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Mahamane IDI ISSA Abdoulahi
Laboratory of Natural Substances and Synthesis Organic, Abdou Moumouni University, Faculty of Science and Technology, Department of Chemistry, BP 10662 Niamey, Niger

Bakasso Sahabi
Laboratory of Natural Substances and Synthesis Organic, Abdou Moumouni University, Faculty of Science and Technology, Department of Chemistry, BP 10662 Niamey, Niger

Hama Garba Rahila
Laboratory of Natural Substances and Synthesis Organic, Abdou Moumouni University, Faculty of Science and Technology, Department of Chemistry, BP 10662 Niamey, Niger

Hama Hamadou Habibou
Laboratory of Natural Substances and Synthesis Organic, Abdou Moumouni University, Faculty of Science and Technology, Department of Chemistry, BP 10662 Niamey, Niger

Hassane Hamidou Zeinabou
Laboratory of Natural Substances and Synthesis Organic, Abdou Moumouni University, Faculty of Science and Technology, Department of Chemistry, BP 10662 Niamey, Niger

Ilagouma Amadou Tidjani
Laboratory of Natural Substances and Synthesis Organic, Abdou Moumouni University, Faculty of Science and Technology, Department of Chemistry, BP 10662 Niamey, Niger

Martin Kiendrebeogo
Laboratory of Applied Biochemistry and Chemistry, University of Ouagadougou, 03 BP 7021 Ouagadougou 03, Burkina Faso

Hassimi Sadou
Nutrition and promotion of agro-resources laboratory , Abdou Moumouni University, Faculty of Science and Technology, Department of Chemistry, BP 10662 Niamey, Niger

Corresponding Author:
Mahamane IDI ISSA Abdoulahi
Laboratory of Natural Substances and Synthesis Organic, Abdou Moumouni University, Faculty of Science and Technology, Department of Chemistry, BP 10662 Niamey, Niger

Phenolics contents and antioxidant activity of six medicinal plants used in the treatment of dentition related ailments in Niger

Mahamane IDI ISSA Abdoulahi, Bakasso Sahabi, Hama Garba Rahila, Hama Hamadou Habibou, Hassane Hamidou Zeinabou, Ilagouma Amadou Tidjani, Martin Kiendrebeogo and Hassimi Sadou

Abstract
The objective of the study is to quantify the secondary metabolists and assess the antioxidant activity of six plants: *Bauhinia rufescens* Lam, *Blepharis linariifolia* PERS, *Chrozophora brocchiana* Vis, *Gardenia ternifolia* Schum and Thonn, *Indigofera astragalina* DC and *Phyllanthus pentandrus* Schumach and Thonn. These plants are used in traditional medicine against diseases linked to dentition.The contents of total polyphenols, total flavonoids and total tannins, were determined by spectrophotometry as well as the antioxidant activity. The highest contents of phenolic compounds were obtained with the extracts of *C. brocchiana* and *P. pentandrus* with respectively 260.05 ± 0.07 and 256.08 ± 1.07 mg GAE/g. For total flavonoids the highest content is obtained with *Chrozophora brocchiana* with 51.60 ± 0.28 mg QE/g. For total tannins, the high content is obtained with the extract of *B. rufescens* with 28.97 ± 0.93 g/L. The best reducing power is obtained with the extract of *C. brocchiana* with 2.32 mmol AAE/g. A good correlation (r = 0.91) was found between the polyphenol content and the reducing power, for flavonoids this correlation is r = 0.84 and r = 0.27 for tannins. The study would justify the traditional use of these plants, also it offers preliminary data for an indepth study.

Keywords: total phenolics, dentition, antioxidant activity

1. Introduction
In many African countries, especially in rural areas, health centers are less developed or even non-existent. Despite the development of generic drugs, many treatments remain financially inaccessible to populations. This is how they continue to turn to nature for essential herbal remedies. Traditional medicine and pharmacopoeia then remain the only possible sources of remedy. To document and thus perpetuate this traditional knowledge, various phytochemical and ethnopharmacological research works have been undertaken. What mothers have in common is the quest for the well-being of their children; they use traditional medicine to take care of infant care. These mothers use plants as a fortifier, in weight gain, teething problems ... This traditional medicine is essentially based on the use of medicinal plants to treat pathologies. In Niger, several studies have focused on the traditional pharmacopoeia and ethnobotanical and pharmacological surveys [1, 2]. However, the investigation of plants used in infant care is not significant; Bilan *et al*., in 2018 reported data on plants used in the treatment of dentition [3]. The aim of this study is to determine the phytochemical composition and to measure the polyphenols of the six plants *Bauhinia rufescens* Lam, *Blepharis linariifolia* PERS, *Chrozophora brocchiana* Vis, *Gardenia ternifolia* Schum and Thonn, *Indigofera astragalina* DC and *Phyllanthus pentumrus* Schum and Thonn, which are commonly used in Niger in the treatment of affections related to the teething of infants.

