

A SCREENING FOR NATURAL COLORANTS IN THE ZONGO VALLEY WITH PROBABLE ANTIOXIDANT AND/OR PHOTO-PROTECTOR ACTIVITIES

BÚSQUEDA DE COLORANTES NATURALES EN EL VALLE DE ZONGO CON POSIBLES PROPIEDADES ANTIOXIDANTES Y/O FOTOPROTECTORAS

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ABSTRACT

Eleven plants were collected in the Zongo Valley following an organoleptic and chimio-taxonomic criteria of collection to find species with colorant and photo-protector properties. *Brachyotum microdon*, *Monnina bridgesii* and *Souroubea fragilis* present promising colorant attributes. In addition, *B. microdon*, *Rumex acetosella* and *Fuchsia boliviana* show important absorptions in the UV-B region while *S. fragilis*, *Orthaea boliviensis*, *Senecio floccosus* and *Baccharis pentlandii* have UV-A and UV-B absorptions. A series of phytochemical tests were performed to learn about the secondary metabolite profile in the collected species. This is the first work done and published for *Souroubea fragilis*, *Orthaea boliviensis* and *Senecio floccosus*.

RESUMEN

Once plantas fueron colectadas en el Valle de Zongo siguiendo un criterio de colecta organoléptico y químio-taxonomico para encontrar especies con propiedades colorantes y fotoprotectoras. *Brachyotum microdon*, *Monnina bridgesii* y *Souroubea fragilis* presentan prometedores atributos como colorantes. Además, *B. microdon*, *Rumex acetosella* y *Fuchsia boliviana* muestran importantes absorciones en la región de UV-B mientras que *S. fragilis*, *Orthaea boliviensis*, *Senecio floccosus* y *Baccharis pentlandii* poseen absorciones en UV-A y UV-B. Una serie de ensayos fitoquímicos fueron realizados para conocer el perfil de metabolitos secundarios en las especies colectadas. Este es el primer trabajo realizado y publicado de *Souroubea fragilis*, *Orthaea boliviensis* y *Senecio floccosus*.

Keywords: Zongo Valley, Photo-protector Properties, UV Absorption, Phytochemical Assays, Colorants, *Brachyotum Microdon*, *Monnina Bridgesii*, *Orthaea Boliviensis*, *Senecio Floccosus* and *Souroubea Fragilis*.

Palabras Clave: Valle de Zongo, Propiedades Fotoprotectoras, Absorción UV, Ensayos Fitoquímicos, Colorantes, *Brachyotum Microdon*, *Monnina Bridgesii*, *Orthaea Boliviensis*, *Senecio Floccosus* y *Souroubea Fragilis*.

1. INTRODUCTION

The “Green Wave” that captivates almost everybody has promoted the research and valorization of some natural products that are used as colorants and the validation of others with possible applications. The number of consumers who wish to eat food with colors coming from nature or people, who wish to have natural pigments in their lotions or cosmetics, increases every day. If antioxidant and/ or photo-protector activities are detected in these natural colorants, the interest in their use will heighten further valorization of our natural resources.

Nowadays, the study of natural antioxidants has an important scientific and economic impact. There are many publications of natural colorants with the mentioned activity. Among them, the work of Malenčioiè [1] with soy beans of different colors, of Seveg [2] with chickpeas of distinct coloring and those of Muntana and Tunnop [3], [4] with rice of different pigmentation stand out. These studies concluded that the most colored species (black, brown, red) present higher antioxidant activity. In addition, it is also important to mention works that report compounds with known antioxidant activity like anthocyanins from purple broccoli [5] or lycopene from a variety of edible and non-edible species [6]. Among the latter species, one plant that stands out to be used in the cosmetic or textile industries is *Rumex acetocella* whose red pigment has antioxidant values [7].

Natural colorants are environment friendly; therefore, many researches on this topic have been launched worldwide. Based on the fact that several pigments protect plants from the ultra violet harmful solar irradiation, like the red pigments found in raspberry and those blue from blueberry [8], several types of natural products have been monitored as possible photo-protectors. Among them we can highlight vegetal extracts from land and marine sources as well as silicates. Among the vegetal extracts there appear those from eucalyptus [9], from avocado, olive tree [8] and from marigold [10]. All these extracts present important protection values in the applied substrate. Among the marine

extracts, *Ishige okamurae* alga stands out because it is specific for UV-B radiation [11]. Among the silicates, we can highlight ocean clay that is more innocuous than the majority of additives used in sunscreen lotions and creams [12].

Bolivia, located in the center of South America, has different ecosystems each of them having a specific climate, altitude and soil. A region in Bolivia that has several ecosystems is the Zongo Valley, located in the northwestern side from the city of La Paz. This valley starts at the high Andean prairie at 4800 m.a.s.l. and it extends to the humid tropical region called Yungas at 800 m.a.s.l. [13], [14]. It has been reported that 109 vegetal families and 158 species exist in the Zongo Valley [13]. This significant plant bio-diversity has captured our attention to evaluate and validate their possible attributes as colorants and photo protectors. Among this wide diversity of plants, we have focused our research in species with colored organs (organoleptic approach) and species that contain polyphenols (chimio-taxonomic approach). Polyphenols, aside from being colored molecules, control the normal oxidation processes in living organisms. In addition, these compounds could be useful as antioxidant additives in case typical metabolic oxidations get out of control. The collected plants were submitted to preliminary phytochemical screenings in order to determine the types of compounds present in each specie. In addition, the ultraviolet absorption profile of each extract was obtained to determine the presence of compounds that could absorb UV-A and/or UV-B radiations. Both radiations, UV-A (320 nm - 400 nm) and UV-B (280 nm -320 nm), constitute part of the solar radiation that arrives to earth and are harmful to living beings because they trigger negative biological reactions in organisms. A screening of compounds that absorb these irradiations would increase their attributes as possible photo-protectors. In addition, thanks to the UV profile of the studied extracts, we can predict the presence of aromatic compounds (phenolics, flavonoids, anthraquinones) which absorb in the region of UV- A and UV-B radiations.

2. EXPERIMENTAL WORK

2.1 General

Ultraviolet studies were done on UV/VIS spectrophotometer Biochrom, model Libra S12. All supports and reagents used in this work were obtained from Merck and Sigma.

2.2 Collection of plant species

Plant species were collected in the Zongo Valley on October 2013. The collection started near the Zongo Dam at altitude 4715 m.a.s.l. (68°05'02'' longitude and 16°15'02'' latitude) and ended near the Huaji Hydroelectric Power Station at 941 m.a.s.l. (67°55'04'' longitude and 16°00'05'' latitude). All species were identify and deposited in the Bolivian National Herbarium, La Paz.

2.3 Extracts preparation

The collected species were air-dried at room temperature, in a dry place protected from the solar radiation. The dried specimens were separated into their different organs, grinded, weighed and extracted with petroleum ether followed by ethanol 96%. The dried extracts were weighed to obtain their yield and later submitted to a series of phytochemical tests to determine their secondary metabolites. The obtained polar and non-polar extracts were also prepared to acquire their UV profiles.

2.4 Phytochemical study

For the preliminary phytochemical test, the obtained extracts were submitted to the following assays:

- Methods to determine the presence of phenolic compounds, flavonoids, flavones; flavonol [15], [16]; isoflavones [17]
- Method to identify tannins [18]
- Method to identify anthocyanins and anthocyanidins [19]
- Methods to detect anthraquinones [17] ; cumarines [15]; chalcones; quinones [20]
- Methods to detect steroids and/or triterpens [16]
- Method to determine carotenoids [21]

The complete phytochemical study was performed on specie depending on the plant's collected amount and their fractions' yields. For the analysis of the chemical composition of each fraction, thin layer chromatographies were carried out in silicagel F₂₅₄ of ½ mm plates. Different solvent systems were tested until an adequate compounds separation was obtained. This information is useful to gain an idea about the chemical complexity of each extract.

2.5 Spectroscopic Study – UV absorptions

For each dried extract, a series of sample concentrations were prepared in solvent mixtures that range from petroleum ether - methylene chloride to methylene chloride - methanol. The concentrations of the prepared samples were 500 ppm, 200 ppm, 100 ppm or 50 ppm. The samples were prepared at all concentrations depending on the plant's collected amount and their fractions' yields. For each study, a target was ran with the solvent system used to dissolve the extract. The area below each absorption curve was obtained from the curve's integration in the UV spectrum following the equation:

$$Area = \sum[(\lambda_1 - \lambda_2) \times \bar{A}]$$

where λ is the wavelength, with $\lambda_1 > \lambda_2$, and \bar{A} the average of studied absorbance.

