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A SCREENING FOR ANTIOXIDANT SPECIES WITH PHOTO-PROTECTOR ACTIVITIES AT THE ZONGO VALLEY (BOLIVIA)

BÚSQUEDA DE ESPECIES ANTIOXIDANTES CON ACTIVIDADES FOTOPROTECTORAS EN EL VALLE DE ZONGO (BOLIVIA)

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ABSTRACT

Eleven plants were collected at the Zongo Valley to evaluate their antioxidant and photo-protector properties. In this paper we report a strong correlation between high antioxidant activity and strong UV-A and/or UV-B absorptions. The most active species, tested at 10µg/ml with the DPPH assay, were *Fuchsia boliviana (leaves)*, *Baccharis pentlandii* (flowers), *Rubus floribundus* (fruits), *Fuchsia boliviana* (flowers and fruits) *and Brachyotum microdon* (flowers). All the mentioned species have important UV- B and/or UV-A absorptions. This DPPH/UV technique could be used to preliminary screen vegetable samples and to select those with DPPH values above 83% and strong UV-A and/or UV-B absorptions. The chosen samples can then be evaluated with other more expensive *in vitro* assay (TEAC, ABTS or FRAP) to finally confirm their activities with the *in vivo* test. To our knowledge, this is the first time that the antioxidant properties of *Distichia muscoides*, *Souroubea fragilis*, *Brachyotum microdon*, *Monnina bridgesii*, *Baccharis pentlandii*, *Thibaudia crenulata*, *Siphocampylus tupaeformis*, *Cobaea scandens*, *Fuchsia boliviana* and *Rubus floribundus* are reported. In addition, this is the first time that *Siphocampylus tupaeformis* and *Thibaudia crenulata* are presented in a publication as well as the study of their photo-protector and antioxidant properties.

Keywords: Zongo Valley, Antioxidant Activity, Photo-Protector Property, UV-A and/or UV-B Absorption, *Distichia muscoides*, *Souroubea fragilis*, *Brachyotum microdon*, *Monnina bridgesii*, *Baccharis pentlandii*, *Thibaudia crenulata*, *Siphocampylus tupaeformis*, *Cobaea scandens*, *Fuchsia boliviana*, *Rumex acetocella* and *Rubus floribundus*.

RESUMEN

Once plantas fueron colectadas en el Valle de Zongo para evaluar sus propiedades antioxidantes y fotoprotectoras. En esta publicación presentamos una fuerte correlación entre una alta actividad antioxidantes y una fuerte absorción UV-A y/o UV-B. Las especies más activas, evaluadas a 10µg/ml con el ensayo DPPH, fueron *Fuchsia boliviana* (hojas), *Baccharis pentlandii* (flores), *Rubus floribundus* (frutas), *Fuchsia boliviana* (flores y frutas) y *Brachyotum microdon* (flores). Todas las especies mencionadas poseen importantes absorciones UV- B y/o UV- A. Esta técnica DPPH/UV puede ser usada para realizar un cernido preliminar de muestras vegetales y seleccionar aquellas con valores de DPPH superiores a 83% y fuertes absorciones UV-A y/o UV-B. Las muestras seleccionadas, luego pueden ser evaluadas con otro ensayo *in vitro* más costoso (TEAC, ABTS o FRAP) para finalmente confirmar sus actividades con el ensayo *in vivo*. A nuestro conocimiento, ésta es la primera vez que las actividades antioxidantes de *Distichia muscoides*, *Souroubea fragilis*, *Brachyotum microdon*, *Monnina bridgesii*, *Baccharis pentlandii*, *Thibaudia crenulata*, *Siphocampylus tupaeformis*, *Cobaea scandens*, *Fuchsia boliviana* y *Rubus floribundus* son reportadas. Adicionalmente, ésta es la primera vez que se presenta una publicación de *Siphocampylus tupaeformis* y *Thibaudia crenulata*, así como el estudio de sus propiedades fotoprotectoras y antioxidantes.

Palabras Clave: Valle De Zongo, Actividad Antioxidante, Propiedad Fotoprotectora, Absorciones UV-A y/o UV-B, *Distichia Muscoides*, *Souroubea Fragilis*, *Brachyotum Microdon*, *Monnina Bridgesii*, *Baccharis Pentlandii*, *Thibaudia Crenulata*, *Siphocampylus Tupaeformis*, *Cobaea Scandens*, *Fuchsia Boliviana*, *Rumex Acetocella* Y *Rubus Floribundus*.

1. INTRODUCTION

Cancer, cardiovascular disease, stroke, Alzheimer's disease, Parkinson's disease, Huntington's disease, neural disorders, neurodegenerative diseases, DNA damage, diabetes, arthritis, alcohol induced liver disease, ulcerative colitis and atherosclerosis are illness that do not differentiate countries, ages, income or medical coverage. Oxidative stress is suggested as the main responsible of these pathologies [1], [2], [3]. Living beings have very well-orchestrated oxidative processes that are vital to cells. In these metabolic processes, a series of reactive molecules intervene e.g.

