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Using *Boscia senegalensis* leaves and fruits as a source of polyphenol, and micronutrients to improve antioxidant activity

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Abstract: *In this study the phytochemical screening of the plant Boscia senegalensis wasperformed.Boscia senegalensis is a shrub from the Sahelian areas belonging to the Capparaceae family.To carry out the practical part of our work, samples of a variety of Boscia senegalensis were obtained in the region of Saint-Louis (Senegal). This qualitative phytochemical screening revealed the presence of polyphenols, flavonoids, saponins, tannins, proanthocyanidin and the absence of alkaloids, cardenolides and steroids. Secondly, the amount of polyphenols, flavonoids, pro-anthocyanidin saponins, minerals and protein are evaluated in the leaves and fruits of this plant.*

Keywords: *Boscia Senegalensis, Extraction, Polyphenols, Flavonoids, Proanthocyanidins, Saponins, Antioxidant,*

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I. Introduction

Senegal is a country located south of the Sahara whose biodiversity is rich in more than 3,500 plant species, but some of them are still little studied[1-3]. Nevertheless, they are well used both in food and in the treatment of certain diseases [4-6]. According to *Dasylva*[7] than 592 species are used in traditional medicine. As health services are extremely limited in rural areas, traditional medicine is dramatically expanding. The lack of a scientific assessment of side effects from the use of plants is a major problem [8]. Depending on the ethnic group, ancestral knowledge is still widely used and often preferred over modern medicine in the treatment of certain diseases such as infectious diseases, bronchitis, inflammation, fevers, snakebites and pain[6-9]. These plants are often used in decoction[10], maceration[11] or infusion[12]without any special precautions. To contribute to a better knowledge and to enhance this heritage, *Boscia senegalensis*, was chosen to estimate secondary metabolites which can explain the therapeutic properties attracted to its leaves and fruits.

II. Material and Methods

II.1 Plant material

Leaves and fruits of *Boscia senegalensis* (BS) were collected from a field in northern Senegal at Dagana (16°31'226" N,15°37'238" E) and dried in the shade for a month. Then the leaves and fruits were finely ground and stored at room temperature, protected from light.

II.2 Extraction

Dried and ground plant materials were extracted separately with water. The mixture was filtered and completed to a fixed volume. The obtained extracts were stored in a refrigerator at 4°C until the performance of analysis.

II.3 Phytochemical screening

Phytochemical screening is a qualitative analysis based on precipitation or colored reactions to confirm or deny the presence or absence of secondary metabolites such as polyphenols, alkaloids, quinones, flavonoids, saponins, tannins, sterols and reducing sugars in the different organs of the plant.

II.3.1 Testing for Alkaloids

This test is based on the ability of alkaloids to combine with heavy metals. The methanolic extract is taken up in a few mL of HCl 6N. The formation of a yellow precipitate, after adding a few drops of *Mayer's* reagent (1.35 g of $HgCl_2$ + 5 g of KI in 100 mL of distilled H_2O), indicates the presence of alkaloids.

II.3.2 Testing for Tannins

Two reactions were used.*Stiasny* reaction: 0.5 g of plant material is added to 5 mL of ammonium acetate (5 M) and 3 to 4 drops of a 2% $FeCl_3$ solution. The presence of gallic tannin is indicated by development of a blue-

black colour. The condensed tannins is confirmed by apparition of a precipitate.*Bate-Smith* reaction: a mixture of 0.5 g of plant material and 1 mL of concentrated HCl was boiled for 5 minutes. A brick red colouris indicative of the presence of catechetic tannins.

II.3.3 Testing for Saponosides

Foam height test: a mixture of 1 g of plant material and 100 mL of distilled water was gentlyboiled for 30 minutes. After filtration and cooling, 1 mL of the extract was introduced into each of the ten test tubes and make up to 10 mL with distilled H_2O if necessary. The test tubes were stopped and manually shaken vigorously for about 30 seconds. It was then allowed to stand for 30 minutes. Development of honeycomb froth is indicative of the presence of saponins.