3. RESULTS AND DISCUSSION

3.1 Collection of plant species

The collected plants belong to twelve different species and to eleven distinct families. The species were collected at one of the following altitudinal stages in the valley: High Andean prairie (from 4200 to 4800 m.a.s.l.), Yungas' Tundra (from 3600 to 4200 m.a.s.l.), Yungas' brow's mountain (from 2800 to 3600 m.a.s.l.) and Yungas (from 800 to 2800 m.a.s.l.) There is only one specimen belonging to the High Andean prairie, three from the Yungas' Tundra, eight found in the Yungas' brow's mountain and two appertain to the Yungas' region. All collected species present colorful organisms (flowers, fruits, leaves or aerial body), Figure 1.

It is relevant to highlight *Brachyotum microdon* and *Cobaea scandens* which have purple flowers and *Monnina bridgesii* for their blue flowers and fruits. These species were previously studied using a chemical reaction that mimics *Plasmodium falciparum*'s infection [7]. Only *Brachyotum microdon* inhibited the chemical infection. The compounds responsible for this activity were β -sitosterol, oleanolic acid, ursolic acid and corosolic acid [7]. In addition, Hultin *et al.* published the isolation of alkaloids from *Cobaea scandens* [22].

The species collected under chemio-taxonomic criterion were *Rumex acetosella* and *Fuchsia boliviana*. *Rumex acetosella* contains gallic acid, a phenolic constituent [23], while *Fuchsia boliviana* has gallic acid as well as anthocyanins that are responsible for the flowers coloring [24]. It is important to emphasize that the presence of phenolic compounds is related to plant coloration and, in some cases, is responsible for biological activities. The species collected based on the chemio-taxonomic information found in their genus were *Fuchsia boliviana*, *Senecio floccosus*, *Monnina bridgesii* and *Baccharis pentlandii*. The genus *Fuchsia* contains a series of highly hydroxylated aromatic rings such as carotenoids, flavonoids, flavonols [25] and anthocyanins [25], [26]. The genus *Senecio* presents a series of phenolic compounds like quinones, acid phenols [27], and flavonoids [27], [28], [29] which include splinter groups like flavonoid glycosides [30], flavones [31], flavonol glycosides [32] and flavonoid alkaloids [33]. Moreover, the *Monnina* genus reports flavonoids (like flavonol glycosides) [34] and xanthenes [35]. Finally, species belonging to the genus *Baccharis* are well studied and present a large quantity and diversity of phenolic compounds responsible for the plant's biological activities and pigmentation [36], [37], [38], [39].

The twelve plants that were collected are shown in Figure 1 while Table 1 presents the taxonomic information (family and specie) and the data acquired for each species at the collection (altitudinal ground, coordinates and altitude).



Figure 1 - Species Collected in the Zongo Valley – La Paz, Bolivia.

TABLE 1 - TAXONOMIC AND COLLECTION INFORMATION OF PLANTS COLLECTED IN THE ZONGO VALLEY

| Code of Collection | Family | Specie | Altitudinal Ground | Latitud (S) | Longitud (W) | Altitude [m.a.s.l.] |
|--------------------|------------------|---|----------------------------|-------------|--------------|---------------------|
| M.Z. 3020 | Juncaceae | <i>Distichia muscoides</i> Nees & Meyen | High Andean prairie | 16°20'39.4" | 068°09'11.8" | 4637 |
| M.Z. 3021 | Polygonaceae | <i>Rumex acetosella</i> L. | Yungas' tundra | 16°12'40.0" | 068°07'24.3" | 4031 |
| M.Z. 3022 | Polygonaceae | <i>Rumex acetosella</i> L. | Yungas' tundra | 16°11'45.7" | 068°07'46.6" | 3782 |
| M.Z. 3023 | Alstroemeriaceae | <i>Bomarea dulcis</i> (Hook.) Beauverd | Yungas' tundra | 16°11'45.7" | 068°07'46.6" | 3782 |
| M.Z. 3024 | Melastomataceae | <i>Brachyotum microdon</i> (Naudin) Triana | Yungas' brow's mountain | 16°10'14.9" | 068°08'02.8" | 3401 |
| M.Z. 3025 | Polygalaceae | <i>Monnina bridgesii</i> Chodat | Yungas' brow's mountain | 16°09'30.8" | 068°07'16.1" | 3142 |
| M.Z. 3026 | Asteraceae | <i>Baccharis pentlandii</i> DC. | Yungas' brow's mountain | 16°09'30.0" | 068°07'14.6" | 3104 |
| M.Z. 3027 | Campanulaceae | <i>Centropogon gloriosus</i> (Britton) Zahlbr | Yungas' brow's mountain | 16°09'16.6" | 068°07'07.7" | 3022 |
| M.Z. 3028 | Polygalaceae | <i>Monnina bridgesii</i> Chodat | Yungas' brow's mountain | 16°09'17.8" | 068°07'08.4" | 3028 |
| M.Z. 3029 | Ericaceae | <i>Orthaea boliviensis</i> B. Fedtsh & Basilevsk | Yungas' brow's mountain | 16°08'50.4" | 068°06'59.7" | 2891 |
| M.Z. 3030 | Onagraceae | <i>Fuchsia boliviana</i> Carrière | Yungas' brow's mountain | 16°08'34.4" | 068°06'57.3" | 2823 |
| M.Z. 3031 | Polemoniaceae | <i>Cobaea scandens</i> Cav. | Yungas | 16°06'47.0" | 068°04'39.2" | 2173 |
| M.Z. 3032 | Marcgraviaceae | <i>Souroubea fragilis</i> de Roos | Yungas | 16°03'44.2" | 068°01'02.3" | 1464 |
| M.Z. 3033 | Compositae | <i>Senecio floccosus</i> Britton | Yungas' brow's mountain | 16°08'55.7" | 068°07'01.2" | 2914 |

3.2 Extracts preparation

A total of seventy vegetal extracts were obtained, thirty five from the ethereal extraction and thirty five with the ethanolic procedure. In some cases, the yields of the obtained extracts were low and a second extraction was required to increase the amount of material to perform all the chemical and spectroscopic studies. Table 2 presents the summary of the extraction codes and the yield of each organ's extract.

3.3 Preliminary phytochemical tests

The extracts were submitted to a series of chemical reactions to identify the compounds' families. We performed two assays for the petroleum ether extracts while seven were ran for the ethanolic extracts. In each assay, between 50 to 100 mg of vegetal extract has been used or as indicated in the methodologies.

a. Preliminary tests for ethereal extracts

From the obtained 35 ethereal extracts only 2 were not assayed. Table 3 displays the results for the detection of flavonoids and carotenoids in the petroleum ether extracts. In this table, a "+" sign exhibits the presence of flavonoids or carotenoids in the studied extracts. The symbol "+/-" points out uncertainty, since the result has a faint coloration or because the initial extract's coloration has the color of the expected positive result. With only one test it is not convenient to claim the presence or absence of a metabolite. The symbol "-" shows a negative result. Finally, the notation NA (not available) shows that the test has not been performed due to the lack of extract