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oxygenated/nitrogenated free radicals or neutral species. These reactive molecules, generated in low concentrations and in normal cell functioning, are involved in important regulatory processes (gene expression, cell proliferation and apoptosis). When free radicals are generated in excess, the body's antioxidant system is overwhelmed by these species which in turns oxidize and damage essential biomolecules (cell proteins, membrane lipids, carbohydrates, enzymes and DNA) triggering the illness mentioned above [4]. Natural antioxidants can be endogenous or exogenous molecules. The endogenous ones are biosynthesized by the organisms and are classified as nonenzymatic antioxidants (glutathione, coenzyme Q, bilirubin, alpha-lipoic acid, metallothionein, l-carnitine, melatonin, albumin, uric acid, ferritin, and antioxidant enzyme cofactors) or enzymatic antioxidants (superoxide dismutase, glutathione peroxidase, glutathione reductase, catalase, thioredoxins, peroxiredoxins and glucose-6-phosphate dehydrogenase). When the endogenous compounds cannot counteract the excessively produced free radicals, it is recommended to obtain antioxidants from foodstuffs, fruits or vegetables (exogenous antioxidants). Among the antioxidants taken as a dietary or preventive form from plants are carotenoids, phenolic acids, flavonoids, tocopherols, tannins, indole compounds, allyl sulfides, vitamins D, A, E, K, C or ascorbic acid [4], [3], [5]. Some of these compounds (like vitamin C and E) are only found in plants therefore its importance in the human diet.

Antioxidants from plants are playing an important role in the search of preventive and therapeutic compounds. Some antioxidant metabolites are present in small amounts in plants as part of their redox homeostasis, but others are produced - and in great amount- when the plant is under stress (high light intensity, heat, drought, pathogen attacks and anoxic conditions). Among the thousands of different types of secondary metabolites; tetraterpenes, carotenoids (belonging to the same family) and phenolic compounds show potent *in vitro* and *in vivo* antioxidant activities [6].

There are several methods to study the antioxidant potential of plants and their phytochemicals. With these methods plants are evaluated for their ability to act as reducing agents, hydrogen donors, singlet oxygen quenchers or metal chelators. Based on these methods, plants and phytochemicals can be classified as primary antioxidants (chain-breaking) or secondary antioxidants (preventive) [6]. Base on the type of inactivation mechanisms, antioxidants' reactions are classified into hydrogen atom transfer (HAT) and electron transfer (ET). HAT- based methods measure the capacity of the antioxidant to trap free radicals by hydrogen donation in order to form a stable molecule. These methods are more relevant to the radical chain breaking antioxidant capacity than the ET methods which measure the ability of an antioxidant to transfer an electron and reduce or stabilize the molecule. Moreover, these methods are classified as *in vitro* and *in vivo* assays. For the *in vitro* assay, there are 10 HAT methods reported [5], [6], [4], 5 ET assays [5], [6], [4], 3 assays that follow both HAT and ET mechanisms, 2 assays related to the chelation power of antioxidants [4], 4 assays based on lipidic mechanistic description [4] and 15 other methods based on chemiluminescence, fluorometry, amperometry, potentiometry or chromatographic techniques where some of the previous reagents are used [5], [4]. Among these *in vitro* methods we highlight those that follow two types of inactivation mechanism (mixed HAT and SET): DPPH (2,2-Diphenyl-1-picrylhydrazyl), ABTS ({2-2'-azinobis-(3-ethyl-benzothiazoline-6-sulphomic acid)}) and TEAC (Trolox Equivalent Antioxidant Capacity) assays. DPPH assay is easy, effective and it provides a rapid way to screen antioxidant samples; however, it is time consuming. ABTS is also a rapid test and it can be used in different media (pH); nevertheless, it is quite expensive [5].

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 at the northwest of La Paz city. 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. [7], [8]. It has been reported that 109 vegetal families and 158 species exist in the Zongo Valley [7]. This significant plant biodiversity has captured our attention to evaluate their possible attributes as antioxidants. Some species in the Zongo Valley were previously study to know their preliminary phytochemical composition [9], antiparasitic activities [10], [11] and photo-protector properties [9]. This publication will complete the information gathered for these species.

2. EXPERIMENTAL WORK

2.1. General

Spectroscopic studies were done on UV/VIS spectrophotometer Biochrom, model Libra S12. DPPH (2, 2-Diphenyl-1-picrylhydrazyl) was obtained from Aldrich. All supports and reagents used were obtained from Merck and Sigma.

2.2. Collection of plant species

Plant species were collected in the Zongo Valley on May 2016. 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 identified 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 solar radiation. The dried specimens were separated into their different organs, grinded, weighed and extracted with petroleum ether followed by ethanol 96%. The polar extracts were then submitted to the DPPH antioxidant assay and to the UV studies.

2.4. DPPH antioxidant assay

A solution of DPPH (2,2-Diphenyl-1-picrylhydrazyl) at 20mg/ml in methanol was prepared. The solution, that has a deep purple color, must be prepared to be used promptly and it must be protected from light because it is very reactive and decomposes with time. The amount of prepared DPPH solution depends on the number of samples to be evaluated. It is important to highlight that the DPPH solution not used must be discarded.

In order to evaluate the plant's antioxidant potential, methanolic extracts from the ethanolic dried extracts were prepared at 3 concentrations: 100µg/ml, 10µg/ml and 1µg/ml. To perform the assay, 1.5ml of DPPH solution is mixed with 750µL of each extract concentration. The mixed sample is incubated at room temperature for 5 minutes and then its absorbance is read at 517nm. The spectrophotometer is calibrated to zero with a methanol: water (2:1) sample. In addition, for each study, the following targets were prepared: a reagent's target with 1.5ml of DPPH and 750µL of unionized water and a sample's target with 750µL of extract and 1.5ml of methanol. The discoloration capacity or the free radical trapping capacity of the plant extract is calculated by the following equation:

$$Discoloration\ capacity = [1 - \frac{(Abs_{extract} - Abs_{sample's\ target})}{A_{reagent's\ target}}\ x\ 100$$

A result value equal to 100 corresponds to the maximum free radical trapping capacity and a value close to zero shows a reduced capacity [12].