II.3.4 Testing for Sterols and polyterpens

Liebermann reaction is used for the research of sterol and polyterpens. The residue (0.5 g) obtained from the extract after evaporation is dissolved in 1 mL of acetic anhydride. 0.5 mL of concentrated sulfuric acid is added to the mixture. The appearance of colouring violet which turns blue then green indicates a positive reaction.

II.3.5 Testing for Flavonoids (Cyanidin)

The methanolic extract is dissolved in 1mL of concentrated HCl and added with a few shavings of magnesium. The appearance of a colour ranging from orange to purple red indicates the presence of flavonoids.

II.4 Optimization of extraction parameters

For each parameter to be determined, the total polyphenols and the antioxidant capacity are assayed by the CUPRAC method [13].

II.4.1 Temperature

To determine the optimum extraction temperature, approximately 0.5 g of plant material is extracted in 50 mL of distilled water at different temperatures (40, 50, 60, 70, 80 and 90°C) for 20 minutes in a bain-marie. After extraction, the extracts are filtered, and the collected filtrate is stored at 4°C until use.

II.4.2 Duration

Once the optimum temperature has been determined, the optimum extraction time is determined. The temperature and the extraction mass are fixed, and the duration is varied from 5 to 30 minutes. Then the filtered extracts are stored at 4°C until use.

II.4.3 Ratio: plant material / water

The temperature and time being determined, the amount of plant material to be extracted is varied in 50 mL of distilled water. The masses to be studied are 0.5; 1; 1.25; 2.5; 3.75 and 5g. The procedure is as above, the filtrates recovered are stored at 4 ° C until use.

II.5 Determination of polyphenols

To measure the total polyphenols, we take 50 µL of our samples which are made up to 200 µL with distilled water. 150 µL of Folin-Ciocalteu reagent and 600 µL of a 20% Na_2CO_3 solution and 2.32 mL of distilled water are added. The mixture is incubated in the dark for 30 minutes before reading the absorbance was read at 760 nm with a Perkin-Elmer UV/Visible spectrophotometer Lambda 365. Gallic acid (GA) was used as a standard. The results were expressed as mg GAE/g ± Standard deviations.

II.6 Determination of flavonoids

To determine the total flavonoids, a total volume of 2.5 mL of sample is taken (dilutions are provided if necessary) to which 2.5 mL of a 2% aqueous aluminium chloride solution is added. The resulting mixture is incubated for 1 hour at room temperature before reading the absorbance at 425 nm with a Perkin-Elmer UV/Visible spectrophotometer Lambda 365. Quercetin (Q) was used as a standard. The results were expressed as mg QE/g ± Standard deviations.

II.7 Determination of saponins

To assay the saponins, to 0.5 mL of extract are added 0.5 mL of 8% vanillin and 5 mL of 72% H_2SO_4. The whole is incubated at 70 ° C for 10 minutes and then cooled rapidly in ice water. The absorbance is read at 560 nm with a Perkin-Elmer UV/Visible spectrophotometer Lambda 365. Aescin was used as a standard and results were expressed as mg AeE/g ± Standard deviations.

II.8 Determination of proanthocyanidins

To 0.5 mL of each extract, 3 mL of 4% vanillin and 1.5 mL of concentrated HCl were added. The mixture is incubated in the dark at room temperature for 15 minutes and then read the absorbance at 500 nm with a Perkin-Elmer UV/Visible spectrophotometer Lambda 365.Catechin (C) was used as standard, and the results are expressed as mg CE/g ± Standard deviations.