TABLE 2 - EXTRACTION CODES AND YIELDS OF PLANT'S EXTRACTS COLLECTED IN THE ZONGO VALLEY

| SPECIE | ORGAN'S CODE | PETROLEUM ETHER EXTRACT'S CODE | YIELD [%] | ETHANOL EXTRACT'S CODE | YIELD [%] |
|---|---------------|--------------------------------|-----------|------------------------|-----------|
| <i>Distichia muscoides</i> Nees & Meyen | MZ 3020AP | DMAP-1-2-EP1 | 0.67 | DMAP-1-5-E1 | 1.2 |
| | MZ 3020R | DMR-1-2-EP1 | 0.1 | DMR-1-5-E1 | 0.80 |
| <i>Rumex acetosella</i> L. | MZ 3022FI | RAFI-1-2-EP1 | 1.30 | RAFI-1-5-E1 | 0.2 |
| | MZ 3022S,L | RAS,L-1-2-EP1 | 0.80 | RAS,L-1-5-E1 | 2.8 |
| <i>Bomarea dulcis</i> (Hook.) Beauverd | MZ 3023FI | BDFI-1-3-EP1 | 0.5 | BDFI-1-5-E1 | 1.1 |
| | MZ 3023L,S | BDL,S-1-3-EP1 | 0.4 | BDL,S-1-5-E1 | 1.5 |
| <i>Brachyotum microdon</i> (Naudin) Triana | MZ 3024FI | BMFI-1-2-EP1 | 0.2 | BMFI-1-5-E1 | 9.9 |
| | MZ 3024L | BML-1-2-EP1 | 0.30 | BML-1-5-E1 | 0.4 |
| | MZ 3024S | BMS-1-2-EP1 | 0.05 | BMS-1-5-E1 | 1.1 |
| <i>Monnina bridgesii</i> Chodat | MZ 3025FI, Fr | MBFI,Fr-1-4-EP1 | 3.3 | MBFI,Fr-1-6-E1 | 7.3 |
| | MZ 3025L | MBL-1-4-EP1 | 0.75 | MBL-1-6-E1 | 4.4 |
| | MZ 3025S | MBS-1-4-EP1 | 0.3 | MBS-1-6-E1 | 3.7 |
| <i>Baccharis pentlandii</i> DC | MZ 3026FI | BPFI-1-2-EP1 | 0.4 | BPFI-1-6-E1 | 1.2 |
| | MZ 3026L | BPL-1-2-EP1 | 2.1 | BPL-1-6-E1 | 1.7 |
| | MZ 3026S | BPS-1-2-EP1 | 1.4 | BPS-1-6-E1 | 1.3 |
| <i>Centropogon gloriosus</i> (Britton) Zahlbr | MZ 3027FI | CGFI-1-3-EP1 | 0.7 | CGFI-1-6-E1 | 1.8 |
| | MZ 3027L | CGL-1-3-EP1 | 0.7 | CGL-1-6-E1 | 6.4 |
| | MZ 3027S | CGS-1-3-EP1 | 0.3 | CGS-1-6-E1 | 0.7 |
| <i>Orthaea boliviensis</i> B. Fedtsh & Basilevsk | MZ 3029Fr | OBFr-1-2-EP1 | 0.4 | OBFr-1-6-E1 | 4.9 |
| | MZ 3029L | OBL-1-2-EP1 | 0.6 | OBL-1-6-E1 | 2.2 |
| | MZ 3029S | OBS-1-2-EP1 | 0.7 | OBS-1-6-E1 | 1.9 |
| <i>Fuchsia boliviana</i> Carrière | MZ 3030FI | FBFI-1-4-EP1 | 2 | FBFI-1-7-E1 | 2.5 |
| | MZ 3030Fr | FBFr-1-4-EP1 | 0.9 | FBFr-1-7-E1 | 1.7 |
| | MZ 3030L | FBL-1-4-EP1 | 0.3 | FBL-1-7-E1 | 0.4 |
| | MZ 3030S | FBS-1-4-EP1 | 0.3 | FBS-1-7-E1 | 0.4 |
| <i>Cobaea scandens</i> Cav. | MZ 3031FI | CSFI-1-3-EP1 | 0.1 | CSFI-1-7-E1 | 2.5 |
| | MZ 3031Fr | CSFr-1-3-EP1 | 0.3 | CSFr-1-7-E1 | 2.1 |
| | MZ 3031L | CSL-1-3-EP1 | 0.9 | CSL-1-7-E1 | 0.7 |
| | MZ 3031S | CSS-1-3-EP1 | 0.6 | CSS-1-7-E1 | 1.6 |
| <i>Souroubea fragilis</i> de Roos | MZ 3032Fr | SoFFr-1-3-EP1 | 2.2 | SoFFr -1-7-E1 | 0.7 |
| | MZ 3032L | SoFL-1-3-EP1 | 1.3 | SoFL-1-7-E1 | 3.4 |
| | MZ 3032S | SoFS-1-3-EP1 | 0.6 | SoFS -1-7-E1 | 1.1 |
| <i>Senecio floccosus</i> Britton | MZ 3033FI | SFFI-1-3-EP1 | 2.8 | SFFI-1-7-E1 | 2.7 |
| | MZ 3033L | SFL-1-3-EP1 | 0.5 | SFL-1-7-E1 | 2.0 |
| | MZ 3033S | SFS-1-3-EP1 | 0.2 | SFS-1-7-E1 | 1.3 |

EP: Petroleum ether, E: Ethanol; AP: Aerial part; R: Root; FI: Flowers; Fr: Fruits; L: Leaves; S: Stems

Analyzing the results obtained from Table 3, the most important specie is *Orthaea boliviensis* whose leaves have both metabolites and their fruits have flavonoids. Other interesting species are: *Monnina bridgesii* whose fruits, flowers and stems present carotenoids and their leaves have flavonoids. It is also important to emphasize the presence of carotenoids in *Cobaea scandens*' leaves and in the flowers of *Senecio floccosus*. Finally, the flowers of *Rumex acetosella*, those of *Brachyotum microdon*, the fruits of *Cobaea scandens* and the stems of *Bomarea dulcis* all have flavonoids.

TABLE 3 - RESULTS OF THE PRELIMINARY PHYTOCHEMICAL TESTS- ETHEREAL EXTRACTS

| SPECIE | ORGAN'S CODE | ASSAY CODE | CAROTENOIDS | FLAVONOIDS |
|------------------------------|---------------|-------------|-------------|------------|
| <i>Distichia muscoides</i> | MZ 3020AP | DMAP-1EP | - | - |
| | MZ 3020R | DMR-1EP | - | NA |
| <i>Rumex acetosella</i> L. | MZ 3022FI | RAFI-1EP | +/- | + |
| | MZ 3022S,L | RAS,L-1EP | +/- | - |
| <i>Bomarea dulcis</i> | MZ 3023FI | BDFI-1EP | - | - |
| | MZ 3023L,S | BDL,S-1EP | +/- | + |
| <i>Brachyotum microdon</i> | MZ 3024FI | BMFI-1EP | - | NA |
| | MZ 3024L | BML-1EP | +/- | + |
| | MZ 3024S | BMS-1EP | - | NA |
| <i>Monnina bridgesii</i> | MZ 3025FI, Fr | MBFI,Fr-1EP | + | +/- |
| | MZ 3025L | MBL-1EP | +/- | + |
| | MZ 3025S | MBS-1EP | + | NA |
| <i>Baccharis pentlandii</i> | MZ 3026FI | BPFI-1EP | - | NA |
| | MZ 3026L | BPL-1EP | +/- | - |
| | MZ 3026S | BPS-1EP | - | - |
| <i>Centropogon gloriosus</i> | MZ 3027FI | CGFI-1EP | - | NA |
| | MZ 3027L | CGL-1EP | - | - |
| | MZ 3027S | CGS-1EP | - | NA |
| <i>Orthaea boliviensis</i> | MZ 3029Fr | OBFr-1EP | - | + |
| | MZ 3029L | OBL-1EP | + | + |
| | MZ 3029S | OBS-1EP | +/- | - |
| <i>Fuchsia boliviana</i> | MZ 3030FI | FBFI-1EP | NA | NA |
| | MZ 3030Fr | FBFr-1EP | - | +/- |
| | MZ 3030L | FBL-1EP | +/- | NA |
| | MZ 3030S | FBS-1EP | - | - |
| <i>Cobaea scandens</i> | MZ 3031FI | CSFI-1EP | - | NA |
| | MZ 3031Fr | CSFr-1EP | - | + |
| | MZ 3031L | CSL-1EP | + | - |
| | MZ 3031S | CSS-1EP | - | NA |
| <i>Souroubea fragilis</i> | MZ 3032Fr | SoFFr-1EP | - | - |
| | MZ 3032L | SoFL-1EP | NA | - |
| | MZ 3032S | SoFS-1EP | - | - |
| <i>Senecio floccosus</i> | MZ 3033FI | SFFI-1EP | + | - |
| | MZ 3033L | SFL-1EP | - | NA |
| | MZ 3033S | SFS-1EP | NA | NA |

EP: Petroleum ether; AP: Aerial Part; R: Root, FI: Flowers; Fr: Fruits; L: Leaves; S: Stems.

b. Preliminary tests for alcoholic extracts

Seven phytochemical tests were performed in the 35 ethanol extracts. These seven tests will cover 13 types of secondary metabolites, some of which are very specific. Table 4 displays the results of the detection of flavonoids, phenolic compounds, anthraquinones, isoflavones, anthocyanins, anthocyanidins, tannins, chalcones, coumarins, flavones, flavonols, quinones and sterols.

In table 4, a “ + ” sign shows the presence of the evaluated secondary metabolite. The symbol “ +/- ” points out uncertainty, since the result has a solution with a faint coloration or precipitate or because the initial extract's appearance is similar to the expected positive result. With only one test it is not convenient to claim the presence or absence of a metabolite. The symbol “ - ” implies a negative result. The notation “ ? ” indicates that is not possible to evaluate the result of the test since the positive result, a yellow solution, is camouflaged or covered by the initial red color of the extract.

