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Original Article

Phytochemical Screening and Antioxidant Activity of the Fruits of *Boscia senegalensis* (Pers.) Lam. e.g. Pear. (Capparaceae)

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ABSTRACT

Objective: This study aims to assess the phytochemical composition and antioxidant activity of the fruits (pulp and seeds) of *Boscia senegalensis* from the Ferlo zone in Northern Senegal. **Material and Methods**: Fruit collection of *Boscia senegalensis* was carried out in three Ferlo's localities: Tessékéré, Labgar and Ranérou. The sample consists of 36 individuals selected randomly from each locality. The major chemical groups were determined by conventional methods using specific general reagents. The determination of total polyphenols and total flavonoids, the 2,2-diphenyl-1picrylhydrazyl (DPPH·) radical scavenging activity and ferric reducing of antioxidant power (FRAP) were evaluated by spectrophotometry. **Results and Discussion**: The results of the photochemical screening revealed the presence of saponins, alkaloids, sterols and triterpenes, flavonoids and polyphenols in both parts of the fruit (pulp and seeds). Assays carried out on extracts of pulp and seed powders reveals higher levels of total polyphenols and flavonoids in the pulp. The study of antioxidant activity has shown that hydro-ethanol extracts of pulp and seeds have a very interesting reducing activity, particularly in the pulp. However, we can see a weak antiradical activity of these extracts. The origin effect has a weak influence on the antiradical and reducing activity of the pulp and seed extracts. **Conclusions**: The biological activity of the harvested *Boscia senegalensis* extracts highlighted in this study could justify the traditional uses of this plant in the treatment of several pathologies. This fruit should be consumed in order to prevent few dietary imbalances or valorized in order to develop new bioactive compounds.

Key Words: Antioxidant activity, *Boscia senegalensis*, Fruits, Phytochemical screening.

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INTRODUCTION

Human beings has always lived with his environment where plants offer him enormous potential for feeding and caring for himself. Natural substances derived from plants have aroused growing interest from nutritionists, food manufacturers and consumers over the last ten years. One of many reasons for this growing interest is the recognition of their antioxidant properties and thus their likely involvement in the prevention of various pathologies associated with oxidative stress. They are widely used therapeutically as vasoconstrictors, anti-inflammatories, enzyme inhibitors, antioxidants and antiradicals, and antimicrobials.[1,2] These natural compounds are essentially secondary metabolites[1] present in all parts of higher plants (fruits, seeds, roots, stems, leaves, flowers, pollens) and are involved in many physiological processes such as cell growth, rhizogenesis, seed germination and fruit ripening.[3]

Secondary metabolites are mainly alkaloids, phenolic components (phenolic acids, flavonoids, tannins, and coumarins) and heterosides.[4,5] Some of these substances cause fewer disorders and are much more easily assimilated by the body than synthetic substances, most of which are questioned because of the potential toxicological risks they may cause. For this reason, new sources of natural antioxidants are being actively sought.[6,7] In recent years, the world of biological and medical sciences has been invaded by a new concept, that of "oxidative stress," a situation in which the cell no longer controls the excessive presence of toxic oxygen radicals.

In this regard, the search for plant sources rich in phenolic compounds to improve human health is pushed to the extreme. It is in this context that we became interested in *Boscia senegalensis*, a shrub of the Capparaceae family.

In the Ferlo zone, *B. senegalensis* is one of the most present woody species after *Balanites aegyptiaca* and *Leptadenia hastate*.[8] It is cited among the food and medicinal plants for the Ferlo populations.[9] The seeds of the plant are also used as a staple food in times of famine in the Sahelian zone in West Africa .[10] In traditional medicine, it is used in the treatment of diabetes [10] and male sexual weakness.[11] The seeds are consumed for its anthelmintic and vermifuge properties.[12]

Given the different medicinal and food uses to which it is put, it is interesting to review the biological activities of some families of compounds it possesses. The present study aims at the phytochemical characterization of Ferlo's fruits and the evaluation of the antioxidant activity of pulp and seed extracts.