II.9 Determination of IC50 and antioxidant activity by DPPH•

To determine the anti-free radical activity, each of the extracts is subjected to a methanolic solution of 0.1 mM $DPPH^{\cdot}$. The solutions are diluted according to the content of the stock solution and 200 µL of each extract are taken to which 3.8 mL of DPPH• were added. The mixture is incubated for 30 minutes in the dark and the absorbance read at 517 nm with a Perkin-Elmer UV/Visible spectrophotometer Lambda 365. TROLOX is used

as standard. Results were expressed as µgTrE/g ± Standard deviations. $DPPH^{\bullet}$ scavenging activity was determined by calculating the percentage of inhibition.

$$Scavenging\ activity = \frac{Absorbance_{control} - Absorbance_{sample}}{Absorbance_{control}} \times 100$$

II.10 Determination of IC50 and antioxidant activity by $ABTS^{\bullet+}$

Like DPPH• assay, we have to determine the TEAC and the IC50. Firstly, we prepare a stock solution by mixing an equal volume of a 3 mM potassium persulfate ($K_2S_2O_8$) solution with an 8 mM $ABTS^{\bullet+}$ solution. The stock solution was kept in the dark during 16h before use. Secondly, we prepare a working solution by diluting the stock solution with a phosphate buffer (0.2 M, pH 7.4, 150 mM NaCl) up to get an absorbance of 1.2 at 734 nm. The different extracts or Trolox solutions (100 µl) were mixed with 2.9 ml of $ABTS^{\bullet+}$ working solution. 30 minutes after incubation we read the absorbance at 735 nm. Next, we made adequate dilution to determine the IC50. Results was expressed as µg TrE/g ± Standard deviations. $ABTS^{\bullet+}$ scavenging activity was determined by calculating the percentage of inhibition:

$$Scavenging\ activity = \frac{Absorbance_{control} - Absorbance_{sample}}{Absorbance_{control}} \times 100$$

II.11 Determination of some mineral elements

5 g of vegetable matter are introduced into a porcelain crucible and heated to 550°Cfor 8 hours in an oven. After cooling, the residue is taken up in 5 mL of concentrated HNO_3 and the resulting suspension is filtered and made up to 1 L with bi-distilled water. The determination of iron, nickel, copper, and zinc is carried out by atomic absorption with a FISCHER apparatus.

III. Results and discussion

III.1 Phytochemical screening

The phytochemical screening carried out on the leaves and fruits of *Boscia senegalensis* (Table 1) revealed in both cases the presence of polyphenols, flavonoids, gallic and catechetic tannins and saponosides. The absence of alkaloid, polyterpenoid, sterol, cardenolide and steroid is noted.

Table 1: Phytochemical screening results

Tests	*Boscia senegalensis*	
	Fruits	**Leaves**
Alkaloids	----	----
polyterpenoids	----	----
Sterols	----	----
Gallic tannins	+	+
Catechetical tannins	++	++
Polyphenols	+++	+++
Flavonoids	++	++
Cardenolides	---	---
Steroids	---	---
Saponosides	+++	+++

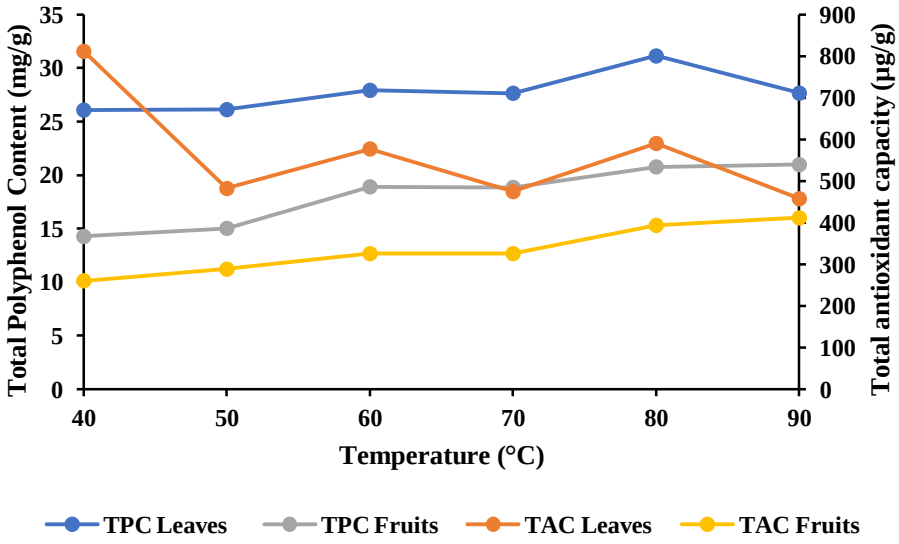
Precipitate or color: +++ strongly present; ++ moderately present; --- absent.