Analyzing the obtained results from Table 4, we highlight the following observations:

- All assayed species present phenolic compounds. It is important to emphasize the results found in *Brachyotum microdon*, *Fuchsia boliviana* and *Senecio floccosus* whose blue solutions suggest the presence of a phenolic compound with a distinctive skeleton.
- The species that have flavonoids are *Brachyotum microdon*, *Monnina bridgesii*, *Baccharis pentlandii*, *Orthaea boliviensis*, *Cobaea scanden*, *Distichia muscoide*, *Fuchsia boliviana* and *Souroubea fragilis*.
- Among the flavonoids, the isoflavones stand out because they have a peculiar structural skeleton compared to the other molecules of this family. Among the studied species, *Bomarea dulcis*, *Orthaea boliviensis*, *Cobaea scanden*, *Fuchsia boliviana*, and *Senecio floccosus* gave positive results on the isoflavones test.
- *Brachyotum microdon* is the only specie that presents anthocyanins. The presence of this molecule is confirmed with the positive results in the phenols and flavonoids tests. This last test detects the presence of the base skeleton of anthocyanins which is close to that found in reduced flavanes in the heterocycle ring.
- The species that contain anthraquinones are *Brachyotum microdon*, *Orthaea boliviensis*, *Cobaea scandens*, *Fuchsia boliviana* and *Souroubea fragilis*. The positive results with the chalcones and/or quinones tests confirm the presence of anthraquinones in the studied plants.

3.4 Spectroscopic studies

To study the photo-protector potential of the plant extracts, spectroscopic studies were carried out using a UV-VIS spectrophotometer and a wave length window between 290 to 500 nm. The maximum absorbance's wave length in each sample was registered for comparison purpose to UV-A and UB-B radiations.

a. UV analysis for ethereal extracts

The 35 ethereal extracts were studied at 200 ppm in petroleum ether-methylene chloride solvent mixtures. Some samples were also ran at 500 and 100 ppm depending on the extracts amount. Table 5 presents the summary of the ethereal extracts' ultraviolet absorptions. In this table, we observe that the flowers of *Senecio floccosus* present maximum absorbances at 290 nm (2.115) and 370 nm (3.001) corresponding to UV-B and UV-A regions, respectively. Other species that have shown important absorptions in the UV-B region are *Distichia muscoides* and *Rumex acetosella*.

b. UV analysis for ethanolic extracts

Based on the work done in the ethereal fractions, we decided to evaluate the UV absorption properties of the ethanol extracts at 100 ppm. Some ethanolic extracts were also ran at 200 ppm to increase their maximum absorbance. Table 6 presents the summary of the ethanol extracts ultraviolet absorptions at 100 ppm.

TABLE 4- RESULTS OF THE PRELIMINARY PHYTOCHEMICAL TESTS- ETHANOL EXTRACTS

| CODE | ANTHRAQUINONES | ISOFLAVONES | PHENOLS | FLAVONOIDS | FLAVONES | ANTHOCYANINS | ANTHOCYANIDINS | TANNINS | COUMARINS | CHALCONES | QUINONES | FLAVONES/ FLAVONOLS | STEROIDS |
|------------|----------------|-------------|---------|------------|----------|--------------|----------------|---------|-----------|-----------|----------|------------------------|----------|
| DMAP -1Et | - | - | + | + | ? | - | - | + | - | - | - | - | - |
| DMR -1Et | - | - | + | - | +/- | - | - | + | - | + | + | +/- | - |
| RAFI -1Et | - | - | + | +/- | - | - | - | + | - | + | + | + | - |
| RASL-1Et | - | - | + | - | +/- | - | - | + | +/- | +/- | +/- | + | - |
| BDFI-1Et | - | + | + | - | +/- | - | - | + | - | + | + | +/- | - |
| BDLS-1Et | - | - | + | - | +/- | - | - | - | - | + | + | +/- | - |
| BMFI-1Et | + | - | + | + | ? | +/- | - | + | - | + | + | + | - |
| BML - 1Et | - | - | + | + | ? | - | - | + | - | - | - | +/- | - |
| BMS - 1Et | - | - | + | - | - | - | - | + | - | + | + | + | - |
| MBFrFl-1Et | - | - | + | + | ? | - | - | - | - | - | - | + | - |
| MBL-1Et | - | - | +/- | - | - | - | - | + | - | + | + | + | - |
| MBS-1Et | - | +/- | + | - | +/- | - | - | + | - | + | + | +/- | - |
| BPFI -1Et | - | - | + | + | ? | - | - | + | - | + | + | +/- | - |
| BPL-1Et | - | - | +/- | - | +/- | - | - | - | - | + | + | - | - |
| BPS - 1Et | - | - | + | - | - | - | - | + | - | + | + | +/- | +/- |
| CGFL -1Et | - | - | + | - | - | - | - | + | - | + | + | +/- | - |
| CGL -1Et | - | - | + | - | - | - | - | + | - | - | - | +/- | - |
| CGS -1Et | - | - | + | - | + | - | - | + | - | + | + | +/- | - |
| OBFr - Et | +/- | + | + | + | ? | - | - | - | + | +/- | +/- | +/- | +/- |
| OBL-1Et | + | - | + | - | - | - | - | + | - | + | + | +/- | +/- |
| OBS - 1Et | - | - | + | + | ? | - | - | + | - | - | - | - | - |
| FBFI - 1Et | + | + | + | - | + | - | - | + | - | +/- | +/- | +/- | - |
| FBFr -1 Et | - | +/- | + | - | - | - | - | - | - | + | + | +/- | - |
| FBL -1Et | - | - | + | + | ? | - | - | + | - | +/- | +/- | + | - |
| FBS -1Et | - | - | + | - | - | - | - | + | - | + | + | + | - |
| CSFI -1Et | + | + | + | - | + | - | - | - | - | + | + | +/- | - |
| CSFr -1Et | - | + | + | + | ? | - | - | + | + | - | - | + | - |
| CSL -1Et | - | - | + | - | - | - | - | + | - | + | + | +/- | +/- |
| CSS -1Et | - | + | + | - | - | - | - | +/- | + | +/- | +/- | +/- | - |
| SoFFr -1Et | - | - | + | - | +/- | - | - | + | - | +/- | +/- | - | - |
| SoFL -1Et | - | - | +/- | - | - | - | - | +/- | - | - | - | - | - |
| SoFS -1Et | + | - | + | + | + | - | - | + | - | +/- | +/- | +/- | +/- |
| SFFI -1Et | - | + | + | - | +/- | - | - | + | - | +/- | +/- | +/- | - |
| SFL -1Et | - | + | + | - | +/- | - | - | + | - | - | - | +/- | - |
| SFS - 1Et | - | - | + | - | - | - | - | + | - | +/- | +/- | + | - |

Et: Ethanol; AP: Aerial Part; R: Root, Fl: Flowers; Fr: Fruits; L: Leaves; S: Stems.

TABLE 5- UV MAXIMUM ABSORPTIONS OF ETHEREAL EXTRACTS AT VARIOUS CONCENTRATIONS

| SPECIE | COLLECTION'S CODE | TEST'S CODE | 500 ppm | | 200 ppm | | 100 ppm | |
|------------------------------|-------------------|-------------------|-----------------|--------------------|-----------------|--------------------|-----------------|--------------------|
| | | | WAVELENGTH [nm] | MAXIMUN ABSORBANCE | WAVELENGTH [nm] | MAXIMUN ABSORBANCE | WAVELENGTH [nm] | MAXIMUN ABSORBANCE |
| <i>Distichia muscoides</i> | MZ 3020AP | DMAP-1EP | 300 | 0.850 | 290 | 0.348 | 290 | 0.007 |
| | MZ 3020R | DMR-1EP | 250-280 | 3.000 | 360 | 0.017 | 330-340 | 0.076 |
| <i>Rumex acetosella</i> | MZ 3020R | RAF1-1EP | NA | NA | 250-280 | 3.000 | 250-280 | 3.000 |
| | MZ 3022FI | RASL-1EP | | | 290 | 0.245 | 340 | 0.056 |
| <i>Bomarea dulcis</i> | MZ 3023FI | BDF1-1EP | | | 290 | 0.541 | 290 | 0.369 |
| | MZ 3023LS | BDLS-1EP | | | 320 | 0.018 | 320-330 | 0.015 |
| <i>Brachyotum microdon</i> | MZ 3024FI | BMF1-1EP | 290 | 0.459* | 290 | 0.487 | 310 | 0.099 |
| | MZ 3024L | BML-1EP | | | 290 | 0.232 | 290 | 1.070 |
| | MZ 3024S | BMS-1EP | | | 290 | 0.490 | 290 | 0.216 |
| <i>Monnina bridgesii</i> | MZ 3025FI,Fr | BMF1Fr-1EP | | | 290 | 0.216 | 330-340 | 0.008 |
| | MZ 3025L | MBL-1EP | | | 290 | 0.407 | 290 | 0.127 |
| | MZ 3025S | MBS-1EP | | | 290 | 0.270 | 290 | 0.229 |
| <i>Baccharis petlandii</i> | MZ 3026FI | BPF1-1EP | 300 | 0.723 | 310 | 0.213 | | |
| | MZ 3026L | BPL-1EP | 290* | 0.846* | 290 | 0.482 | 290 | 0.402 |
| | MZ 3026S | BPS-1EP | | | 290 | 0.605 | | |
| <i>Centropogon gloriosus</i> | MZ 3027FI | CGFL-1EP | | | 290 | 0.465 | | |
| | MZ 3027L | CGL-1EP | | | 330-340 | 0.007 | 290 | 0.270 |
| | MZ 3027S | CGS-1EP | 290 | 0.350 | 290 | 0.148 | | |
| <i>Orthaea boliviensis</i> | MZ 3029Fr | OBFr-1EP | | | 290 | 0.118 | 295 | 0.422 |
| | MZ 3029L | OBL-1EP | | | 320 | 0.017 | 360 | 0.061 |
| | MZ 3029S | OBS-1EP | | | 290 | 0.068 | 350 | 0.012 |
| <i>Fuchsia boliviana</i> | MZ 3030FI | FBF1-1EP | | | 290 | 0.034 | 326 | 0.073 |
| | MZ 3030Fr | FBFr-1EP | | | 290 | 0.073 | 390 | -0.057 |
| | MZ 3030L | FBL-1EP | | | 290 | 0.320 | 320 | 0.103 |
| | MZ 3030S | FBS-1EP | | | 290 | 0.282 | 290 | 0.330 |
| <i>Cobaea scandens</i> | MZ 3031FI | CSF1-1EP | NA | NA | NA | NA | NA | NA |
| | MZ 3031Fr | CSFr-1EP | | | 290 | 0.102 | 340 | 0.083 |
| | MZ 3031L | CSL-1EP | | | 400 | 0.015 | 400 | 0.025 |
| | MZ 3031S | CSS-1EP | | | | | 300 | 0.126 |
| <i>Souroubea fragilis</i> | MZ 3032Fr | SoFFr-1EP | | | 290 | 0.281 | 296 | -0.137 |
| | MZ 3032L | SoFL-1EP | | | 290 | 0.196 | 320 | 0.197 |
| | MZ 3032S | SoFS-1EP | | | 290 | 0.038 | | |
| <i>Senecio floccosus</i> | MZ 3033FI | SFF1-1EP | | | 370 | 3.001 | 290 | 2.115 |
| | MZ 3033L | SFL-1EP | NA | NA | NA | NA | NA | NA |
| | MZ 3033S | SFS-1EP | NA | NA | NA | NA | NA | NA |

