bis 6- (1-dimethylethyl) -4-methyl-phenol	85.0	0.01
2	42.63	3 ', 8,8', trimethoxy-3-piperidine-2,2'-binaphthalene-1,1 ', 4,4'tetrone	70.0	0.01
3	47.70	3,5-di-tert-butyl-4-hydroxyanisole	69.5	98.42
	F4-2PAA			

1	10.91	Copaene	88.50	2.73
2	12.12	Cariopilene	93.20	12.09
3	14.65	1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1- (1-methylethyl) (1S-cis) -naphthalene	91.20	3.08
4	14.77	1,3,3,4-tetrahydro-1,6-dimethyl-4-81-methletiy) - (1Scis) -naphthalene	86.60	1.02
5	14.94	3-methoxymethyl.2,5,58a-tetramethyl-6,7,8,8a-tetrahydro-5-chromene)	76.00	1.65
6	16.33	(-) espatulenol	88.60	7.32
7	16.44	cariopilene oxide	87.90	2.71
8	16.56	Globulol	53.00	0.91
9	16.83	8S, 14-cedran-diol,	77.60	1.35
10	17.86	Cubenol	85.50	0.59
11	18.11	espatulenol	79.90	1.43
12	18.90	a-cadinol	81.70	0.70
13	19.60	a-N-normetadol	64.20	0.51
14	21.65	Is oaromadendreno epoxide	84.10	1.11
15	42.63	Diisooctyl 1,2-benzenedicarbilate	92.30	1.81
16	47.22	13-docosenamide (Z)	88.30	29.13
17	47.61	3,5-di-tert-butyl-4-hydroxyanisole	69.30	25.07
18	49.91	7-Acetoxy-3-methoxy-2- (3,4-dimethoxy penyl) -4-chromen-4-one)	68.40	0.80
	F5-2PAA			
1	19.62	1,9,5-cycloheptatriene, 6-methyl-1- (6-methyl) -1,3,5-cycloeptatriene-il)	82.00	0.61
2	42.63	Diisooctyl 1,2-benzenedicarboxylate	94.10	1.75
3	47.68	3,5-di-tert-butyl-4-hydroxyanisole	69.60	97.65
	F6-2PAA			
1	12.12	Cariofillene	87.40	52.31
2	14.65	2,3,5,6,8a-hexahydro-4,7-dimethyl-1- (1-methyl ethyl) - (1S-cis) or cadine-3,9-diene-naphthalene	82.40	10.54
3	16.34	Espatulene	88.00	37.15

Table 6. Molecules found by CG-'MS in the methanol fractions of the leaves of 12PAA specie (Caryocar glabrum)

	F2-12 PAA			
N°	Retention time, min	Molecules	Prob. %	Area
1	11.17	7,8-epoxylanstan-ol, 3-acetoxy	63.1	0.95
2	11.172	4,3-ethyl-5-octadecane	66.9	1.79
3	14.624		72.1	0.62
4	18.872	1,2,8-trimethyl-4-propenyl- (E) -naphthalene	80.9	0.70
5	19.64	1,1"bisphenyl, 2,2 ', 5,5'-tetramethyl	81.3	1.78
6	20.939	1,1'-dodecylidene bis (methyl) -benzene	65.4	1.78
7	42.638	Mono (2-ethyl hexyl) 1,1'-benzenedicarboxylate	98.1	93.19
	F3 - 12PAA			
1	14.6	2,4-bis (1,1-dimethyl-ethyl-phenol)	73.3	1.37
2	42.6	diisooctyl 1,2-benzene dicarboxylate	96.6	98.6
	F4-12PAA			
1	14.61	3,5-bis (1,1-dimetyl ethyl)-phenol	78.9	2.10
2	43.63	diisooctyl 1,2-benzene dicarboxylate	96.5	97.0
	F5-12PAA			
1	14.61	7,8-epoxylanostan-11-ol, 3 acetoxy	60.3	3.71
2	16.8	chlorotetracycline	62.2	3.62
3	42.6	Diisoctyl 1,2-benzenedicarboxylate	95.7	92.59
	F6-12PAA			
1	23.7	9 H-fluorene, 9-methylene	95.9	11.23
2	30.7	fluoranthene	95.7	33.14
3	32.12	pyrene	93.2	24.65
4	41.5	benzantrene	90.3	6.48
5	42.63	Diisoctyl 1,2-benzenedicarboxylate	94.2	24.47

Table 7. Molecules found by CG-'MS in the methanolic fractions of the leaves of the 14PAA specie (Tapirira guianensis)