III.2 Optimization of water'sextract

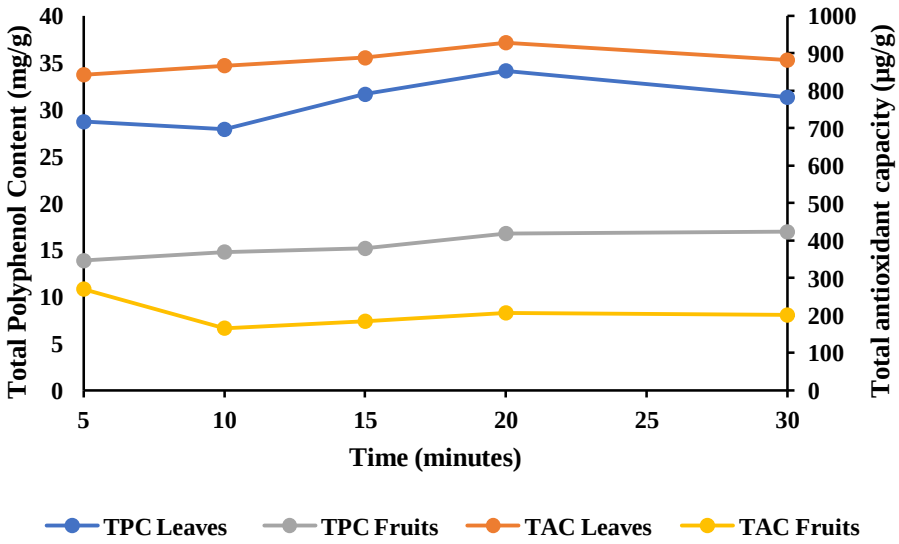
Optimization of the extraction parameters in water is followed by the total polyphenol content (TPC) and the total antioxidant capacity (TAC). The study of the influence of temperature (Fig. 1a) on the polyphenol extraction rate shows that this rate increases and reaches a maximum at **80°C** for both leaves and fruits. It can be noted that the TPC level remains constant for fruits at 90°C while it decreases for leaves at this temperature. The decrease in TPC at higher temperatures is probably due to the thermal decomposition of these molecules which are thermosensitive as reported in the literature[14,15]. In fact, some authors[14-17] have shown that even if the mass of the extracted product increases with temperature, the TPC decreases due to the decomposition of the polyphenol molecules. The results agree with the total antioxidant capacity (TAC) measured as a function of temperature. For the leaves and for the fruits the total antioxidant capacity is maximum at 80°C. TAC remains

constant at 90°C for fruits while it decreases for leaves at this temperature (Fig. 1a). These remarks are in accordance with the observations for temperature optimization. In conclusion, it was decided to perform all the extractions at 80°C for the leaves and for the fruits to obtain the best extraction yields. The study of the influence of the extraction time shows (Fig. 1b) that the best results (TAC and TPC) are obtained at **20 minutes** for both leaves and fruits extracted at 80°C. The ratio is studied by setting the temperature at 80°C and the time optimized at 20 min for each part of the plant material. The figure (Fig. 1c) shows that the **ratio of 1%** (plant / water) gives the best values of TPC and TAC for leaves and for fruits. Indeed, it has been reported that the lower the ratio, the better the kinetics of the extraction is[14].For the rest of the work, all the extractions will be done for the leaves and for the fruits at 80°C for 20 minutes with a ratio of 1%.