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MATERIALS AND METHODS

Biological materials

Boscia senegalensis is a species of perennial woody plant (Figure 1A) of the genus *Boscia* belonging to the Capparaceae family (Capparales). It is a shrub or bush 1 to 5 m high, conical in shape, with a very dense crown and small diameter trunks with many branches. The leaves of the plant are small and solid. The plant produces fruits grouped in small clusters (Figure 1B), in the form of spherical yellow berries that are yellow at maturity and up to 8 mm in diameter. These fruits contain 1 to 4 whitish nuts (Figure 1C) and the edible kernel is greenish when ripe.[11]

Sampling

The samples were taken in three Ferlo's localities: Tessékéré, Labgar and Ranérou. The choice of the 3 localities was motivated by the availability and the importance given to this species from the nutritional, therapeutic and fodder point of view. The collection of fruits of *B. senegalensis* was carried out in July 2018 in the natural stands of the species. The sample consists of 36 individuals per locality. From each individual we collected as many ripe fruits as possible from different branches. Three composite batches, intended for phytochemical analyses, were then constituted per site.

Sample preparation

The composite fruit samples were first removed from their epicarp by hand and then pulped in distilled water using a Moulinex mixer. After filtration, the aqueous pulp extract obtained is evaporated using a rotary evaporator and then dried in an oven under ventilation to obtain a dry pulp residue. The seed samples are dried in the shade and then in an oven at 50°C, then shelled, crushed and sieved to obtain almond powder for analysis.

For the study of the antioxidant activity of seeds and pulp, 20 g of seed or pulp powder was decocted with 200 mL of ethanol-water mixture (80v/20v) under reflux for 30 min. The resulting filtrate was evaporated under vacuum using a rotary evaporator until a dry residue of hydro-ethanol extract was obtained.

Phytochemical screening

The phytochemical study consisted of researching the chemical families of seeds and pulp. This research was carried out, using the classical methods of identification of the major chemical families (alkaloids, saponosides, flavonoids, tannins, cardiotonic heterosides, anthracenes, sterols and triterpenes) by general reagents.[13]

Determination of total polyphenols

The determination of total polyphenols is based on the redox reaction using the Folin Denis reagent which is a mixture of sodium tungstate ($Na_2WO_4.2H_2O$) and ($12MoO_3.H_3PO_4$) phosphomolybdic acid.[13]

The determination of phenolic compounds is preceded by an extraction step carried out with a methanol-water mixture. The extraction of phenolic compounds by maceration consists of mixing 0.4 g of seed or pulp grind with 20 ml of 60% methanol. The mixtures are then subjected to magnetic stirring for one hour and filtered on filter paper. The extracts are centrifuged at 5000 rpm for 10 min and the supernatant is recovered.

The actual assay is performed with 2 mL of extract plus 2 mL of Folin Denis reagent. The mixture is homogenized with vigorous stirring. After 3 min incubation, 2 mL of a Na_2CO_3 solution (12.4% in water) is added to the mixture, stirred and incubated for 2 hours at room temperature in the dark. The sample is then centrifuged at low speed until the particulate matter is deposited. The reaction produces a blue coloration whose absorbance is read at 725 nm against a distilled water blank.[13] The calibration range is performed with a tannic acid standard. The results are expressed in mg tannic acid equivalent per g dry matter.

Figure 1: *Boscia senegalensis*: (A) adult individual; (B) cluster fruit; (C) fruit nuts

Determination of total flavonoids

Total flavonoids were determined using Marinova's method.[14] 50 mL of distilled water was added to 5 g of seed or pulp powder. After 5 min of homogenization with a magnetic stirrer, the solution was filtered with cotton wool. A volume of 2.5 mL of this filtrate is placed in a 25 mL flask and 0.75 mL sodium nitrite ($NaNO_2$) 5% is added. 0.75 mL of 10% aluminum chloride ($AlCl_3$) is added to the mixture. The resulting solution is incubated in the dark for 6 minutes.

After incubation, 5 mL sodium hydroxide solution (NaOH; 1N) is added. The volume is then made up to 25 mL with distilled water. After vigorous stirring of the mixture, the absorbance is measured at 510 nm (BioSystems BTS-3500). For each sample, the tests were repeated 3 times. The proportion of total flavonoids is then evaluated from a calibration curve established with rutin. The results are expressed in mg rutin equivalent per g dry matter.