2.5. Spectroscopic Study – UV absorptions

For each ethereal or ethanolic dried extract, a series of sample concentrations were prepared in solvent mixtures that range from petroleum ether to methylene chloride - methanol to methanol - water. The concentrations of the prepared samples were 100 ppm, 50 ppm or 25 ppm. The samples were prepared at all concentrations depending on the collected amount of the plant and their fractions' yields. For each study, a target was run 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:

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

where λ: wavelength, in which 1>2, $\bar{A}$: average of studied absorbances.

3. RESULTS AND DISCUSSION

3.1 Collection of plant species

The collected plants belong to eleven different species and 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 mountain brow (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, two from the Yungas' Tundra, six found in the Yungas mountain brow and two appertain to the Yungas' region. All collected species present colorful organisms (flowers, fruits, leaves or aerial body).

The eleven plants collected are shown in Figure 1 while Table 1 shows the taxonomic information (family and specie) and the data acquired at the collection site (altitudinal ground, coordinates and altitude) for each specie. Table 2 presents a summary of the bibliographic information of each of the collected specie as well as their traditional uses.

3.2 Extract preparation

A total of sixty-two vegetal extracts were obtained, thirty-one from the ethereal extraction and thirty-one with the ethanolic procedure. Table 3 shows the summary of the extraction codes and the yield of each organ's extract. It is important to highlight that for the antioxidant assays only the ethanolic extract were tested since the ethereal extracts were insoluble in the DPPH media. In addition, the tested extracts of *Brachyotum microdon* flowers where those obtained in a previous collection (2014) since in the collection trip for this publication (2016) we were unable to find this plant with inflorescence.

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

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

Code of Collection	Family	Specie	Altitudinal Ground	Latitude (S)	Longitude (W)	Altitude [m.a.s.l.]
R 4643	Juncaceae	*Distichia muscoides* Nees & Meyen	High Andean Praire	*16°20'39.4"*	068°09'11.8"	4452
R 4645	Polygonaceae	*Rumex acetosella* L.	Yungas' Tundra	*16*°11*'45.7"*	068°07'46.6"	3949
R 4646	Campanulaceae	*Siphocampylus tupaeformis* Zahlbr	Yungas' Tundra	*16*°09*'16.6"*	068°07'07.7"	3882
R 4649	Melastomataceae	*Brachyotum microdon* (Naudin) Triana	Yungas Mountain Brow	*16*°10*'14.9"*	068°08'02.8"	3401
R 4650	Polygalaceae	*Monnina bridgesii* Chodat	Yungas Mountain Brow	*16*°09*'620'*	068°07'362'	3162
R 4651	Compositae	*Baccharis pentlandii* DC.	Yungas Mountain Brow	*16*°09*'516'*	068°07'230'	3105
R 4652	Ericaceae	*Thibaudia crenulata* E. J, Remy	Yungas Mountain Brow	*16*°08*'643'*	068°06'970'	2966
R 4653	Onagraceae	*Fuchsia boliviana* Carrière	Yungas Mountain Brow	*16*°08*'583'*	068°06'955'	2821
R 4654	Rosaceae	*Rubus floribundus* Weihe	Yungas Mountain Brow	*16*°08*'583'*	068°06'955'	2821
R 4655	Polemoniaceae	*Cobaea scandens* Cav.	Yungas	*16*°07*'378''*	068°05'253'	2322- 2165
R 4656	Marcgraviaceae	*Souroubea fragilis* de Roon	Yungas	*16*°03*'679'*	068°01'061'	1450

TABLE 2- BIBLIOGRAPHIC INFORMATION OF THE COLLECTED SPECIES AT THE ZONGO VALLEY

Family	Specie	Collection Criterion	Traditional Use	Bibliographic Information
Juncaceae	*Distichia muscoides* Nees & Meyen	Altitudinal	- In Peru it is used as fuel [10].	• Geographical distribution and botanical, morphological and reproductive characteristics [11]. • It is under extinction danger due to global heating [12]. • Preliminary phytochemical information [13].
Compositae	*Baccharis pentlandii* DC.	Chemio-taxonomic	- It is used as an anti-inflammatory plant in South America [14]. - Leaves are used against cough, rheumatism and to heal scars. They produce pers-pirations and are antiseptic [15].	• Inactive against both *Plasmodium falciparum* test and against Ferriprotoporphyrin Biocrystalli-zation Inhibition test (FBIT), a chemical reaction related to antiplasmodial activity [15]. • Isolation of 3 highly oxygenated flavonoids with probable cytotoxic and apoptotic properties [16]. • Preliminary phytochemical information. Their flower and leaves absorb both UV B and UV A radiation [13].