(a)



(b)



(c)

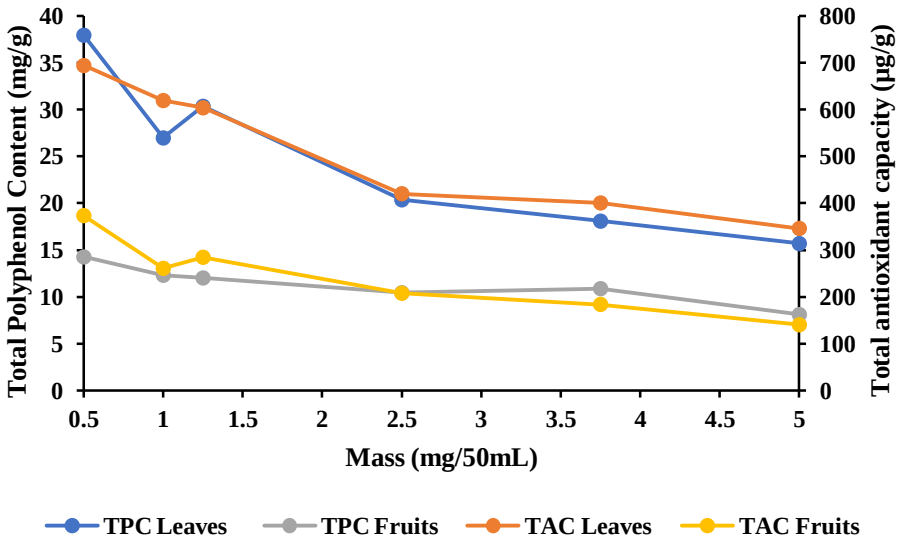


Figure 1: (a) optimization of the extraction temperature; (b) optimization of extraction time; (c) optimization of the extraction ratio.

III.3 Total Phenolic and Total Flavonoid Content

Quantitative determination of the total polyphenol content is carried out for leaves and fruits in various solvents: water, methanol, and acetone. For the leaves, the TPC varies from 75.06 to 0.81 mg EAG / g while for the fruits the TPC is between 36.21 and 2.10 mg EAG / g (Table 2). In both cases the methanolic extract is richer in polyphenol followed by the aqueous extract, while the acetone extracts are less rich with exceptionally low polyphenol levels. It is observed that the degree of extraction of polyphenols depend on the polarity of the solvents used. In fact, polyphenol molecules are polar and are compatible with polar solvents. Overall, extractions with various solvents show that the leaves are richer in polyphenols than the fruits. It is only in the case of acetone, which has the lowest extraction rate, that the TPC of the fruits is observed to be higher than the TPC of the leaves.

On the other hand, for flavonoids, the extraction rate is much lower in water than in other organic solvents, both for leaves and for fruits. For the leaves, the total flavonoid content (TFC) varies from 78.05 to 17.75 mg EQ / g while for the fruits the TFC is between 16.48 to 3.00 mg EQ / g (Table 2). Methanol extract is richer in flavonoids followed by ethanol and then acetone for the leaves and fruits. This observation can be explained by the low dipole moment of these molecules which reduces the solubility in solvents with high dipole moment. In fact, water has an extremely high dielectric constant (ε= 80), while for methanol, ethanol and acetone the ε values are respectively 33, 24.5 and 21. We also note that the leaves are much richer[7] in flavonoid than the fruits whatever the solvent used.

Table 2: Total polyphenol content and total flavonoid content.