	F1 -14PAA			
Nº	Retention time, min	Molecules	Prob. %	Area
1	14.02	decahydro-4a-methyl-1-methylene-7- (1-methylethylidene) -4 a-trans-naphthalene	80.7	6.7
2	14.81	2,4-bis (1,1-dimethylethyl) -phenol	76.2	11.19
3	19.65	naphthalene, 1,2,3-trimethyl-4-propanil	70.5	5.84
4	47.27	Vitamin E	80	76.17
F2-14PAA				
1	10.93	copaene	81.2	12.41
2	13.83	2-isopropyl-4a, 8-dimethyl-1,2,3,4,4a, 5,6,7-octahydronaphthalene	86.2	8.62
3	13.94	Eudesna-4 (14), 11dieno	67.8	2.89
4	14.03	1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7- (1-methylethyl enyl) - [1R - (1a, 7a, 8a, a)] - naphthalene (synonimous 2-valenceno)	96.8	33.67
5	14.73	a-panasinsene	89.3	42.42
F3-14 PAA				
1	11.58	2,4-bis (1,1-dimethylethyl) -phenol	69.2	2.944
2	14.83	α -patchulene	83.4	9.13
3	13.93	valenceno	88.6	27.87
4	14.03	a-panasinseno	88.6	39.596
5	14,72	diisooctylphthalate	95.0	20.407
F4 - 14PAA				
1	13.859	α -patchulene	74.2	2.011
2	14.058	(+) valencene	91.4	4.086
3	42.659	diisooctylphthalate	96	93.903

4. Discussion

Test for antioxidant activity.

Based on the results presented in Table 2, it can be stated that of the 31 species studied, 9.7% exceeded 60% inhibition, while 16.1% exceeded 45%. The families with better results, besides those mentioned, of inhibition were Annonaceae, Apocynaceae and a species of Euforbiaceae, with inhibition greater than 40%. These results are within the expected, since researchers who worked with 25 plants from Colombia, found similar or higher percentages of inhibition, and those who also consider that the Euforbiáceaes have good antioxidant qualities (Mosquera et al. 2007). According to these results, the three with the best activities were selected: Virola sebifera (Myristaceae). Caryocar glabrum (Caryocarceae) and Tapirira guianensis (Anacardiaceae), which present an inhibition percentage of 81.08%, 74.25%, and 79.41% respectively, at the concentration of 5.0 mg/ml and the IC_{50} of each of them is 1.5 mg/ml 2.2 mg/ml, and 2.8 mg/ml, respectively.

Phenols and total alkaloids.

Several authors agree that the high concentration of phenolic compounds in a plant species has high antioxidant activity, this correlation is given for wine, which at higher phenolic concentration improves antioxidant activity. This is the case for tea (Camellia sinensis) (Benzie & Szeto 1999), from lime (Tilia argéntea) (Yildrim et al., 2000), among others. According to Table 3, it can be observed that the concentration of phenolic compounds is high in the species Virola sebifera 18580.87 g/100g followed by Caryocar glabrum 15180.71 mg/100g and Tapirira guianensis 11568.78 mg/100g. It is worth mentioning the very similar concentration of flavonoids and the high presence of anthocyanins, proven compounds with a high antioxidant effect. In the case of C. glabrum, researchers found molecules in the bark of this species, such as coumarins, Aladul et al., (2007), as well as Rodríguez et al., (2017), who found several anthocyanins in T. guianensis, such as quercitin, and derivatives thereof.

It is observed that the concentration of alkaloids is present only in the species Virola sebifera and Tapira guianensis, but not in the Caryocar glabrum; indicative that these substances do not play an important role in this species, as antioxidants. Reducing its activity with greater certainty to the phenolic compounds present.

On the other hand, in the other species Virola sebifera and Tapira guianensis, an appreciable concentration of these substances is observed, and according to Chávez et al., (1996), they indicate that many alkaloids of different types of structures have been shown to be powerful inhibitors of singlet oxygen. Many of these compounds proved to be better inhibitors than the tertiary amine 1,4-diaza [2.2.2] bicycloctane (DABCO). Likewise, Ibarra et al., (2011) found a high antioxidant activity in the alkaloidal fractions of Erytrina americana, and when isolating the molecule erisodin, found that it had an IC_{50} of 150 μg/mL.