2. Materials and Methods
2.1. Plant Material
The samples of the six (6) plants were collected (*Bauhinia rufescens* Lam, *Indigofera astragalina* DC, *Phyllanthus pentandrus* Schumach & Thonn, *Chrozophora brocchiana* Vis, *Gardenia ternifolia* Schum and Thonn and *Blepharis linariifolia* PERS).
The plant material consists of the leaves of *Bauhinia rufescens* Lam. For *Indigofera astragalina* DC, *Phyllanthus pentandrus* Schumach & Thonn and *Chrozophora brocchiana* Vis, the whole plants were used. For *Blepharis linariifolia* PERS, it is the whole plant without the seeds and the fruit of *Gardenia ternifolia* Schum & Thonn. The samples from each plant were washed and then allowed to dry in an airy room at room temperature; the dry samples

were ground using a mechanical grinder and the ground material (powder) obtained was stored in plastic bags at room temperature, in a dry place and away from moisture and the light.

2.2. Extraction
50 g of powder obtained from each plant material were put in a flask containing 500 ml of distilled water. The mixture is heated at a temperature of 60 ° C for 1 hour. After cooling, the mixture is filtered in a beaker using a funnel and filter paper. The sand bath was used to evaporate the water (obtaining a dry extract).

2.3. Spectrophotometric assay
2.3.1. Determination of total polyphenols
The total polyphenol contents of the various aqueous extracts were determined using FCR (Folin Ciocalteu reagent). To 0.5 ml of each aqueous extract of concentration 1 mg/ml, 2.5 ml of Folin Ciocalteu reagent diluted 10 times and 2 ml of sodium carbonate solution (75 g/L) were added. The mixture is stirred and incubated in the dark at room temperature for 30 minutes and the absorbance was measured at 760 nm using a UV-visible Helios β spectrophotometer. The calibration curve was plotted using 1 mL of gallic acid solution of concentrations: 0, 20, 40, 60, 80 and 100 mg/L. The results are expressed in mg gallic acid equivalent per g of dry vegetable matter by referring to the calibration curve of gallic acid. The absorbance was measured to determine the contents of total polyphenols using the following formula:

$$C = \frac{C1\ X\ V}{m}$$

C being the content of total polyphenols expressed in mg equivalent gallic acid/g of dry matter, C1 the concentration of gallic acid established from the calibration curve in mg/L, V the volume of extract in L and m the weight of the plant extract in g [4, 5].

2.3.2. Determination of total tannins
The assay is based on the property of proanthocyanidins to transform, by cleavage of the interflavane bond in an acid medium and at 100 ° C., into colored anthocyanidins (yellow-green) absorbing mainly at 550 nm. This reaction is commonly called the Bate-Smith reaction. To 2 ml of aqueous extract of each plant (1 mg/ml) placed in a hydrolysis tube (glass tube), 3 ml of hydrochloric acid (12N or 37%) was added. The tube is then closed using a plug fitted with a Teflon seal and placed in a water bath at 100 ° C for 30 min. At the same time, a control tube containing the same solution is left at room temperature. After the hydrolyzed tube has cooled, the optical density is read at 550 nm. The total tannin contents were calculated according to the following formula:
C = 19.33 (Doh - Dot)
C being the content of total tannins expressed in g/L, Doh the optical density of the hydrolyzed tube and Dot the optical density of the control tube [6, 7, 8].