Determination antioxidant activity

Two methods are used to evaluate the antioxidant activity of pure or extracted phenolic compounds. The first method is the reducing power estimation which measures the ability of extracts to reduce metal ions (FRAP = ferric reducing antioxidant power). The second takes into account the antiradical power by measuring the capacity of neutralization of a radical ($DPPH^{\cdot}$ or $ABTS^{\cdot+}$) by the antioxidants present in the samples.[15,16]

Scavenging of the 2,2-diphenyl-1picrylhydrazyl ($DPPH^{\cdot}$) Radical

The chemical compound 2,2-diphenyl-1-picrylhydrazyl (α, α -diphenyl-βpicrylhydrazyle) was one of the first free radicals used to study the antioxidant structure-activity relationship of phenolic compounds.[17]

The antioxidant effect of extracts vis-à-vis the DPPH radical is evaluated according to the method described by Molyneux.[18] The method consists of dissolving 4mg of DPPH powder in 100 ml of ethanol. The resulting solution is stored in a dark place for 12 hours. Then in a series of 10 test tubes containing 200 µl of hydro-ethanol extract at different concentrations, 800 µl of the DPPH solution is added. After 30 min incubation in the dark and at room temperature, the absorbance is read at a wavelength of 517 nm. Three determinations were carried out for each concentration tested (n=3). The results are expressed as percentage inhibition (PI) according to the following formula:

$$PI = (A0-Ae)*100/A0$$

With Ao absorbance of DPPH alone and Ae absorbance of the extract at a given concentration

Reduction of ferric iron: Frap (ferric reducing ability of plasma)

As the name suggests, this technique was developed to measure the ability of plasma to reduce ferric iron Fe(III) to ferrous iron Fe(II).

The method used is that described by Bassène.[13] Indeed, 400 µl of different concentrations of the pulp or seed extract (9.375 - 18.75 - 37.5 - 75 - 150 - 300 µg/ml) diluted in ethanol are mixed with 1 ml of phosphate buffer solution (0.2 M; pH 6.6) and 1 ml of potassium hexacyanoferrate. The mixtures were incubated at 50°C for 30 min. Then 1 ml of 10% trichloroacetic acid solution is added to each tube and centrifuged at 3000 rpm for 10 min. To 1 ml of the supernatant obtained were added 1 ml of distilled water and 200 µl of FeCl3 (0.1% m/v). The absorbance was measured at 700 nm using a UV/Visible spectrophotometer (BTS350).

A reactive blank, where the extract is replaced by ethanol, is treated in the same way as the extract. Ascorbic acid used as a positive control in this method has also been tested at the same concentrations. The reducing power is obtained by the following formula:

$$PR = [(Ae-Ab) / Ae] \times 100$$

With Ae: absorbency of the extract and Ab: absorbency of the white

STATISTICAL ANALYSIS

The experiments on the determination of polyphenols and the measurement of the antioxidant activity of the extracts were carried out in triplicate. The results were expressed as mean ± standard deviation. Statistical analyses were performed with the Fischer's test and values of $p<0.05$ were considered statistically significant.

RESULTS

Phytochemical screening

Table 1 presents the results of phytochemical screening tests of hydro-ethanol extracts of *B. senegalensis* pulp and seeds.

Phytochemical tests carried out on *B. senegalensis* fruit extracts have revealed the presence of saponosides, alkaloids, sterols and/or triterpenes, phenolic compounds including flavonoids. However, anthracenosides, tannins and cardiotonic heterosides are absent in both parts of the fruit.

Polyphenol contents

The total polyphenol contents of the various *B. senegalensis* powder extracts examined are expressed in terms of tannic acid equivalent (TAE/) from the standard equation of the ascorbic acid calibration curve (y = 1,1288x + 0,0136; R2= 0,9888) and expressed in ascorbic acid equivalent. Figure 2 shows the results obtained.

Analysis of Figure 2 shows that the pulp of *B. senegalensis* fruit concentrates six times more polyphenols than the seed. The polyphenol contents evaluated in the pulp and seed extracts are respectively 680.90 ± 6.90 and 118.63 ± 0.60 mg TAE/g DM at Tessékéré; 670.27 ± 7.40 and 104.85 ± 0.203 mg EAT/g DM at Ranérou; and 664.51 ± 2.77 and 103.22 ± 0.20 mg EAT/g DM at Labgar. Statistical analysis of these values shows that they are significantly different at the 5% cut-off between localities.

Flavonoid content

The flavonoid contents of the various extracts of pulp and seed powder, expressed as milligram rutin equivalent per gram dry matter (mg RE /g DM), were obtained from the calibration curve of equation y = 0.0062x; R^2= 0.9878. Flavonoid concentrations in *B. senegalensis* extracts are shown in Figure 3.

The results obtained indicate that the flavonoid content of the pulp is much higher than that of the seed. The flavonoid contents measured in the pulp and the seed respectively are of the order of 17.04 ± 0.10 and 1.151 ± 0.125 mg RE/g DM at Tessékéré; 17.04 ± 0.18 and 0.98 ± 0.16

Table 1: Phytochemical screening of hydro-ethanolic extracts of pulp and seeds.