TABLE 2 CONTINUED - BIBLIOGRAPHIC INFORMATION OF THE COLLECTED SPECIES AT THE ZONGO VALLEY

Ericaceae	*Thibaudia crenulata* E. J, Remy	Altitudinal	- None	• None
Campanulaceae	*Siphocampylus tupaeformis* Zahlbr	Altitudinal	- Leaves are antiseptic. Flowers and leaves are used against cardiac pain, palpitations and to heal infected wounds. Stems are used to treat nervous seizures [15].	• None
Marcgraviaceae	*Souroubea fragilis* de Roon	Organoleptic	- None	• Preliminary phytochemical information. Their fruits, stems and leaves absorb UV B radiation. Their leaves absorb both UV B and UV A radiation [13].
Melastomataceae	*Brachyotum microdon* (Naudin) Triana	Organoleptic	- Against venereal diseases [15].	• Active against *Plasmodium falciparum* test and against Ferriprotoporphyrin Biocrystallization In-hibition test (FBIT), a chemical reaction related to antiplasmodial activity [15]. • Identification of fatty alcohols and terpenoids (β-sitosterol, oleanolic acid, ursolic acid and corosolic acid) with FBIT activity [17]. • Preliminary phytochemical information. Their flower and stems absorb UV B radiation [13].
Polygalaceae	*Monnina bridgesii* Chodat	Organoleptic	- Leaves are used against dysentery. Together leaves and flowers are used against boils and for rheumatism [15].	• Inactive against both *Plasmodium falciparum* test and against Ferriprotoporphyrin Biocrystalli-zation Inhibition test (FBIT), a chemical reaction related to antiplasmodial activity [15]. • Preliminary phytochemical information [13].
Rosaceae	*Rubus floribundus* Weihe	Organoleptic	- It is used for the treatment of Mellitus' diabetes [18].	• Hypoglycemic activity and preliminary phytochemical assays [18]. • Hypoglycemic activity and identification of vitamin B5 as responsible for this activity [19]. • Physicochemical studies of the fruits and their changes when exposed to ultrasound processes and temperature [20]. • Flowers are used to treat cough and inflammations [21].
Polemoniaceae	*Cobaea scandens* Cav.	Organoleptic	- None	• Active against Ferriprotoporphyrin Biocrystalli-zation Inhibition test (FBIT), a chemical reaction related to antiplasmodial activity. Inactive against *Plasmodium falciparum* [15]. • Preliminary phytochemical information [13].
Onagraceae	*Fuchsia boliviana* Carrière	Chemio-taxonomic	- None	• Inactive against both *Plasmodium falciparum* test and against Ferriprotoporphyrin Biocrystalli-zation Inhibition test (FBIT), a chemical reaction related to antiplasmodial activity [15]. • Preliminary phytochemical information. Their leaves absorb UV B radiation [13].

TABLE 2 CONTINUED - BIBLIOGRAPHIC INFORMATION OF THE COLLECTED SPECIES AT THE ZONGO VALLEY

Polygonaceae	*Rumex acetosella* L.	Chemio-taxonomic	- It is used as astringent, spasmolytic and for its colagogenic action [22]. - Along with other herbs to detoxify and strengthen the body. As an antioxidant, anti-inflammatory and anti-cancer extract. As a stimulator of the immune system [23]. - Edible, leaves are cooked in soup in Poland [24], in Belarus [25] and in Turkey [26] [27]. - Leaves are used against hepatic infections. They are antiseptic, scar healer and relieve bruises and boils [15]. - Aerial parts are used against jaundice in Asia [28], Iran [29], and as febrifuge [30] [28]. - It is used to treat hypertension and diabetes [31]. - In Macedonia, to fill pies and in salads [32] while in Turkey the young leaves are eaten directly [33] [34]. - Used as an antioxidant, antifungal, antibacterial and anticancer treatment [35] [36].	• Anti-enzyme activity [37]. • Hepatoprotective activity [38]. • Nutritional studies and presence of heavy elements in their leaves [39]. • Allergic sensibility and its relationship with rhinitis and asthma [40]. • Total phenolic compounds [37] [41]. • Preliminary phytochemical information. Their flowers absorb UV B radiation [13]. • Antioxidant activity [42] [43]. • Presents a moderated anticoagulant activity [44]. • Related to the production of ethylene [45]. • Used to synthesize bio-nanoparticles and to evaluate its toxicity [46]. • Presents flavonoids, chalcons and phenolic acids. Shows antioxidant and antidiabetic activities. There are studies for the treatment of obesity [43]. • Isolation of Hyperin, a flavonoid, with antiulcer, antihyperglycemic, antiviral, cardioprotective, hepatoprotective, gastric-mucosal protective activities [47].

TABLE 3 - EXTRACTION CODES AND YIELDS OF PLANT'S EXTRACTS COLLECTED AT THE ZONGO VALLEY

FAMILY	GENUS	SPECIE	ORGAN CODE	YIELD OF ETHEREAL EXTRACT [%]	YIELD OF ETHANOLIC EXTRACT [%]
Juncaceae	Distichia	*Distichia muscoides* Nees & Meyen	DMAP	0.7	1.0
			DMR	0.3	1.6
Polygonaceae	Rumex	*Rumex acetosella* L.	RAFl	0.1	3.8
			RASL	1.0	4.7
Campanulaceae	Siphocampylus	*Siphocampylus tupaeformis* Zahlbr	STFl	0.8	2.5
			STL	1.8	2.6
			STS	0.8	4.8
Melastomataceae	Brachyotum	*Brachyotum microdon* (Naudin) Triana	BMFl*	0.2	9.9
			BML	0.6	5.4
			BMS	0.2	2.5
Polygalaceae	Monnina	*Monnina bridgesii* Chodat	MBFlFr	0.5	6.8
			MBL	0.8	3.3
			MBS	0.2	3.6
Compositae	Baccharis	*Baccharis pentlandii* DC	BPFl	1.4	3.1
			BPL	2.3	4.1
			BPS	0.8	0.9
Ericaceae	Thibaudia	*Thibaudia crenulata E. J, Remy*	TCFl	0.5	4.1
			TCS	0.1	3.9
			TCL	0.9	4.7
Onagraceae	Fuchsia	*Fuchsia boliviana* Carrière	FBFlFr	0.8	2.0
			FBL	0.3	0.7
			FBS	0.2	0.6
Rosaceae	Rubus	*Rubus floribundus Weihe*	RFFr	2.5	4.4
			RFL	1.4	8.9
			RFS	0.4	2.7
Polemoniaceae	Cobaea	*Cobaea scandens* Cav.	CSFl	1.1	2.9
			CSFr	3.2	3.8
			CSL	0.8	2.1
			CSS	0.2	2.6
Marcgraviaceae	Souroubea	*Souroubea fragilis* de Roon	SoFL	2.1	16.2
			SoFS	0.9	2.2