Molecules identified by CG-MS

The molecules of interest found in the methanolic fractions were a) V. sebifera: folic acid, 3,5-diterbutio-4-hydroxyanisole (phenolic), caryophyllene (bicyclic sesquiterpene) and spatulenol (alcoholic sesquiterpene); b) C. glabrum: 2,4-bis (1,1-dimethylethyl) -phenol, 1,1'-benzenedicarboxylate of mono (2-ethyl hexyl), 9 H-fluorene, 9-methylene, fluoranthene and pyrene (these last two, aromatic compounds) and c) T. guianensis: vitamin E, copaene (tricyclic sesquiterpene), patchulene and valencene both (sesquiterpenes). Likewise, observing the compounds currently recognized as antioxidants, there is an anisole derivative in fractions F2, F4 and F5 of V. sebifera, such as 3,5-di-tert-butyl-4-hydroxyanisole in fraction F3, F4 C. glabrum, phenolic derivatives, such as 2,4-bis (1,1-dimethyl-ethyl-phenol) and 3,5-bis (1,1-dimethyl-ethyl) -phenol and in the F1 of T. guianensis, the Vitamin E.

5. Conclusions

According with the results, the species Virola sebifera, Cariocar glabrum and Tapira guianensis, present excellent antioxidant activity and the chemical analysis showed several molecules of interest, for this activity.

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References

Duke, J.; Vasquez, R. (1994) Amazonian Ethnobotanical Dictionary. CRC Press Book Taylor & Francois Group. 224 p.

Gobierno Regional de Loreto (2011). RESOLUCIÓN DIRECTORAL Nº 003-2010-GGR/PROCREL-DE. Plan Maestro del Área de Conservación Regional "Comunal Tamshiyacu Tahuayo" 2011-2015. Belén.

Shoobridge D., Manrique C., Moran M.; Carreón, G.; Macahuachi, R., (2004). Evaluación Socioambiental de la Zona del Yavarí-Yavarí-Mirín y Tamshiyacu-Tahuayo. Parks Watch, Strengthening Parks to Safeguard Biodiversity. 99 p

Ibarra, E.; Pacheco, M.; García, M. San Miguel Chávez, R., 2011. Actividad antioxidante de alcaloides de Erythrina americana Miller. Rev. Fitotec. Mex. 34 (4): 241 – 246.

Coronado M.; Vega y León, S.; Gutiérrez, R.; Vázquez, M.; Radilla, C. (2015). Antioxidantes: perspectiva actual para la salud humana. Rev Chil Nutr 42:2:206-212.

Sotero, V.; Silva, L.; Merino, C.; et al. (2011). Evaluación de la actividad antioxidante de seis frutales amazónicos: anona, castaña, chope, huasaí, huito y uvilla. Folia Amazonica.20(1-2):53-58.

Valls, J.; Lampreave, M.; Nadal, M.; Arola, L.; (2000) Importancia de los compuestos fenólicos en la calidad de los vinos tintos de crianza. Alimentación, 119-124.

Shamsa, F., H.; Monsef, R., Ghamooshi, Verdian-rizi, M. (2008). Spectrophotometric determination of total alkaloids in some Iranian medicinal plants. Thai J. Pharm. Sci. 32: 17-20.

Sotero, V.; Suarez, P.; Vela, J.E.; Garcia de Sotero, D.; Fujii, Y. (2016). Allelochemicals of three amazon plants identified by GC-MS. International Journal of Engineering and Applied Sciences. 3(2): 1-3.

Mosquera, O.; Correa, Y.; Buitrago, D.: Niño, J. (2007). Antioxidant activity from Colombian Flora Mem Inst Oswaldo Cruz. 102(5): 631-634.

Benzie, I.F. and Szeto, Y.T. (1999). Total antioxidant capacity of teas by the ferric reducing/ antioxidant power assay. J. Agric. Food Chem. 47: 633-636.

Yildrim, A. Mavi, A. Oktay, M.; Kara, A.; Algur, O. and V. Bilaloglu. (2000). Comparison of antioxidant and antimicrobial activities of Tilia (Tilia argentea Desf. Ex DC), Sage (Salvia triloba L.), and Black tea (Camellia sinensis) extracts. J. Agric. Food Chem. 48: 5030-50

Alabdul Magid, A.; Voulquenne-Nazabadioko, L.; Moroy, G.; Moretti, C. (2007). Dihydroisocoumarin glucosides from stem bark of Caryocar glabrum Phytochemistry. 68: 2439-2443.

Rodríguez, A.M; Guimarães, D.O; Konno, T.U; Tinoco, L.W.; Barth, T.; Aguiar, F.A.; Lopes, N.P.; Leal, I.C.; Raimundo, J.M.; Muzitano, M.F. (2017). Phytochemical Study of Tapirira guianensis Leaves Guided by Vasodilatory and Antioxidant Activities. Molecules. 22: 304-319.

Chávez, R.; Plaza, A.; Lock, O. (1996). Antioxidantes de origen vegetal. Revista de Química. PUC-Lima. 10(1): 71-101.