EP: Petroleum ether; AP: Aerial Part; R: Root, FI: Flowers; Fr: Fruits; L: Leaves; S: Stems.

*: Samples tested at 400 ppm. NA: Not assayed due to lack of extract

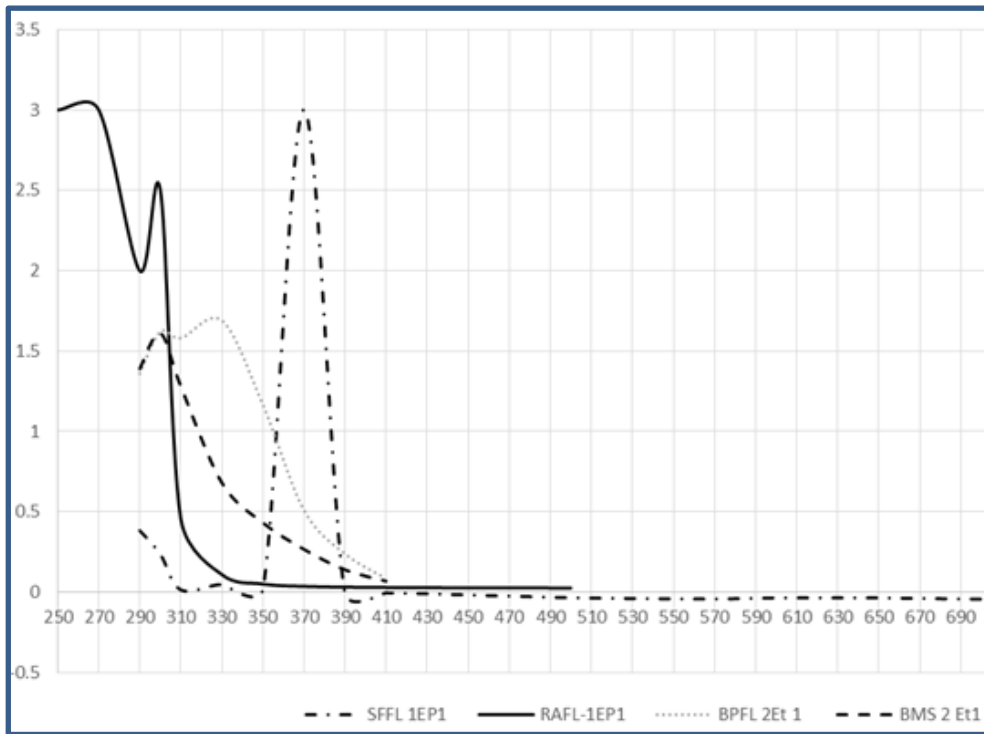
Analyzing the results obtained from Table 6, we observed that the species with important absorbances in the region of UV-B are: the flowers of *Rumex acetosella*, the flowers and stems of *Brachyotum microdon* whose flowers have greater absorbance; the flowers of *Baccharis pentlandii*, the stems of *Orthaea boliviensis*; the leaves of *Fuchsia boliviana* and the three organs of the studied *Souroubea fragilis* whose leaves and stems have greater absorbances. The species that absorb near the region of UV-A are: the leaves and flowers of *Baccharis pentlandii*; the leaves of *Souroubea fragilis*; the flowers and leaves of *Senecio floccosus* and the leaves of *Orthaea boliviensis*. Of these plants, the most promising species are the leaves of *Baccharis pentlandii* and the flowers of *Senecio floccosus* because their extracts absorb the dangerous UV-A radiation with a high absorbance (3.000 at 100 ppm). Among the analyzed plants we highlight the leaves of *S. fragilis* and the flowers of *B. pentlandii* because they present maximum absorbances in both studied wave lengths (UV-A and UV-B).

TABLE 6 - UV MAXIMUM ABSORPTIONS OF ETHANOLIC EXTRACTS AT 100 PPM

| SPECIE | COLLECTION'S CODE | TEST'S CODE | 100 ppm | |
|------------------------------|-------------------|-------------------|-----------------|--------------------|
| | | | WAVELENGTH [nm] | MAXIMUN ABSORBANCE |
| <i>Distichia muscoides</i> | MZ 3020AP | DMAP-1Et | 290 | 0.768 |
| | MZ 3020R | DMR-1Et | 290 | 0.608 |
| <i>Rumex acetosella</i> | MZ 3020R | RAF1-1Et | 290* | 3.000* |
| | MZ 3022FI | RASL-1Et | 320 | 0.783 |
| <i>Bomarea dulcis</i> | MZ 3023Fl | BDF1-1Et | 350 | 0.540 |
| | MZ 3023LS | BDLS-1Et | 340 | 0.569 |
| <i>Brachyotum microdon</i> | MZ 3024Fl | BMF1-1Et | 250-270 | 3.000 |
| | MZ 3024L | BML-1Et | 340* | 0.122* |
| | MZ 3024S | BMS-1Et | 300* | 1.610* |
| <i>Monnina bridgesii</i> | MZ 3025Fl,Fr | BMF1Fr-1Et | 340 | 0.244 |
| | MZ 3025L | MBL-1Et | 340 | 1.058 |
| | MZ 3025S | MBS-1Et | 362 | 0.266 |
| <i>Baccharis petlandii</i> | MZ 3026Fl | BPF1-1Et | 300/330* | 1.620/1.690* |
| | MZ 3026L | BPL-1Et | 320 | 3.000 |
| | MZ 3026S | BPS-1Et | 296 | 1.085 |
| <i>Centropogon gloriusus</i> | MZ 3027Fl | CGFL-1Et | 302 | 0.417 |
| | MZ 3027L | CGL-1Et | 362 | 0.141 |
| | MZ 3027S | CGS-1Et | 235 | 1.761 |
| <i>Orthaea boliviensis</i> | MZ 3029Fr | OBFr-1Et | 330 | 0.607 |
| | MZ 3029L | OBL-1Et | 333 | 1.970 |
| | MZ 3029S | OBS-1Et | 270-280 | 3.000 |
| <i>Fuchsia boliviana</i> | MZ 3030Fl | FBF1-1Et | 362 | 0.367 |
| | MZ 3030Fr | FBFr-1Et | 362 | 0.114 |
| | MZ 3030L | FBL-1Et | 290* | 3.000* |
| | MZ 3030S | FBS-1Et | 290* | 0.315* |
| <i>Cobaea scandens</i> | MZ 3031Fl | CSF1-1Et | 270 | 1.239 |
| | MZ 3031Fr | CSFr-1Et | 360 | 0.113 |
| | MZ 3031L | CSL-1EtP | 270 | 2.017 |
| | MZ 3031S | CSS-1Et | 330 | 0.595 |
| <i>Souroubea fragilis</i> | MZ 3032Fr | SoFFr-1Et | 280 | 1.551 |
| | MZ 3032L | SoFL-1Et | 310-350/400 | 3.000/1.787 |
| | MZ 3032S | SoFS-1Et | 260-280 | 3.000 |
| <i>Senecio floccosus</i> | MZ 3033Fl | SFF1-1Et | 330-340 | 3.000 |
| | MZ 3033L | SFL-1Et | 330 | 2.475 |
| | MZ 3033S | SFS-1Et | 300 | 0.250 |