PE: Petroleum ether, E: Ethanol; AP: Aerial part; R: Root; Fl: Flowers; Fr: Fruits; L: Leaves; S: Stems. *Organ collected in 2014; the specie collected in 2016 did not have flowers.

3.3 DPPH antioxidant assay

The ethanolic extracts were evaluated with the DPPH test at 3 concentrations: 100, 10 and 1µg/ml. Table # 4 shows the results of these antioxidant tests. As it can be seen, most of the extracts present antioxidant activity at 100µg/ml; however, the selection of interesting samples must be done at lower concentrations as compared with the control (ascorbic acid). Therefore, the extracts with interesting results (with activities close to the control at 10µg/ml) are: *Baccharis pentlandii* (flowers with 91.3% of free radicals trapping capacity), *Rubus floribundus* (fruits, 90.2%), *Fuchsia boliviana* (flowers and fruits, 86.7%), *Brachyotum microdon* (flowers, 82.5%) and *Thibaudia crenulate* (leaves, 82.1%). Nevertheless, *Fuchsia boliviana* (leaves) present a higher activity (93.2%) compared to the control (ascorbic acid with 90.0%). These species could have their minimum free radical trapping concentration between 10 and 1µg/ml presenting promising antioxidant activities. In addition, *Rumex acetosella*, *Thibaudia crenulate* and *Fuchsia boliviana* present good antioxidant activities in various organs. Among these species, the genus *Baccharis* has reported important antioxidant activities which validates our assays and presents this genus as a natural control for antioxidant tests [48], [49], [50], [51], [52], [53].

3.4 Spectroscopic studies

It has been reported that HPLC and spectrophotometric techniques (UV-Vis) are applied to monitor assays used to analyze antioxidant capacities [4]. Based on this work, we have studied the absorption potential of the collected plants and their photoprotective activities in the regions of 280 to 320nm (UV-B) and between 320 to 400nm (UV-A). These spectroscopic studies were carried out using a UV-VIS spectrophotometer and a wavelength window between 200 to 500 nm. Table # 5 presents the samples that have important absorptions in the UV-A and UV-B regions. Some samples were run at a smaller window than 200 to 500 nm and the new window is specified in Table #5. For exemplification purposes, the UV-B absorptions are presented in blue while the UV-A ones are in red.

a. UV analysis for ethereal extracts

The 31 ethereal extracts were studied at 100 ppm in petroleum ether-methylene chloride solvent mixtures. As shown in Table # 5, the species that present important UV-B absorptions are *Rumex acetosella* (flowers and stems), *Brachyotum microdon* (leaves and stems), *Monnina bridgessi* (flowers/fruits), *Fuchsia boliviana* (flowers/fruits), *Thibaudia crenulata* (leaves) and *Rubus floribundus* (fruits).

b. UV analysis for ethanolic extracts

The 31 ethanolic extracts were studied at 100 ppm, some of the samples were also tested at 50 and 25 ppm depending on the plant's collected amount. As shown in Table # 5, it is important to highlight *Fuchsia boliviana* (flowers/fruits) absorbing the UV-A and UV-B radiations. Other important species are *Baccharis pentlandii* and *Thibaudia crenulata* whose flowers absorb the dangerous UV-A radiation. The species that presents interesting UV-B absorptions are: *Brachyotum microdon* (flowers), *Monnina bridgessi* (flowers/fruits), *Siphocampylus tupaeformis* (flowers and leaves), *Fuchsia boliviana* (leaves), *Souroubea fragilis* (stems), *Cobaea scandens* (flowers, fruits, leaves and stems) and *Rubus floribundus* (fruits, leaves and stems).

3.5 Analysis of global results (Antioxidant activity and Spectroscopic data)

For comparative purposes, Table #6 presents a summary of the global results of the samples with antioxidant activity and their corresponding UV absorptions. As can be seen in this table, there is a strong correlation between the antioxidant activity of a sample and its UV absorptions. Samples at 10µg/ml with free radical trapping capacities above 83% present intense UV- A or/and UV- B absorptions. In Table #6 and for exemplification purposes, DPPH activities above 83% are highlighted in green, UV-B absorptions in blue and UV-A absorptions in red. We can highlight the leaves of *Fuchsia boliviana* and the flowers of *Baccharis pentlandii* that present the highest DPPH activity and the highest UV-B and UV-A absorptions, respectively. The fruits of *Rubus floribundus* are also important for having a DPPH activity above 90% and a high abortion (3.000) in the UV-B region. Figures #2 and #3 show the absorption spectra of the active samples. In Figure #2 we can appreciate the spectra of *Fuchsia boliviana* (leaves), *Baccharis pentlandii* (flowers), *Rubus floribundus* (fruits) and *Brachyotum microdon* (flowers) all run at 100 ppm. Figure #3 presents the UV absorptions of *Fuchsia boliviana* (flowers and fruits) assessed at 100ppm and 50ppm. This specie also has a very high DPPH value (91%) and strong absorptions in both UV-B and UV-A regions. Finally, Table #7 presents the areas under the absorption curves of the most active plant extracts at 100 ppm.