2.3.3. Determination of total flavonoids
The total content of flavonoids is determined by the Dowd method adapted by Arvouet-Grand *et al*. (1994) [9]. The total flavonoid contents of the various aqueous extracts of the six plants were determined through the reactions with aluminum trichloride and sodium hydroxide. Aluminum trichloride forms a yellow complex with the flavonoids. As for the sodium hydroxide, it forms with these compounds a pink complex which absorbs in the visible at 415 nm. One (1) mL of the aqueous extract of each plant (1 mg/mL) is mixed with 1 mL of $NaNO_2$ (5%), 1 mL of the aluminum trichloride solution (20 g/L) $AlCl_3$ and 2 mL NaOH (4%). After incubation in the dark for 30 minutes at room temperature, the absorbance of the mixture was measured at 415 nm using a UV-visible Helios β spectrophotometer against a blank using distilled water. The total flavonoid contents in the extracts are calculated from a linear calibration curve, established with concentrations 5; 10; 15; 20; 25 and 30 mg / L of quercetin as a reference standard, under the same conditions as the sample. The flavonoid contents of the six plants are calculated according to the following formula:

$$C = \frac{C1\ X\ V}{m}$$

C being the content of total flavonoids expressed in mg quercetin equivalent /g of dry matter, C1 the concentration of quercetin established from the calibration curve in mg/L, V the volume of extract in L and m the weight of the plant extract in g.

2.4. Ferric (Fe III) Reducing Antioxidant Power (FRAP) Assay
The FRAP (Ferric reducing antioxidant power) method is based on the ability of extracts to reduce the ferric ion (Fe^{3+}) to ferrous ion (Fe^{2+}). The total antioxidant capacity of each plant extract was determined by the method described by Hinneburg *et al*., in 2006 used by Bakasso, in 2009; Bakasso *et al*., in 2013, Compaoré *et al*., in 2016 [10, 5, 11, 12]. Thus, 0.5mL of an aqueous solution of each extract (2 mg/mL diluted to obtain 1mg / mL) of ascorbic acid, were mixed with 1, 25 mL of phosphate buffer (0.2 M pH 6.6) and 1.25 mL of an aqueous solution (1%) of potassium hexacyanoferrate ($K_3Fe(CN)_6$). After 30 minutes of incubation at 50 ° C, 1.25 mL of trichloroacetic acid (10%) was added. The mixture was then centrifuged at 3000 rpm for 10 min. 0.625 mL of the supernatant was mixed with the same volume of water and 0.125 mL of a freshly prepared aqueous solution of $FeCl_3$ (0.1%) was added. For all samples, the measurements were carried out in three tests. The absorbances were read at 700 nm against a calibration curve obtained from ascorbic acid (0-200 mg/L). The reducing power was expressed in ascorbic acid equivalents (AAE) (mol ascorbic acid/g of dry extract) and was calculated according to the following formula:

$$C = {}^{c \times D}/_{M \times Ci}$$

C = concentration of reducing compounds in mmol AAE/g of dry extract;
c = concentration of the sample read; D = dilution factor of the mother solution;
Ci = concentration of the stock solution; M = molar mass of ascorbic acid (176.1 g/mol).

2.5. Data analysis
All data were analyzed using Excel to calculate means, standard deviation and obtain the graph and correlations. The ANOVA from the XLSTAT software was used to measure the statistical difference.

3. Results and discussion

3.1. Results of the spectrophotometric assay of six plant samples

Figure 1 shows the standard calibration curve for gallic acid (y = 0.0118x + 0.1202 with R^2 = 0.9841) and Figure 2 shows the standard calibration curve for quercetin (y = 0.0294x + 0.0271 with R^2 = 0.9914).