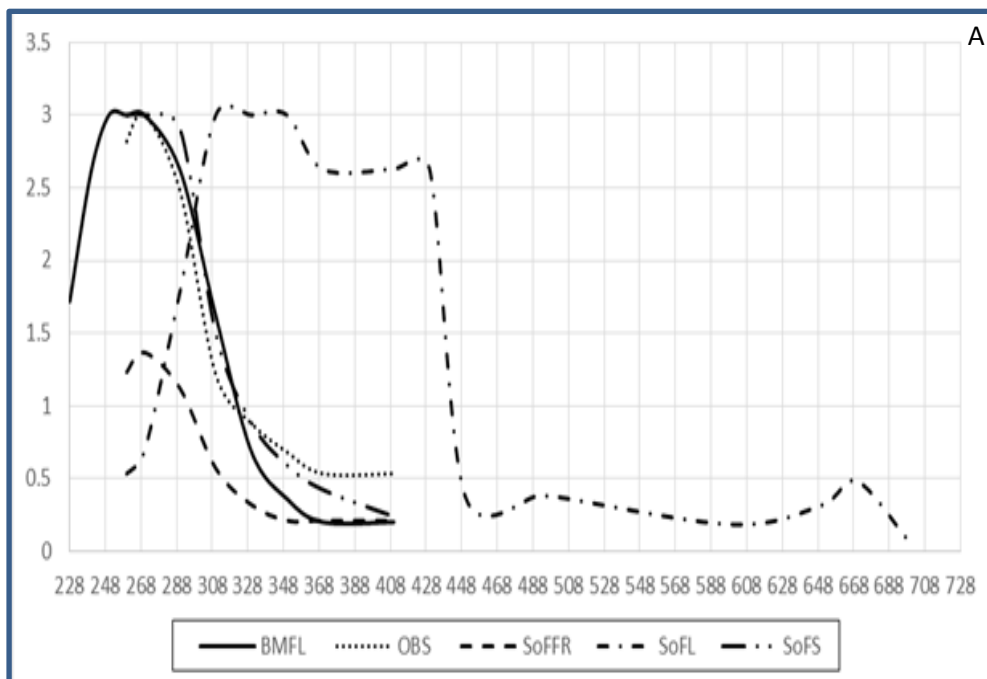
Et: Ethanol; AP: Aerial Part; R: Root, Fl: Flowers; Fr: Fruits; L: Leaves; S: Stems. *: Samples tested at 200 ppm

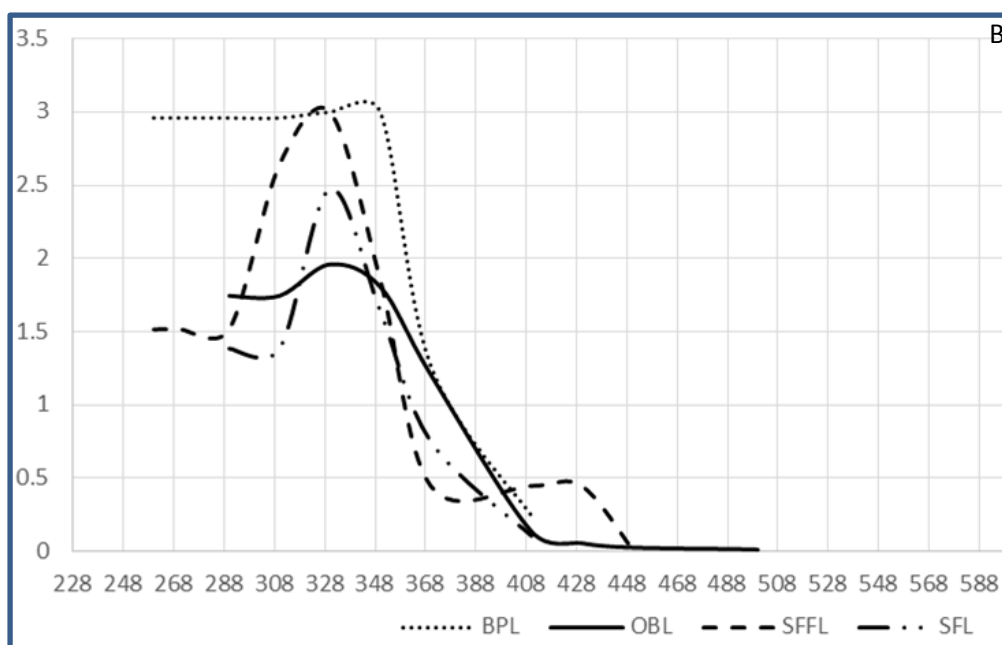
Figure 2 presents the absorption spectra of the important samples ran at 200 ppm. In this figure, the flowers of *Rumex acetosella* and *Senecio floccosus* stand out with the highest absorbances in the UV-B and UV-A regions, respectively.



EP: Petroleum ether extract; Et: Ethanolic extract; Fl: Flowers; S: Stems
Figure 2 - Comparison of the UV spectra of the studied extracts al 200 ppm.

The absorption spectra of the important samples ran at 100 ppm is shown in Figure 3A-B. In Figure 3 we can highlight the flowers of *Brachyotum microdon* absorbing at UV-B, the leaves of *Baccharis pentlandii* and the flowers of *Senecio floccosus* (both absorbing at UV-A), the stems of *Souroubea fragilis* and *Orthaea boliviensis* (both absorbing at UV-B) and the leaves of *Souroubea fragilis* absorbing at both UV- B and UV-A regions.





Fl: Flower; S: Stems; Fr: Fruits; L: Leaves

Figure 3 - Comparison of the UV spectra of the studied ethanolic extracts at 100 ppm.

Tables 7 and 8 show the areas under the absorption curves of the important extracts ran at 200 ppm and 100 ppm, respectively.

TABLE 7 - AREAS UNDER THE ABSORPTION CURVES OF THE PLANT EXTRACTS AT 200 PPM

| SPECIES | ORGAN | TOTAL (280- 400 nm) | UV B (280- 320 nm) | UV A (320- 400 nm) |
|-----------------------------|---------|------------------------|-----------------------|-----------------------|
| <i>Rumex acetosella</i> | Flowers | 42.91 | 42.91 | - |
| <i>Brachyotum microdon</i> | Stems | 49.05 | 49.05 | - |
| <i>Baccharis pentlandii</i> | Flowers | 108.89 | 30.9 | 77.99 |
| <i>Senecio floccosus</i> | Flowers | 64.51 | 4.34 | 60.17 |

TABLE 8- AREAS UNDER THE ABSORPTION CURVES OF THE PLANT EXTRACTS AT 100 PPM

| SPECIES | ORGAN | TOTAL (280- 400 nm) | UV B (280- 320 nm) | UV A (320- 400 nm) |
|-----------------------------|---------|------------------------|-----------------------|-----------------------|
| <i>Brachyotum microdon</i> | Flowers | 235.68 | 235.68 | - |
| <i>Orthaea boliviensis</i> | Stems | 120.75 | 120.75 | - |
| <i>Souroubea fragilis</i> | Fruits | 63.65 | 63.65 | - |
| | Leaves | 255.15 | 138.87 | 116.28 |
| | Stems | 156.84 | 156.84 | - |
| <i>Baccharis pentlandii</i> | Leaves | 310.3 | - | 310.3 |
| <i>Orthaea boliviensis</i> | Leaves | 104.89 | - | 104.89 |
| <i>Senecio floccosus</i> | Flowers | 169.65 | - | 169.65 |
| | Leaves | 103.71 | - | 103.71 |

3.5 Analysis of global results (Phytochemical tests, Chromatographic study, Spectroscopic data)

For comparative purposes, Table 9 presents a summary of the global results of the ethereal extracts of the plants collected in the Zongo Valley, while Table 10 shows those for the ethanolic extracts.