Total polyphenol content (mg EAG / g)				
	Water	Methanol	Ethanol	Acetone
Leaves	30.49 ± 1.01^{a}	75.06 ± 0.12^{c}	22.75 ± 0.90^{e}	0.81 ± 0.46^{g}
Fruits	11.29 ± 1.10^{b}	36.21 ± 0.74^{d}	13.36 ± 2.29^{f}	2.10 ± 0.21^{h}
Total Flavonoids content (mg EQ/g)				
Leaves	17.75 ± 0.57^{a}	78.05 ± 4.46^{c}	58.92 ± 0.70^{d}	48.26 ± 3.37^{f}
Fruits	3.00 ± 0.25^{b}	16.48 ± 3.75^{a}	8.58 ± 0.42^{e}	6.04 ± 0.07^{g}

Values with different letters in superscript in column showed the significant differences at the significance level of p-value of 0.05.

III.4 Saponins and Proanthocyanidins Content

The various extracts show that the saponin content varies from 5.46 mg AeE / g to 54.81 mg AeE / g for the leaves, while for the fruits this content varies from 1.41 mg AeE / g to 21.16 mg AeE / g (Table 3). Acetone provides the best results followed by methanol, ethanol, and water for the leaves. For fruits, ethanol offers the lowest extraction rate followed by water, methanol, and acetone. Either way, acetone is the best solvent for extracting saponins from this plant. The leaves are very much richer in saponins than the fruits.

A significant change is observed in the rate of extraction of proanthocyanidin in the leaves when switching from acetone (170.46 mg EC / g) to ethanol (57.31 mg EC / g). The extraction rates are similar for water (95.50 mg EC / g) and methanol (93.34 mg EC / g). Unlike leaves, proanthocyanidins in fruits are best extracted in water (69.32 mg EC / g) then in organic solvent: acetone (20.82 mg EC / g), methanol (13.36 mg EC / g) and ethanol (7.79 mg EC / g) (Table 3). It is likely that the leaves and fruits of Boscia S do not contain the same types of proanthocyanidin molecules.

Table 3: Content of saponin and proanthocyanidin.

Saponins content (mg AeE/g)				
	Water	Methanol	Ethanol	Acetone
Leaves	5.46±0.83[a]	25.66±1.34[c]	11.08±1.48[d]	54.81±2.47[f]
Fruits	2.13±0.38[b]	5.25±1.34[a]	1.41±0.49[e]	21.16±4.94[g]
Proanthocyanidins content (mg EC/g)				
Leaves	95.50±7.11[a]	93.34±1.62[a]	57.31±5.35[d]	170.46±4.36[f]
Fruits	69.32±5.90[b]	13.36±4.60[c]	7.79±1.84[e]	20.82±4.03[g]

Values with different letters in superscript in column showed the significant differences at the significance level of p-value of 0.05.

III.5 Antioxidant Capacity and IC50

Since polyphenols are known for their ability to scavenge free radicals, a study was conducted for determining the antioxidant and IC50 properties of the leaves and fruits of *Boscia senegalensis*. The stable $DPPH^{\bullet}$ radical is suitablefor studying the anti-free radical capacity of the leaves and the fruitsof the *Boscia senegalensis*. The results summarized in table 4 show that the anti-free radical capacity of the fruits is much greater than that of the leaves with a value of 1.73 ± 0.02 TEAC (mg / g) against 5.62 ± 0.09 TEAC (mg / g) for aqueous extracts. These samples are not sufficiently active against $DPPH^{\bullet}$ to allow the determination of an IC50. The methanolic extracts have less favourable anti-free radical capacities than the aqueous extracts, as can be seen in Table 4. Using $ABTS^{\bullet+}$, the aqueous, ethanolic and acetone extracts of the fruits exhibit better anti-free radical activity than the corresponding extracts of the leaves. On the other hand, the methanolic extract of the leaves has a better anti-free radical activity than the methanolic extract of the fruits with respective values of 53.94 ± 1.69 TEAC (mg / g) and 68.59 ± 0.30 TEAC (mg / g). Both for the fruits and for the leaves, the ethanolic extract is much more active against the $ABTS^{\bullet+}$ radical with respective values of 8.30 ± 0.29 TEAC (mg / g) and 9.08 ± 0, 13 TEAC (mg / g). Acetone extracts are the least active with values of 70.04 ± 0.49 TEAC (mg / g) for fruits and 71.61 ± 1.38 TEAC (mg / g) for leaves. The IC50 determined in the case of the study of leaves with ABTS gives 0.25 mg / mL for the aqueous extract and 0.53 mg / mL for the methanolic extract.