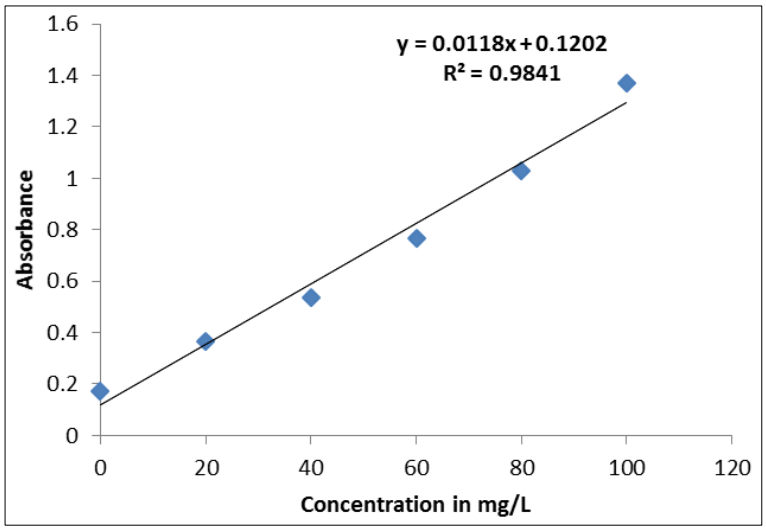

Fig 1: Standard curve for gallic acid

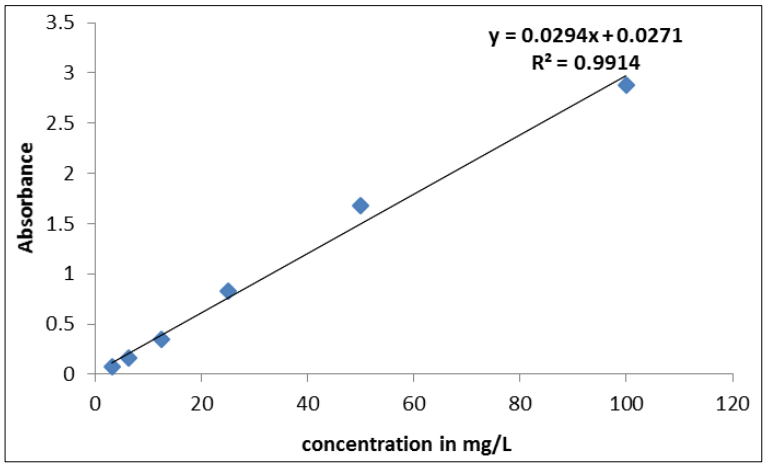

Fig 2: Standard curve for quercetin

Table 1: Results of the spectrophotometric assay of six plant samples

Species	Total polyphenols in mg GAE/g	Total tanins in g/L	Total Flavonoids in mg QE/g
B. rufescens	203,44±0,37^{d}	28,97±0,93^{a}	39,55±0,98^{c}
B. linariifolia	154,72±0,48^{e}	5,31±0,21^{d}	44,92±0,65^{b}
C. brocchiana	260,05±0,07^{a}	4,26±0,89^{d}	51,60±0,28^{a}
G. ternifolia	100,33±0,98^{f}	7,75 ±0,03^{bc}	11,79±1,01^{d}
I. astragalina	206,24±0,73^{c}	6,71±0,33^{c}	45,01±0,73^{b}
P. pentandrus	256,08±1,07^{b}	8,27±0,08^{b}	47,68±0,63^{b}

The data in the column are statistically different letters (p <0.05), except the data with the same letters in superscript (a-f). Data are obtained by performing triplicate tests (n = 3).

The results of the determination of total polyphenols are expressed in gallic acid equivalent and vary from 100.33 ± 0.98 to 260.05 ± 0.07 mg GAE/g of the dry extract (see Table I). *C. brocchiana* and *P. pentandrus* are the plants which contain more total polyphenols with values of 260.05 ± 0.07 and 256.08 ± 1.07 mg GAE/g respectively. The low content of total polyphenols is obtained with the extract of *G. ternifolia*. The tannin contents for the dosed extracts vary between 4.26 ± 0.89 and 28.97 ± 0.93 g/L. The highest content of total tannins is obtained with the extract of *B. rufescens* with 28.97 ± 0.93 g/L (see Table I). The low value is obtained with the extract of *C. brocchiana*. The contents of total flavonoids obtained vary from 11.79 ± 1.01 mg QE/g to 51.60 ± 0.28 mg QE/g; the highest content is obtained with *Chrozophora brocchiana* which gave the highest content in total polyphenols and the low content with *Gardenia ternifolia* (see Table I).

Polyphenols are antioxidants and are involved in several biological activities. Flavonoids are attributed with properties that increase capillary resistance and decrease membrane permeability, as well as anti-inflammatory, anti-allergic and antioxidant activities. As antioxidants, flavonoids are able to inhibit the process of carcinogenesis implemented by mutations caused by DNA damage by free radicals. These

mutations are even more serious when they affect critical genes such as oncogenes and tumor suppressor genes [13, 4].

One of the most recognized properties of tannins is their ability to form very stable complexes with proteins, which explains their use in the tanning of hides. Tannins are recognized for their antiseptic, bactericidal and astringent properties [14]. These characteristics give them many biological properties exploited in particular by the pharmaceutical and food industries. Barlow in 2002, revealed that a majority of health professionals and parents, associate the following symptoms with teething: gingival inflammation, hyper salivation, irritability, restlessness, insomnia and fever [15]. Thus Coreil *et al*., in 1995 established a link between diarrhea and teething [16]. In general, this dental diarrhea is considered less dangerous for the child.