TABLE 4 - ANTIOXIDANT ACTIVITY OF ETHANOLIC EXTRACTS FROM PLANTS COLLECTED AT THE ZONGO VALLEY

SAMPLE / [µg/ml]		**FREE RADICAL TRAPPING CAPACITY [%]**		
		[100]	[10]	[1]
Distichia muscoides	DMAP	57.3	5.8	0.8
	DMR	47.3	2.3	-
Rumex acetosella	*RAFl*	92.9	72.7	15.3
	RAS,L	95.6	78.2	3.3
Souroubea fragilis	*SoFS*	92.1	31.4	5.5
	SoFL	90.4	66.8	9.7
Brachyotum microdon	*BMFl*	86.7	82.5	32.5
	BML	91.7	23.9	2.8
	BMS	94.1	46.6	3.3
Monnina bridgesii	*MBFlFr*	83.4	10.5	-
	MBL	94.7	51.6	1.6
	MBS	93.3	37.0	2.8
Baccharis pentlandii	*BPFl*	94.2	91.3	26.5
	BPL	87.3	25.7	6.5
	BPS	93.2	41.2	-
Thibaudia crenulata	*TCFl*	NT	NT	NT
	TCL	92.1	82.1	3.1
	TCS	93.9	74.2	11.5
Siphocampylus tupaeformis	*STFl*	21.7	-	-
	STL	15.8	1.6	-
	STS	32.3	7.8	-
Cobaea scandens	*CSFl*	74.5	23.4	-
	CSFr	40.0	13.1	10.4
	CSL	47.7	11.6	12.8
	CSS	22.4	-	-
Fuchsia boliviana	*FBFlFr*	95.0	86.7	14.5
	FBL	96.3	93.2	44.6
	FBS	94.9	38.3	11
Rubus floribundus	*RFFr*	93.9	90.2	20.7
	RFL	89.6	47.5	3.9
	RFS	NT	NT	NT
Ascorbic Acid (control)	AA	98.2	90.0	8.6

Coding: R: Roots; Fl: Flowers; L: Leaves; S: Stems.

TABLE 5 - EXTRACTS FROM PLANTS COLLECTED AT THE ZONGO VALLEY WITH IMPORTANT ABSORPTION IN THE UVB AND UVA REGIONS

		ETHEREAL EXTRACTS			ETHANOLIC EXTRACTS		
SPECIE	**ASSAY CODE**	**CONCENTRATION**	WAVE LENGHT [nm]	MAXIMUM ABSORBANCE	**CONCENTRATION**	WAVE LENGHT [nm]	MAXIMUM ABSORBANCE
					100 ppm	329 - 331 – 333-350$^{\circ,+}$	3 – 3 – 2.890-2.100
Baccharis pentlandii	BPFl				50 ppm	332	0.827
	RAFl	100 ppm	280 - 295	3 -1.355			
Rumex acetosella L.	RAS,L	100 ppm	256 – 276*	3			
	BMFl				100 ppm	308 - 310$^{\circ,+}$	1.177 – 1.143
	BML	100 ppm	200-280; 300*	3; 1.051			
Brachyotum microdon Naudin T	BMS	100 ppm	280 – 295	3 - 1.555			
Monnina bridgesii Chodat	MBFlFr	100 ppm	220 - 280 -295*	3 – 3 - 1.354	100 ppm	290-296^{+}	3
					100 ppm	290$^{+,\circ\circ}$	3
					50 ppm	298 - 300	1.123 – 1.000$^{\neq}$
	FBL				25 ppm	300	1.175
		100 ppm	225 – 280 – 290**	3 – 3 – 1.881	100 ppm	289- 290$^{+,\circ\circ}$	3 – 2.994
					50 ppm	250-298^{+}	1.988- 1.134
Fuchsia boliviana Carrière	FBFlFr				25 ppm	400$^{++,a}$	1.994
Souroubea fragilis	SoFS				50 ppm	280	1.810
	TCFl				100 ppm	330	0.857
Thibaudia crenulata E. J. Remy	TCL	100 ppm	230 – 275 – 280 -295	3 – 3 – 3 – 1.507			

TABLE 5 CONTINUED - EXTRACTS FROM PLANTS COLLECTED AT THE ZONGO VALLEY WITH IMPORTANT ABSORPTION IN THE UVB AND UVA REGIONS

	CSFl				50 ppm	280 - 300	1.851 – 1.068
	CSFr				50 ppm	280 - 300	1.834 – 0.850
	CSL				50 ppm	280	1.821
Cobaea scandens Cav.	CSS				50 ppm	280 - 298	1.783 – 1.050
	STFl				50 ppm	280[+]	1.800
Siphocampylus tupaeformis Z	STL				50 ppm	280 -300[+]	1.891 – 1.139
		100 ppm	220 – 280 - 295*	3 – 3 – 1.312	100 ppm	290	3.00
	RFFr				50 ppm	280 - 300	1.779 – 1.153[≠]
	RFL				50 ppm	280 - 300	1.813 – 1.004
Rubus floribundus Weihe	RFS				50 ppm	280 - 298	1.780 – 0.962

Coding: R: Roots; Fl: Flowers; L: Leaves; S: Stems.* Extract tested until 465 nm; ** Extract tested until 500 nm; [+] Extract tested until 400 nm; [++] Extract tested until 420 nm; °Extract tested from 300 nm; °°Extract tested from 289 nm; [≠] Approximate data, the absorption is found at the drop of the curve of the maximum absorption; [a] Extract at 100ppm and 50ppm were tested until 380nm.

