

ANTIOXIDANTS FROM AMAZONIAN VEGETABLES: A POTENTIAL SOURCE OF NATURAL MEDICINE

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Abstract: The Amazon rainforest is home to a vast diversity of plant species, many of which have been used for medicinal purposes by indigenous peoples for centuries. This study investigated the antioxidant activity of plant species collected from the Tamshiyacu-Tahuayo Communal Regional Conservation Area (ACR CTT) in Peru. The antioxidant activity of the plant extracts was determined using the DPPH assay. The results showed that the plant extracts had significant antioxidant activity, with some extracts exhibiting more activity than commercial antioxidants. The findings of this study suggest that the plant species from the ACR CTT have potential as sources of natural antioxidants.

Keywords: Amazon rainforest, Medicinal plants, Antioxidant activity, DPPH assay, Natural antioxidants

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, arteriosclerosis 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,

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 exsiccates were deposited in the Herreense herbarium of the Research Institute of the Peruvian Amazon (IIAP).

Evaluation of antioxidant activity. with the samples of the dried and powdered leaves, the methanolic 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 μ L of the methanolic extract and 975 μ L of 0.1 mmol DPPH solution were added, then the absorbance was measured at a wavelength of 517 nm, 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 = $[(Ac - Am)/Ac] \times 100$

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 | <i>Sapium sp.</i> | 18M 0714538 | 9555251 |
| 2 PAA | Magnoliales/ Myristicaceae | <i>Virola sebifera</i> | 18M 0714538 | 9555249 |
| 3 PAA | Magnoliales/ Annonaceae | <i>Oxandra sp.</i> | 18M 0714501 | 9555241 |

| | | | | |
|--------|-------------------------------|-----------------------------------|-------------|---------|
| 4 PAA | Magnoliales/ Myristicaceae | <i>Virola sebifera</i> | 18M 0714495 | 9557062 |
| 5 PAA | Magnoliales/ Myristicaceae | <i>Iryanthera cf. laevis</i> | 18M 0714497 | 9555242 |
| 6 PAA | Magnoliales/ Annonaceae | <i>Cymbopetalum cf. longipes</i> | 18M 0714451 | 9555237 |
| 7 PAA | Rosales/ Moraceae | <i>Ficus cf. americana</i> | 18M 0704508 | 9560836 |
| 8 PAA | Fabales/ Fabaceae | <i>Parkia cf. multijuga</i> | 18M 0704522 | 9560848 |
| 9 PAA | Rosales/ Moraceae | <i>Brosimum parinaroides.</i> | 18M 0704985 | 9561001 |
| 10 PAA | Gentianales/ Apocynaceae | <i>Couma macrocarpa.</i> | 18M 0705215 | 9561180 |
| 11 PAA | Magnoliales/ Annonaceae | <i>Xylopia cf. benthamii</i> | 18M 0705289 | 9561177 |
| 12 PAA | Malpighiales/ Caryocaraceae | <i>Caryocar glabrum</i> | 18M 0705951 | 9558757 |
| 13 PAA | Magnoliales/ Annonaceae | <i>Guatteria cf. hyposericea</i> | 18M 0705964 | 9558755 |
| 14 PAA | Sapindales/ Anacardiaceae | <i>Tapirira guianensis</i> | 18M 0705988 | 9558712 |
| 15 PAA | Magnoliales/ Myristicaceae | <i>Virola cf. surinamensis</i> | 18M 0706268 | 9559258 |
| 16 PAA | Gentianales/ Apocynaceae | <i>Llasmelleasp.</i> | 18M 0711608 | 9557071 |
| 17 PAA | Magnoliales/ Annonaceae | <i>Guatteria cf. flabellata</i> | 18M 0711617 | 9557063 |
| 18 PAA | Fabales/ Fabaceae | <i>Dialium cf. guianense</i> | 18M 0711612 | 9557072 |
| 19 PAA | Rosales/ Moraceae | <i>Helicostylis cf. tomentosa</i> | 18M 0711610 | 9557062 |
| 20 PAA | Rosales/ Moraceae | <i>Helicostylis cf. turbinata</i> | 18M 0711623 | 9557049 |
| 21 PAA | Santalales/ Olacaceae | <i>Mnquartia guianensis</i> | 18M 0711855 | 9556913 |
| 22 PAA | Laurales / Siparunaceae | <i>Siparuna cf. sessiliflora</i> | 18M 0711854 | 9556909 |
| 23 PAA | Rosales/ Moraceae | <i>Ficus sp.</i> | 18M 0711842 | 9556901 |
| 24 PAA | Alismatales/ Araceae | <i>Dracontium cf. amazonense</i> | 18M 0711830 | 9556904 |
| 25 PAA | Malpighiales/ Clusiaceae | <i>Vismia cf. macrophylla</i> | 18M 0711852 | 9556899 |
| 26 PAA | Caryophyllales/ Nyctaginaceae | <i>Neea cf. divaricata</i> | 18M 0711864 | 9556887 |
| 27 PAA | Fabales/ Fabaceae | <i>Zygia cf. macribridei</i> | 18M 0711868 | 9556888 |
| 28 PAA | Fabales/ Fabaceae | <i>Dialium sp.</i> | 18M 0706244 | 9561454 |
| 29 PAA | Gentianales/ Apocynaceae | <i>Malouetia cf. naias</i> | 18M 0706538 | 9561642 |
| 30 PAA | Magnoliales/ Annonaceae | <i>Unonopsis cf. sitipitata</i> | 18M 0706764 | 9561927 |
| 31 PAA | Ranunculales/ Menispermaceae | <i>Curarea cf. toxicofera</i> | 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 nm and 374 nm 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 nm.