Some flavonoids are recognized for their ability to inhibit 5-lipoxygenase and therefore the synthesis of leukotrienes which are mediators of inflammation and allergic manifestations. Thus flavonoids inhibit cyclooxygenase and platelet aggregation. These properties partly explain the anti-inflammatory and antiallergic activities usually attributed to various plants known to contain flavonoids. Flavonoids have anti-inflammatory and possibly antiallergic, hepatoprotective, cholesterol-lowering, diuretic, antibacterial properties [17].

The presence of phenolic compounds, in the broad sense including tannins and flavonoids in these plants, gives them significant therapeutic power, which would justify their use in traditional medicine for the treatment of diseases developing fever, pain and inflammation therefore in the case of affections related to dentition.

3.2. Reducing power of extracts from six plants samples

The reducing power of the extracts results from the capacity of their compounds to give electrons, therefore to participate in reduction reactions. Thus, the antioxidant activity is expressed in mmol equivalent of ascorbic acid/g of extract. The concentrations were determined using a regression line : y = 0.0073x + 0.1221 r = 0.98. It was established from an ascorbic acid standard (200 mg/L stock solution). The values obtained with the aqueous extracts of the plants vary from 0.69 to 2.32 mmol AAE/g (Table V). The best antioxidant activity is obtained with extracts of *C. brocchiana*.

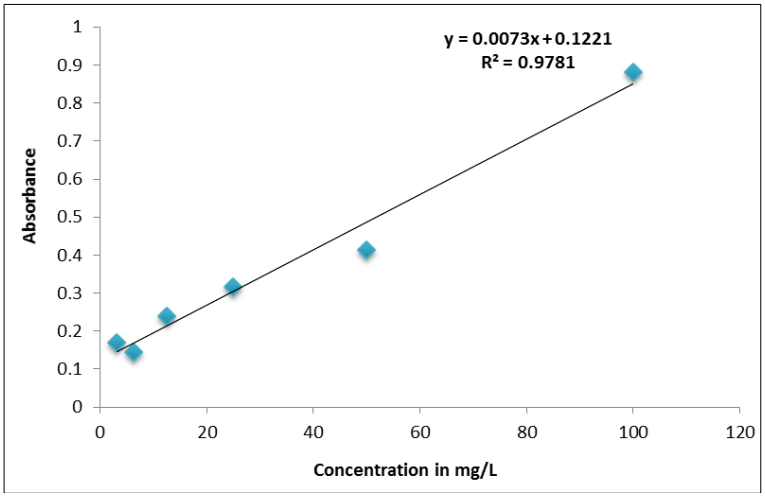

Fig 1: Ascorbic acid standard curve

Table 2: Reducing power of extracts from six plant samples

Species	Reducing power in mmol EAA/g
B. rufescens	1,62±0,07^{b}
B. linariifolia	1,78±0,04^{b}
C. brocchiana	2,32±0,01^{a}
G. ternifolia	0,69±0,03^{c}
I. astragalina	1,82±0,28^{b}
P. pentandrus	2,21±0,02^{a}

The data in the column are statistically different letters (p <0.05), except the data with the same letters in superscript (a-c). Data are obtained by performing triplicate tests (n = 3).

Polyphenols attract considerable attention and enthusiasm and many of their biological properties are the subject of numerous non-exhaustive studies; one of the primary reasons is the recognition of their antioxidant properties, as well as their implications in the prevention of various pathologies associated with oxidative stress [18]. Free radicals are the cause of several pathologies among which we can cite: arthrosis, asthma, cancer, diabetes, heart disease, atherosclerosis [19, 20]. The use of natural products (fruits, vegetables) with a good antioxidant content could play an important role in the prevention of these diseases [21].

Interest in research on naturally occurring antioxidants has increased significantly in recent years because they may be involved in the fight against some diseases caused by free radicals [22]. Free radicals are suspected in the process of fever and inflammatory reactions [23]. Using antioxidant-rich products could limit the damage these radicals can cause. Fever and inflammation are symptoms involved in several childhood ailments, the antioxidant activity of the plants studied could justify their uses in the treatment of infant diseases.

Correlation between the contents of total polyphenols, flavonoids, tannins and antioxidant activity by the FRAP method of extracts from six plant samples

The regression equations and correlation coefficients between total polyphenols, total tannins, total flavonoids and antioxidant activity by the FRAP method are shown in the figure.