TABLE 6 – SUMMARY OF THE GLOBAL RESULTS OF THE SAMPLES WITH ANTIOXIDANT ACTIVITY AND THEIR CORRESPONDING UV ABSORPTIONS

SPECIE	ORGAN	SAMPLE CODE	FREE RADICAL RAPPING CAPACITY [%] at 10µg/ml	UV ABSORPTIONS					
				100 ppm		50 ppm		25 ppm	
				Wavelength [nm]	Maximum Absorbance	Wavelength [nm]	Maximum Absorbance	Wavelength [nm]	Maximum Absorbance
Rumex acetosella	Flowers	RAFL	72.7	350	0.158[b]	350	0.116	NT	NT
	Stem and Leaves	RAS,L	78.2	350	0.446[b]	350	0.215	NT	NT
Souroubea fragilis	Leaves	SoFL	66.8	259[+]	0.028	ND	ND	ND	ND
Brachyotum microdon	Flowers	BMFl	82.5	308- 310	1.177- 1.143	-	-	NT	NT
Monnina bridgesii	Leaves	MBL	51.6	296[+]	0.296	296	0.236	NT	NT
Baccharis pentlandii	Flowers	BPFl	91.3	329- 331- 333- 350[°, +]	3- 3- 2.890- 2.100	325; 350	0.250; 0.602	NT	NT
Thibaudia crenulata	Flowers	TCFl	72.6	330[°, ++]	0.857	330	0.600	NT	NT
	Leaves	TCL	82.1	299[++]	0.358	ND	ND	ND	ND
	Stems	TCS	74.2	299[++, °°]	0.316	-	-	NT	NT
Fuchsia boliviana	Flowers and Fruits	FBFlFr	86.7	289- 290[a, °°]	3- 2.994	250- 300[a]	1.988- 0.993	400[++, a]	1.994
	Leaves	FBL	93.2	290[+, °°]	3	298- 300	1.123- 1.000[≠]	300	1.175
Rubus floribundus	Fruits	RFFr	90.2	290[++, °°]	3	280- 300	1.779- 1.153[≠]	ND	ND

[b] Spectrum ran until 470nm.; NT: Not tested since the previous assay did not give a significant absorption. [+] Extract tested until 400nm. [°] Extract tested from 300nm. [++] Extract tested until 420nm. [°°] Extract tested from 289nm. [a] Extracts at 100 and 50 ppm were tested until 380nm. [≠] Approximate data, the absorption is found at the drop of the curve of the maximum absorption. ND: Not defined since the equipment presented fluctuations.

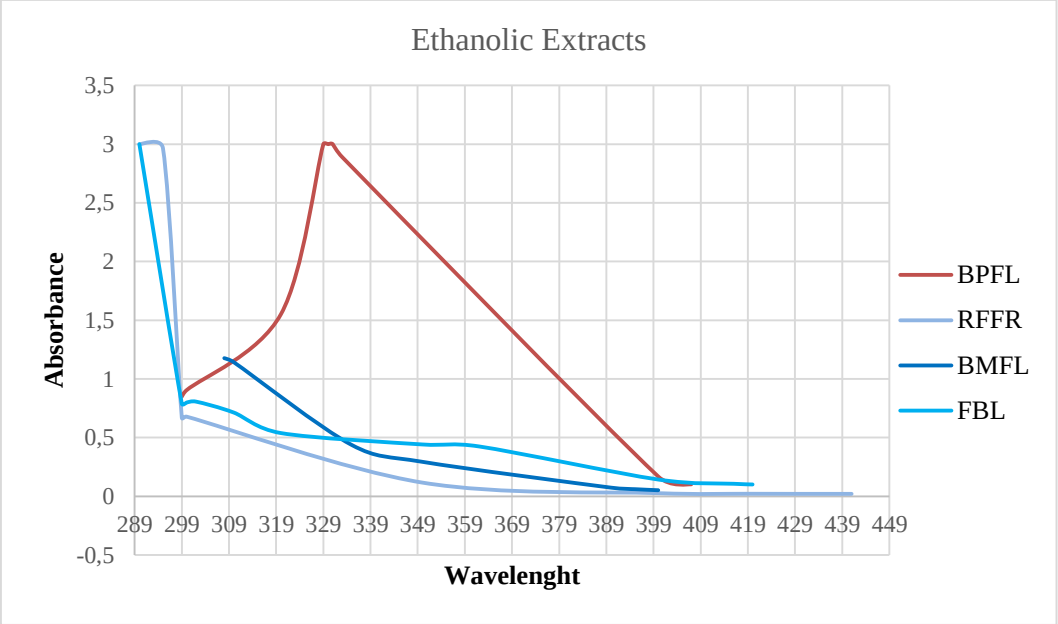
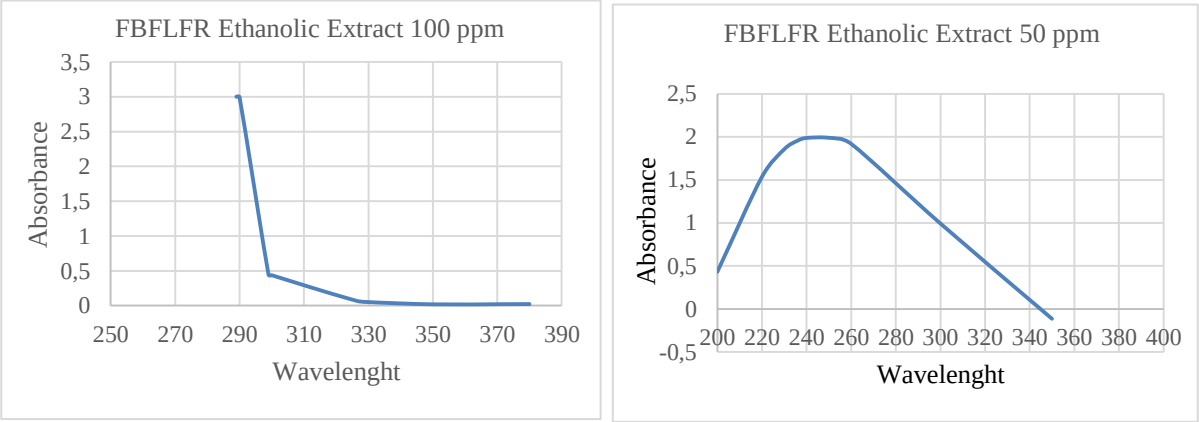