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-Ciocalteu 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 nm. 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 °C, 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 filled to volume with chloroform, in this solution is proceeded to perform the absorbance reading at 470 nm. 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 7890^a ® 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 sebifera*, *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 | <i>Oxandra sp.</i> | 32,59 | 10,01 | 12,11 | 18,35 | 18,37 | 17,67 |
| Annonaceae | <i>Guatteria hyposericea</i> | 44,42 | 10,37 | 5,55 | 3,51 | 3,17 | 2,80 |
| Annonaceae | <i>Cymbopetalum longipes</i> | 26,19 | 0,05 | 0 | 0 | 0 | 0 |
| Annonaceae | <i>Xylopia benthamii</i> | 43,70 | 9,46 | 0,51 | 0,43 | 0 | 0 |
| Annonaceae | <i>Guatteria flabellata</i> | 45,56 | 26,74 | 7,10 | 3,68 | 2,40 | 1,51 |
| Annonaceae | <i>Unonopsis sitipitata</i> | 3,15 | 0 | 0 | 0 | 0 | 0 |
| Apocynaceae | <i>Couma macrocarpa</i> | 19,48 | 7,18 | 4,10 | 3,26 | 0,55 | 1,78 |
| | | | | | | | |
| | | | | | | | |
| | | | | | | | |
| | | | | | | | |