Phytochemical groups	Seeds	Pulp
Alkaloids	+	+
Saponosides (foam index)	500	500
Hydrolysable tannins	-	-
Condensed tannins	-	-
Flavonoids	+	+
Cardiotonic heterosides	-	-
Anthracenosides	-	-
Sterols et triterpenes	+	+

(-): negative reaction; (+): positive reaction

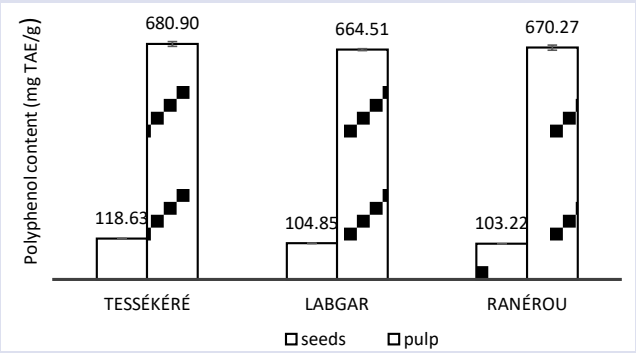

Figure 2: Polyphenol contents of seed powder extracts and pulp.

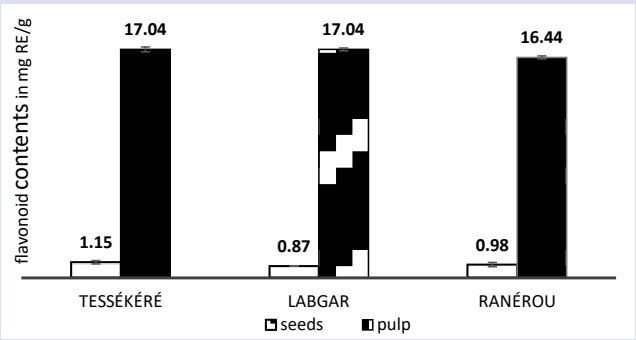

Figure 3: Flavonoid contents of seed powder extracts and pulp.

mg RE/g DM at Ranérou; 16.44 ± 0.11 and 0.871 ± 0.02 mg RE/g DM at Labgar. The results obtained indicate a slight variability between sites.

Antioxidant and reducing activity of the fruit of *B. senegalensis*

The tests carried out have demonstrated the antiradical activity of hydro-ethanol extracts of pulp and seeds (Figures 4, 5 and 6) as well as their reducing power (Figures 7, 8 and 9). The overall results obtained with the different concentrations used showed significant inhibition and reduction potentials of the extracts in a dose-dependent manner ($p<0,05$ versus negative control).

Antiradical properties of *B. senegalensis* fruit extracts

Tests carried out by the DPPH radical reduction method show that the antiradical activity of hydro-ethanol extracts from pulp and seeds is relatively weak compared to the reference standard (ascorbic acid). Compared to each other, the pulp extract is slightly more active than the seed extract at the different concentrations tested (Figure 4). The origin effect has a small influence on the antiradical activity of the fruits. Our results indicate a non-significant variability between sites at the seed and pulp level. However, it can be noted that extracts from Labgar seeds show a higher activity (Figure 5) and inhibition related to the activity of Ranerou a pulp extracts seems to be more important compared to the other sites (Figure 6).

Reducing properties (frap) of extracts of the fruit of *B. senegalensis*

It is based on the ability of the extracts to reduce ferric iron Fe(III) to ferrous iron Fe(II). The results indicate that the reducing activity of the pulp and seed extracts is much less than the control (ascorbic acid). However, the reducing power of the pulp extract is much greater than that of the seed (Figure 7). Overall, Labgar fruit was more reducing compared to other sources (Figures 8 and 9). Statistical analysis of these different values indicates that they are significantly different at the 5% threshold between localities.