Figure 2: UV spectra of the most active antioxidant samples run at 100ppm.



*Spectra run until 350nm.

Figure 3: UV spectra of *Fuchsia boliviana* (flowers and fruits) assessed at 100 and 50ppm*.

TABLE 7 - AREAS UNDER THE ABSORPTION CURVES OF THE MOST ACTIVE PLANT EXTRACTS AT 100 PPM

SPECIES	ORGAN	TOTAL (280- 400 nm)	UV B (280- 320 nm)	UV A (320- 400 nm)
Brachyotum microdon	Flowers	14.27	2.32	11.95
Baccharis pentlandii	Flowers	204.61	23.14	181.47
Fuchsia boliviana	Flowers and Fruits	32.12	30.10	2.02
Fuchsia boliviana	Leaves	65.03	33.23	31.80
Rubus floribundus	Fruits	45.71	18.28	27.43

4. CONCLUSIONS

Eleven plants were collected at the Zongo valley that provided sixty two vegetal extracts, thirty one from the ethereal extraction and thirty one with the ethanolic procedure. The ethanolic extracts were essayed with the DPPH test in order to find antioxidant samples. In addition, the UV absorptions of all extracts were also evaluated. Twelve extracts showed free radical trapping capacity above 50% when tested at 10µg/ml. The most active samples (activities above 83%) presented strong UV-B (280- 320nnm) and/or UV-A (320- 400nm) absorptions. It is important to highlight the leaves of *Fuchsia boliviana* with 93.2% in the DPPH assay and with a strong absorption (3.000 A) in the UV-B region (290nm); the flowers of *Baccharis pentlandii* (91.3% DPPH, 329-331nm/3A); the fruits of *Rubus floribundus* (90.2% DPPH, 290nmn/3A); the flowers and fruits of *Fuchsia boliviana* (86.7% DPPH, 289-291nm/3A; 400nm/2A) and the flowers of *Brachyotum microdon* (82.5% DPPH, 308nm/1.2A). The ultraviolet absorptions of many of the species submitted in this paper were previously reported [13]. Comparing the results from both publications we can define the best season to collect the important species. For instance, *Brachyotum microdon* (flowers) should be collected in October (spring) while *Baccharis pentlandii* and *Fuchsia boliviana* in May (fall). Plants collected in the mentioned season have higher UV-A and/or UV-B absorption. In addition, all the active species present phenolic compounds, flavonoids, flavones and tannins in their composition [13]. The antioxidant activities of these types of compounds were previously presented in the work of Pisoschi et. al. [4]. Moreover, *Brachyotum microdon* (flowers) also presents anthraquinones, anthocyanins, chalcones and quinones while *Baccharis pentlandii* (flowers) and *Fuchsia boliviana* (flowers and fruits) have chalcones and quinones. Finally, *Fucshia boliviana* (flowers and fruits) also has anthraquinones and isoflavones [13]. These compounds could have biological activities since their molecular structures are close to the active flavonoids and their biosynthetic pathways are those of phenolic compounds (derived from shikimate) [54].

Most of the plants have antioxidant compounds as their main defense mechanisms; therefore, it is not surprising to find many positive results in antioxidant evaluations in vitro [6]; however, if the samples are evaluated at low concentrations (10 or 1µg/ml) the most powerful antioxidants can be detected. In this paper we report a strong correlation between antioxidant activity evaluated by DPPH and UV absorptions. This DPPH/UV technique could be used to preliminary screen vegetable samples and select those with DPPH values above 83% and strong UV-A or UV-B absorptions. The chosen samples, then can be evaluated with other more expensive in vitro assay (TEAC, ABTS or FRAP) to finally confirm their activities with the in vivo tests.

To our knowledge, this is the first time that the antioxidant properties of *Distichia muscoides*, *Souroubea fragilis*, *Brachyotum microdon*, *Monnina bridgesii*, *Baccharis pentlandii*, *Thibaudia crenulata*, *Siphocampylus tupaeformis*, *Cobaea scandens*, *Fuchsia boliviana* and *Rubus floribundus* are reported. In addition, this is the first time that *Siphocampylus tupaeformis* and *Thibaudia crenulata* are presented in a publication.

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