Table 4:Antioxidant capacity and IC50 of *B. senegalensis*

Leaves								
	Eau		Methanol		Ethanol		Acetone	
	TEAC (mg/g)	CI50 (mg/mL)	TEAC (mg/g)	CI50 (mg/mL)	TEAC (mg/g)	CI50 (mg/mL)	TEAC (mg/g)	CI50 (mg/mL)
$DPPH^{\bullet}$	5.62 ± 0.09	n/d	11.35 ± 0.95	n/d	n/d	n/d	n/d	n/d

$ABTS^{\cdot+}$	35.18 ± 0.09	0.25	53.94 ± 1.69	0.53	9.08 ± 0.13	n/d	71.61 ± 1.38	n/d
Fruits								
$DPPH^{\cdot}$	1.73 ± 0.02	n/d	n/d	n/d	n/d	n/d	n/d	n/d
$ABTS^{\cdot+}$	19.08 ± 0.67	n/d	68.59 ± 0.30	n/d	8.30 ± 0.29	n/d	70.04 ± 0.49	n/d

III.6 Mineral content

Metals, known as trace elements, mainly provided through human food by fruits and vegetables, integrate into certain biomolecules to generate enzymes or coenzymes. These enzymes and coenzymes help control certain biological functions in the body. These trace elements also contribute to the protection of health thanks to their contributions to the antioxidant properties of these foods. *Boscia senegalensis*, the fruits of which are consumed regularly by the populations in rural zones, contributes a lot to the nutritional requirements of trace elements (Table 5). It also appears that the leaves,which are not used in human alimentation, are quite rich in oligo-elements. The concentration of the calcium in the fruits (280.50 ± 0.10 mg / 100 g) is higher than those of the leaves (100.30 ± 0.10 mg / 100 g).

These materials are less rich in magnesium with concentrations of 0.85 ± 0.01 mg / 100 g for fruits and 5.84 ± 0.01 mg / 100 g for leaves. It can be noted that the fruits are also richer in copper and nickel than the leaves. On the other hand, the latter have higher iron and zinc levels than in fruits. The common use of the fruit could be combined with the use of the leaf extracts in the form of an infusion as a source of minerals.

Table 5: Mineral Content of leaves and fruit of *Boscia Senegalensis.*

Cations	Leaves	Fruits
Ca (mg/100 g)	100.30 ± 0.10	280.50 ± 0.10
Mg (mg/100 g)	5.84 ± 0.01	0.85 ± 0.01
Fe (µg/g)	159.57 ± 0.59	127.63 ± 0.25
Ni (µg/g)	0.50 ± 0.02	0.72 ± 0.03
Cu (µg/g)	144.83 ± 0.76	158.27 ± 0.64
Zn (µg/g)	15.43 ± 0.40	1.99 ± 0.08

IV. Conclusion

Boscia senegalensis is a wild plant that is widespread throughout the Senegalese territory and in certain regions of sub-Saharan Africa. While only the fruits of this tree are consumed in human food, the results obtained in this work show that in the leaves there is also the presence of polyphenols, flavonoids, proanthocyanidins, saponins and oligo-elements. The optimization of the aqueous extraction conditions shows that these conditions comply with the preparation of infusions. These results obtained on fruits and leaves constitute an interesting prospect for the development of new products in the fields of food and human nutrition.

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