TABLE 9- GLOBAL RESULTS OF SPECIES COLLECTED IN THE ZONGO VALLEY. ETHEREAL EXTRACTS

| SPECIES | SAMPLE CODE | CAROTENOIDS | FLAVONOIDS | ABSORPTION UV B (200 ppm) [nm/Abs] | ABSORPTION UV B (100 ppm) [nm/Abs] | ABSORPTION UV A (200 ppm) [nm/Abs] | TLCs INFORMATION |
|------------------------------|-------------|-------------|------------|------------------------------------|------------------------------------|------------------------------------|-------------------------------|
| <i>Distichia muscoides</i> | DMAP-1EP | | | | | | |
| | DMR -1EP | | | 250 - 280/ 3.000* | | | Resolved |
| <i>Rumex acetosella</i> | RAFI -1EP | | + | 250-280/ 3.000; 300/2.5 | 250 - 280/ 3.000 | | Resolved |
| | RASL-1EP | | | | | | |
| <i>Bomarea dulcis</i> | BDFI-1EP | | | | | | |
| | BDLS-1EP | | + | | | | Resolved |
| <i>Brachyotum microdon</i> | BMFI-1EP | | | | | | |
| | BML - 1EP | | + | | | | Resolved, complex mixture |
| | BMS - 1EP | | | | | | |
| <i>Monnina bridgesii</i> | MBFIFr-1EP | + | +/- | | | | Resolved, possible flavonoids |
| | MBL-1EP | | + | | | | Resolved, complex mixture |
| | MBS-1EP | + | | | | | Resolved, complex mixture |
| <i>Baccharis pentlandii</i> | BPFI -1EP | | | | | | |
| | BPL-1EP | | | | | | |
| | BPS - 1EP | | | | | | |
| <i>Centropogon gloriosus</i> | CGFL -1EP | | | | | | |
| | CGL -1EP | | | | | | |
| | CGS -1EP | | | | | | |
| <i>Orthaea boliviensis</i> | OBFr - EP | | + | | | | Resolved, possible flavonoids |
| | OBL -1EP | + | + | | | | Resolved, possible flavonoids |
| | OBS - 1EP | | | | | | |
| <i>Fuchsia boliviana</i> | FBFI -1 EP | | | | | | |
| | FBFr -1 EP | | | | | | |
| | FBL -1EP | | | | | | |
| | FBS -1EP | | | | | | |
| <i>Cobaea scandens</i> | CSFI -1EP | | | | | | |
| | CSFr -1EP | | + | | | | Resolved, possible flavonoids |
| | CSL -1EtP | + | | | | | Not resolved |
| | CSS -1EP | | | | | | |
| <i>Souroubea fragilis</i> | SoFFr -1EP | | | | | | |
| | SoFL -1EP | | | | | | |
| | SoFS -1EP | | | | | | |
| <i>Senecio floccosus</i> | SFFI -1EP | + | | | 290/2.115 | 370/3.001 | Resolved |
| | SFL -1EP | | | | | | |
| | SFS - 1EP | | | | | | |

EP: Petroleum ether; AP: Aerial Part; R: Root, Fl: Flowers; Fr: Fruits; L: Leaves; S: Stems. *: Samples tested at 500 ppm

In Tables 9 and 10, the UV-A and UV-B absorptions with maximum absorbance above 1.5 can be appreciated, as well as the TLC (Thin Layer Chromatography) information of the relevant extracts and the phytochemical tests that gave clear positive results. In some cases, the results that gave “ +/- ” or “ ? ” data were corroborated by a positive result in the flavonoid test or by TLC.

Analyzing Table 9, the most important species are: *Rumex acetosella* (flowers) that presents a UV-B absorption and has flavonoids and *Senecio floccosus* (flowers) that absorb at both UV-A and UV-B regions and has carotenoids.

Analyzing Table 10, the most important species based on their UV-B absorption properties are: *Rumex acetosella* (flowers), *Brachyotum microdon* (flowers and stems), *Baccharis pentlandii* (flowers), *Orthaea boliviensis* (stems), *Fuchsia boliviana* (leaves) and *Souroubeo fragilis* (fruits, leaves and stems). Among the species that absorb in UV-A, we mention *Baccharis pentlandii* (flowers and leaves), *Orthaea boliviensis* (leaves), *Souroubea fragilis* (leaves) and *Senecio floccosus* (flowers and leaves). At this point, it is important to outline *B. pentlandii*, *O. boliviensis* and *S. fragilis* since they absorb both types of UV radiations (UV-A and UV-B). All species contain phenols and flavonoids that could be responsible for the registered absorbance. It is important to highlight *Brachyotum microdon* and *Orthaea boliviensis* because they have anthraquinones, molecules with orange coloration.

Table 11 presents a summary of the important plants along with their phytochemical information, type of UV absorption, and TLC data. We include *Monnina bridgesii* in this table because their fruits have a blue colorant that tinted paper and cardboard.

The plants presented in this work and were previously reported are *Brachyotum microdon*, *Monnina bridgesii*, *Rumex acetosella*, *Baccharis pentlandii*, *Fuchsia boliviana*, *Distichia muscoides*, *Cobaea scandens* and *Centropogon gloriosus* [40], [7]. In these publications, the plants' activity against *Plasmodium falciparum*, *Leishmania* sps., *Trypanosoma cruzi* and their response on the ferriprotoporphyrine bio-crystallization inhibition test (FBIT) were evaluated. Among these plants *B. microdon* and *R. acetosella* inhibited the mentioned crystallization and showed activity against *P. falciparum*. While *Monnina bridgesii* had important IC₅₀ values against the tested *Leishmania* species. In addition, the *Rumex acetosella*'s antioxidant activity was previously published as well as the presence of phenolic compounds, flavonoids and anthocyanins [41], [23]. Flavonoids were also found in *B. pentlandii* [37] and in *F. boliviana* [42], this last plant also has anthocyanins [24], [42]. Finally, *Bomarea dulcis* has only one taxonomic publication [43].


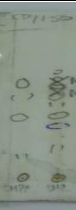

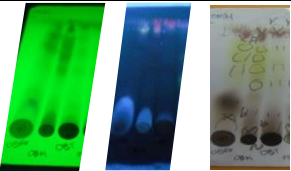



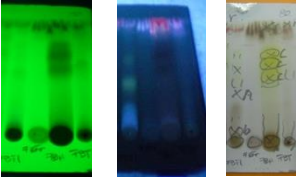

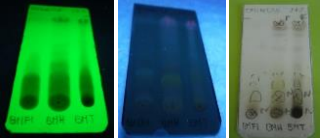

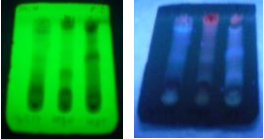

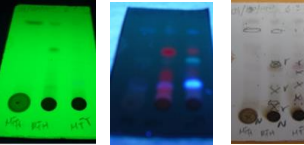

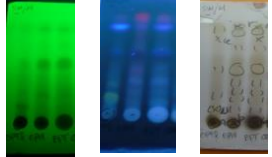

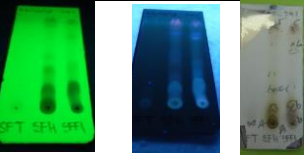
TABLE 10 - GLOBAL RESULTS OF SPECIES COLLECTED IN THE ZONGO VALLEY - ETHANOLIC EXTRACTS