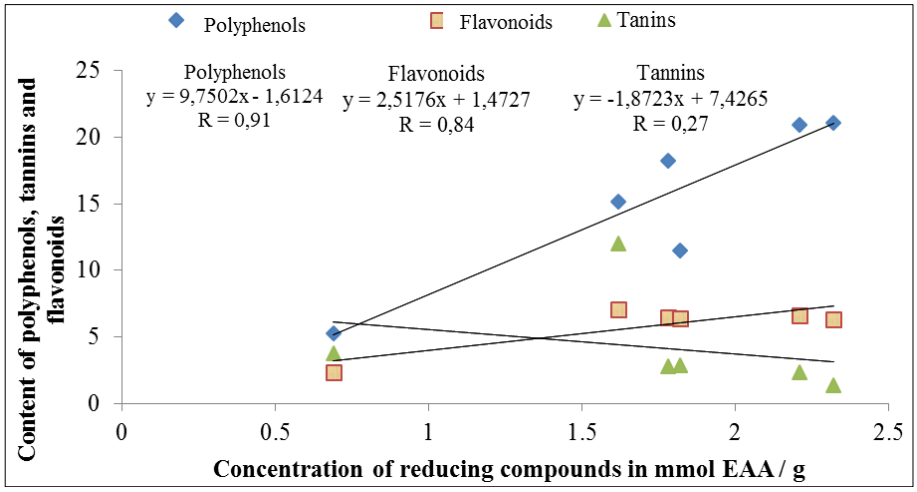

Fig 4: Correlation between total polyphenols, flavonoids, tannins and antioxidant activity by the FRAP method.

The correlation coefficients obtained are 0.91 for total polyphenols; 0.84 for flavonoids and 0.27 for tannins. Some authors have found a correlation between antioxidant activity and the concentration of total polyphenols while others have found no correlation. Wamtinga, in 2006; Bakasso, in 2009; Bakasso *et al*., in 2013; Compaoré *et al*., in 2016 found a strong correlation between antioxidant activity and the concentration of total phenolics in plant extracts [24, 5, 11, 12]. Phenolic compounds in general constitute the class of compounds having a better contribution to the antioxidant activity of plants, fruits, cereals and other products of plant origin [25]. However, Kahkonen *et al*., in 1999 found no correlation between the content of total phenolics and the anti-radical activity of plant extracts [26].

Polyphenols have multiple biological activities, including antioxidant activity [27]. In this study, a good correlation between the antioxidant activity and the concentration of total polyphenols in the extracts of the six species studied. The content of total polyphenols in the extracts largely contributes to their antioxidant activity. With flavonoids the correlation is lower than that of total polyphenols and a weak correlation has been observed with tannins (Figure). However, the *B. rufescens* plant has the best polyphenol content (203.445 ± 0.37 mg GAE/g) than *B. linariifolia* (154.72 ± 0.48 mg GAE/g) and it is the latter which has the better reducing power; considering the flavonoid contents, *B. linariifolia* has the best content with 44.925 ± 0.65 mg QE/g; this content is 39.55 ± 0.98 mg QE/g for *B. rufescens*. The reducing power of these two plants, could be attributed to flavonoids; thus this could be justified by the diversity of the structures of polyphenols and flavonoids which do not have the same antioxidant power. In addition, *B. linariifolia*, which has a low tannin content (5.315 ± 0.21 g/L) has a higher antioxidant activity than that of *B. Rufescens*, which has the highest tannin content among the six plants studied (28.97 ± 0.93 g/L). This could be explained by the weak correlation between the tannin content and the reducing power.

Conclusion

The present study has shown the content of phenolics which could justify the traditional use of these plants. This study constitutes a contribution to a better knowledge of the phytochemistry of these plants and also to the promotion of medicinal plants. The spectrophotometric assay shows that the *C. brocchiana* and *P. pentandrus* plants have the highest levels of polyphenols and flavonoids. The highest content in total tannins is obtained with the extract of *B. rufescens* with 28.97 ± 0.93 g/L. There is a significant correlation between total polyphenols and antioxidant activity and a good specific contribution of flavonoids has been obtained. The significant presence of these compounds gives these plants properties sought-after in human therapy. It should therefore be emphasized that other studies targeting the pharmacological aspect are necessary for better valorisation of these plants.

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