| | | | | | | | |
|----------------|-------------------------------|-------|-------|-------|-------|-------|-------|
| Apocynaceae | <i>Llasmelleasp.</i> | 42,11 | 17,09 | 10,75 | 10,90 | 10,90 | 10,50 |
| Apocynaceae | <i>Malouetia. nalias</i> | 27,84 | 9,52 | 13,27 | 21,55 | 0 | 0 |
| Anacardiaceae | <i>Tapirira guianensis</i> | 79,41 | 23,53 | 20,59 | 11,76 | 11,76 | 8,82 |
| Araceae | <i>Dracontium amazonense</i> | 25,82 | 12,69 | 7,72 | 7,11 | 7,08 | 8,50 |
| Caryocaraceae | <i>Caryocar glabrum</i> | 74,25 | 29,41 | 23,53 | 14,71 | 11,76 | 5,88 |
| Clusiaceae | <i>Vismia macrophylla</i> | 23,87 | 10,43 | 7,27 | 6,23 | 5,83 | 6,32 |
| Euphorbiaceae | <i>Sapium sp.</i> | 40,56 | 7,03 | 11,98 | 10,78 | 11,88 | 16,04 |
| Fabaceae | <i>Parkia multijuga</i> | 6,82 | 1,40 | 2,81 | 1,50 | 0,85 | 0,10 |
| Fabaceae | <i>Dialium guianense</i> | 11,41 | 3,17 | 2,08 | 0,71 | 0,26 | 0 |
| Fabaceae | <i>Zygia macribridei</i> | 11,49 | 0 | 0,27 | 0 | 0 | 0 |
| Fabaceae | <i>Diaium sp.</i> | 30,48 | 5,55 | 4,09 | 2,11 | 2,18 | 2,42 |
| Menispermaceae | <i>Curarea toxicofera</i> | 22,85 | 2,91 | 1,37 | 0 | 0 | 0 |
| Moraceae | <i>Brosimum parinaroides.</i> | 17,89 | 3,92 | 0,84 | 1,33 | 1,46 | 0,36 |
| Moraceae | <i>Helicostylis turbinata</i> | 22,21 | 12,94 | 13,78 | 2,36 | 2,85 | 4,39 |
| Moraceae | <i>Ficus americana</i> | 9,85 | 0 | 0 | 0 | 0 | 0 |
| Moraceae | <i>Helicostylis tomentosa</i> | 13,10 | 1,99 | 4,71 | 4,56 | 4,67 | 4,22 |
| Moraceae | <i>Ficus sp.</i> | 34,48 | 23,94 | 24,97 | 24,72 | 18,53 | 19,23 |
| Myristicaceae | <i>Virola. sebifera</i> | 81,08 | 40 | 32,43 | 24,32 | 8,11 | 5,41 |
| Myristicaceae | <i>Virola sebifera</i> | 43,16 | 12,25 | 9,96 | 6,52 | 0,94 | 0,12 |
| Myristicaceae | <i>Iryanthera laevis</i> | 35,22 | 6,42 | 5,87 | -2,54 | -5,89 | -6,06 |
| Myristicaceae | <i>Virola surinamensis</i> | 46,19 | 16,80 | 13,06 | 7,44 | 8,52 | 6,57 |
| Nyctaginaceae | <i>Neea divaricata</i> | 10,33 | 0 | 0,98 | 0 | 0 | 0 |
| Olacaceae | <i>Mnquartia guianensis</i> | 42,25 | 8,38 | 4,20 | 1,65 | 1,41 | 2,00 |
| Siparunaceae | <i>Siparuna sessiliflora</i> | 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 |
| <i>Virola sebifera</i> | 71,38 | 143,90 | 18580,87 | 0,16 |
| <i>Caryocar glabrum</i> | 93,95 | 144,33 | 15180,71 | 0,18 |
| <i>Tapirira guianensis</i> | 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 |
|----------------------------|------------------------|--------------------------|
| <i>Virola sebifera</i> | 36,03 | 3,60 |
| <i>Caryocar glabrum</i> | n.d | n.d |
| <i>Tapirira guianensis</i> | 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 | | | | |
|-----------------|---------------------|--|---------|-------|
| Nº | Retention time, min | Molecules | Prob. % | Area |
| 1 | 9.99 | (5a) preganane-3,20a-diol, 14a- [4-methyl-3-oxo- (1-oxa-4azabuten-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) (1Scis) -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-5chromene) | 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 | isoaromadendreno epoxide | 84.10 | 1.11 |
| 15 | 42.63 | Diisooctyl 1,2-benzenedicarboxylate | 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-dimethoxyphenyl)-4-chromenone | 68.40 | 0.80 |
| F5-2PAA | | | | |
| 1 | 19.62 | 1,9,5-cycloheptatriene, 6-methyl-1-(6-methyl)-1,3,5-cycloheptatriene-yl | 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) - (1Scis) 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 | | | | |
|-------------------|---------------------|--|---------|-------|
| Nº | 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-dimethyl 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-epoxy lanostan-11-ol, 3 acetoxo | 60.3 | 3.71 |
| 2 | 16.8 | chlorotetracycline | 62.2 | 3.62 |
| 3 | 42.6 | Diisooctyl 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 | benzanthrene | 90.3 | 6.48 |
| 5 | 42.63 | Diisooctyl 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, 5,6,7octahydronaphthalene | 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 2valenceno) | 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 Euforbiaceae 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* (Caryocaraceae) 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 IC₅₀ 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 argentea*) (Yildirim 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 quercetin, 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] bicyclooctane (DABCO). Likewise, (Ibarra et al., 2011) found a high antioxidant activity in the alkaloidal fractions of *Erythrina americana*, and when isolating the molecule erisodin, found that it had an IC₅₀ 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-4hydroxyanisole (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, 9methylene, 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-dimethylethyl-phenol) and 3,5-bis (1,1-dimethyl-ethyl) -phenol and in the F1 of *T. guianensis*, the Vitamin E.

5. Conclusion

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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7. References

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