| SAMPLE CODE | ANTRAQUINONS | ISOFLAVONES | PHENOLS | FLAVONOIDS | FLAVONES | ANTHOCYANINS | TANNINS | COUMARINS | CHALCONES | QUINONES | FLAVONES/FLAVONOLS | ABSORPTION UV B (200 ppm) [nm/Abs] | ABSORPTION UV B (100 ppm) [nm/Abs] | ABSORPTION UV A (200 ppm) [nm/Abs] | ABSORPTION UV A (100 ppm) [nm/Abs] | TLCs INFORMATION |
|-------------|--------------|-------------|---------|------------|----------|--------------|---------|-----------|-----------|----------|--------------------|------------------------------------|------------------------------------|------------------------------------|------------------------------------|------------------|
| DMAP -1Et | | | + | + | ? | | + | | | | | | | | | R, Flv |
| DMR -1Et | | | + | | +/- | | + | | + | + | +/- | | | | | R, Flv |
| RAFI -1Et | | | + | +/- | | | + | | + | + | + | 290/ 3.00 | | | | R, Flv |
| RASL-1Et | | | + | | +/- | | + | +/- | | | + | | | | | R, Flv |
| BDFI-1Et | | + | + | | +/- | | + | | + | + | +/- | | | | | R, Flv |
| BDLS-1Et | | | + | | +/- | | | | + | + | +/- | | | | | R, Flv |
| BMFI-1Et | + | | + | + | ? | +/- | + | | + | + | + | | 250-280-/3.00 | | | PR, MC, Flv |
| BML - 1Et | | | + | + | ? | | + | | | | | | | | | PR, MC, Flv |
| BMS - 1Et | | | + | | | | + | | + | + | + | 300/1.61 | | | | PR, MC, Flv |
| MBFrFl-1Et | | | + | + | ? | | | | | | + | | | | | PR, MC, Flv |
| MBL-1Et | | | +/- | | | | + | | + | + | + | | | | | PR, MC, Flv |
| MBS-1Et | | +/- | + | | +/- | | + | | + | + | +/- | | | | | PR, MC, Flv |
| BPFI -1Et | | | + | + | ? | | + | | + | + | | 300/1.62 | | 330/1.69 | | R, Flv |
| BPL-1Et | | | +/- | | +/- | | | | + | + | | | | | 330-350/3.00 | R, Flv |
| BPS - 1Et | | | + | | | | + | | + | + | +/- | | | | | R, Flv |
| CGFL -1Et | | | + | | - | | + | | + | + | | | | | | R |
| CGL -1Et | | | + | | | | + | | | | | | | | | R |
| CGS -1Et | | | + | | + | | + | | + | + | +/- | | | | | R |
| OBFr - Et | | + | + | + | ? | | | + | | | | | | | | R, MC, Flv |
| OBL -1Et | + | | + | | | | + | | + | + | +/- | | | | 333/1.97 | R, MC, Flv |
| OBS - 1Et | | | + | + | ? | | + | | | | | | 270-280 /3.00 | | | R, MC, Flv |
| FBFI - 1Et | + | + | + | | + | | + | | | | +/- | | | | | R, MC, Flv |
| FBFr -1 Et | | +/- | + | | | | | | + | + | +/- | | | | | NR, MC, Flv |
| FBL -1Et | | | + | + | ? | | + | | | | + | 290/3.00 | | | | R, MC, Flv |
| FBS -1Et | | | + | | | | + | | + | + | + | | | | | NR, MC, Flv |
| CSFI -1Et | + | + | + | | + | | | | + | + | +/- | | | | | NR, MC, Flv |
| CSFr -1Et | | + | + | + | ? | | + | + | | | + | | | | | NR, MC, Flv |
| CSL -1Et | | | + | | | | + | | + | + | +/- | | | | | NR, MC, Flv |
| CSS -1Et | | + | + | | | | | + | | | +/- | | | | | NR, MC, Flv |
| SoFFr -1Et | | | + | | | | + | | | | | | 270/1.37 | | | NR, MCP |
| SoFL -1Et | | | | | | | | | | | | | 310-330 /3.00 | | 330-350/3.00 | NR, MCP |
| SoFS -1Et | + | | + | + | + | | + | | | | +/- | | 260-280 /3.00 | | | NR, MCP |
| SFFI -1Et | | + | + | | +/- | | + | | | | +/- | | | | 325/3 | NR, MCP |
| SFL -1Et | | + | + | | +/- | | + | | | | +/- | | | | 330/2.48 | NR, MCP |
| SFS - 1Et | | | + | | | | + | | | | + | | | | | NR, MCP |

Flv: possible presence of flavonoids or related compounds; R: resolved TLC; PR: partially resolved TLC; MC: Complex mixture; MCP: polar complex mixture.

A SCREENING FOR NATURAL COLORANTS IN THE ZONGO VALLEY...

TABLE 11 - SUMMARY OF RESULTS OF IMPORTANT SPECIES COLLECTED AT THE ZONGO VALLEY

| SPECIES | PHYTOCHEMICAL INFORMATION | REGION OF UV ABSORPTION (100 ppm) | TLC | SPECIES | PHYTOCHEMICAL INFORMATION | REGION OF UV ABSORPTION (100 ppm) | TLC |
|--|--|-----------------------------------|--|---|---|-----------------------------------|---|
|  <i>Distichia muscoides</i> | Flavonoids, Phenols, Chalcones, Quinones | UV B ¹ |  EP/isoProp 8:2, Visualized: H ₂ SO ₄ |  <i>Orthaea boliviensis</i> | Carotenoids, Flavonoids, Phenols, Chalcones, Quinones, Anthraquinones, Isoflavones, Coumarins | UV B and UV A |  DMM 8:2 Visualiz: a 254 nm 365nm H ₂ SO ₄ |
|  <i>Rumex acetosella</i> | Flavonoids, Phenols, Flavones, Flavonols, Chalcones, Quinones | UVB ² |  EP/isoProp 8:2, Visualized: H ₂ SO ₄ |  <i>Fuchsia boliviana</i> | Anthraquinones, Flavonoids, Phenols, Isoflavones, Flavones, Tannins, Chalcones, Quinones, Flavonols | UV B ² |  DMM 8:2 Visualiz: a 254 nm 365nm H ₂ SO ₄ |
|  <i>Brachyotum microdon</i> | Flavonoids, Phenols, Tannins, Anthraquinones, Chalcones, Quinones, Flavones, Flavonols, Anthocyanins | UVB |  DM/Ace/W 1:5:4 Visualiz: a 254 nm 365nm H ₂ SO ₄ | | | | |
|  <i>Monnina bridgesii</i> | Carotenoids, Flavonoids, Phenols, Chalcones, Quinones, Flavones, Flavonols | - |  DMM 8:2 Visualiz: a 254 nm 365nm |  <i>Souroubea fragilis</i> | Anthraquinones, Flavonoids, Phenols, Flavones | UV B and UV A |  Chl/isoProp/W 5:3:2 Visualiz: a 254 nm 365nm H ₂ SO ₄ |
|  <i>Baccharis pentlandii</i> | Phenols, Flavonoids, Chalcones, Quinones | UV B ² and UV A |  DM/M 9.5:0.5 Visualiz: a 254 nm 365nm H ₂ SO ₄ |  <i>Senecio floccosus</i> | Carotenoids, Phenols, Isoflavones, Flavones, Flavonols | UV B and UV A |  Ace/isoProp/W 5:4:1 Visualiz: a 254 nm 365nm H ₂ SO ₄ |

EP: Petroleum ether; isoProp: isopropyl alcohol; DM: Methylene chloride; Ace: acetone; W: water; M: methanol, Chl: chloroform; *¹: 500 ppm *²: 200 ppm

4. CONCLUSIONS

Eleven plants were collected in the Zongo valley that could be used as colorants. Thirty five ethereal extracts and 35 ethanolic extracts were obtained and submitted to several assays to study their photo-protector potentials and their phytochemical composition.

The species that presented stain properties because they dye paper or cardboard are: the flowers of *Brachyotum microdon* (purple), the fruits and flowers of *Monnina bridgesii* (blue) and the stems of *Souroubea fragilis* (brown). Among these species, *Monnina bridgesii* tinted more easily the cellulose than the other plants. In addition, this plant has flavonoids and carotenoids that could present antioxidant properties. The flowers of *Brachyotum microdon* have anthocyanins that explain the color change, from purple to light blue, when the sample is exposed to different temperatures. Moreover, the flowers and stems of *B. microdon* have important UV-B absorptions and the presence of flavonoids shows a possible antioxidant property. Finally, this plant presents interesting molecules like anthraquinones and anthocyanins that could have biological and photo-protector activities, respectively. The entire plant of *Souroubea fragilis* presents important UV-B absorptions; however, their leaves stand out since they also have compounds that absorb UV-A radiation. Something peculiar is that the preliminary phytochemical tests of the leaves of *S. fragilis* do not show the typical compounds for the mentioned absorption (anthocyanins, flavonoids, anthraquinones) which shows a molecule not covered in our screening. It should not be ruled out that conjugated tannins could be responsible for this property. Another interesting organ in *S. fragilis* is the stem whose resin gets oxidized to orange when it is exposed to air. This behavior is found in antioxidant compounds, which get oxidized avoiding other molecules to get so. This property could be confirmed with the presence of flavonoids, flavones, flavonols and/or anthraquinones. The TLCs of these three plants present several compounds with difficult resolution. Among the three studied plants, *S. fragilis* is the most interesting for its possible photo-protector and antioxidant activities, however further studies must be performed.

Other species that could be further studied due to their photo-protector and possible antioxidant activities are: *Orthaea boliviensis*, *Senecio floccosus*, *Rumex acetosella*, *Baccharis pentlandii* and *Fuchsia boliviana*.

To our knowledge, there are no publications for *Souroubea fragilis*, *Senecio floccosus* and *Orthaea boliviensis* being this work the first one done and published for these species.

San Martin *et al.* have published studies of UV absorptions and phytochemical assays in *Baccharis genistelloides* [44]. This publication supports and validates our methods and results since in the studied *Baccharis pentlandii* we found the same absorption regions and phytochemical constituents as in the reported *Baccharis genistelloides*.

The colorant properties as well as the antioxidant activities of the most important plants reported here (*Brachyotum microdon*, *Monnina bridgesii* and *Souroubea fragilis*) are now being studied in our research group. With this work we encourage the evaluation, valorization, and further study of our natural resources with possible colorant, photo-protector and antioxidant activities.

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To Bolivian National Herbarium -La Paz (HNB).

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