DISCUSSION

Phytochemical tests carried out on the pulp and seeds of *B. senegalensis* indicate the presence of saponosides, alkaloids, sterols and/or triterpenes and phenolic compounds, particularly flavonoids. Saponosides and alkaloids were also characterized in *B. senegalensis* seed extracts by Adam

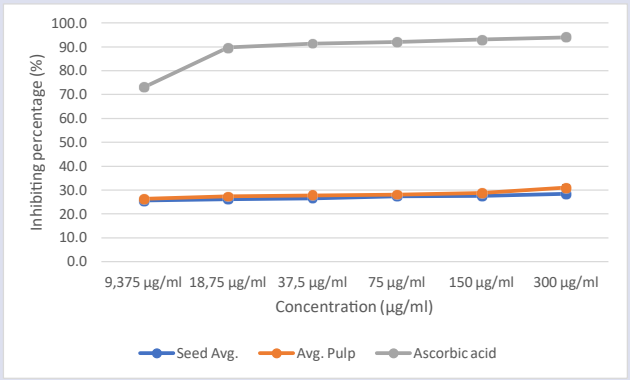

Figure 4: Anti-radical activity of pulp and seed extracts and comparison with control (ascorbic acid).

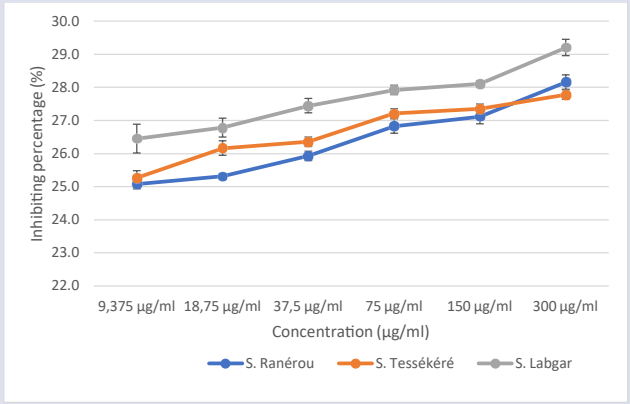

Figure 5: Antiradical activity of seed extracts: Variability between sites.

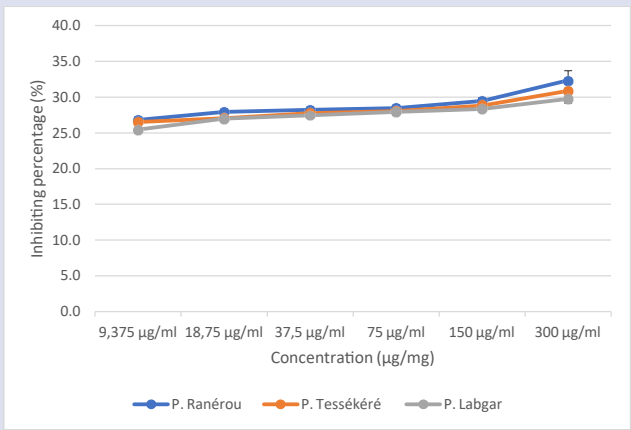

Figure 6: Antiradical activity of pulp extracts: Variability between sites.

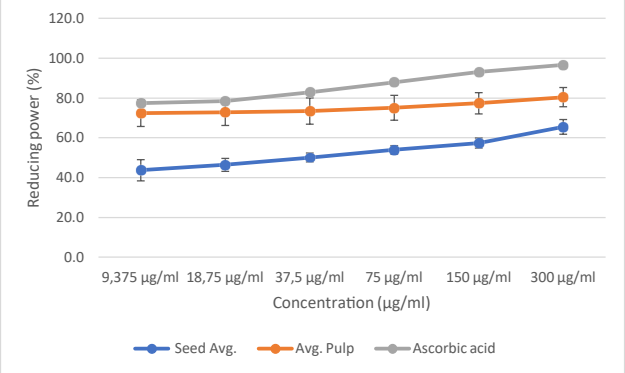

Figure 7: Reducing activity of pulp and seed extracts and comparison with control (ascorbic acid).

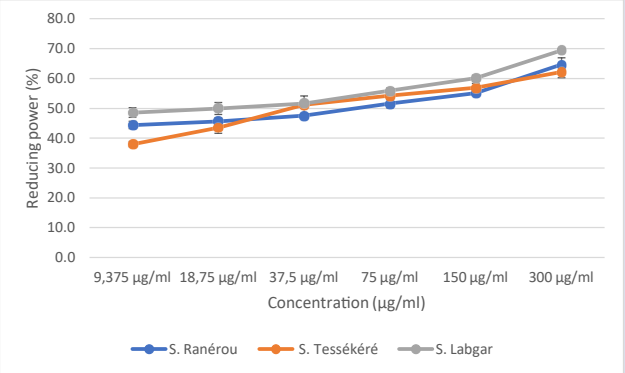

Figure 8: Reducing activity of seed extracts: Variability between sites.

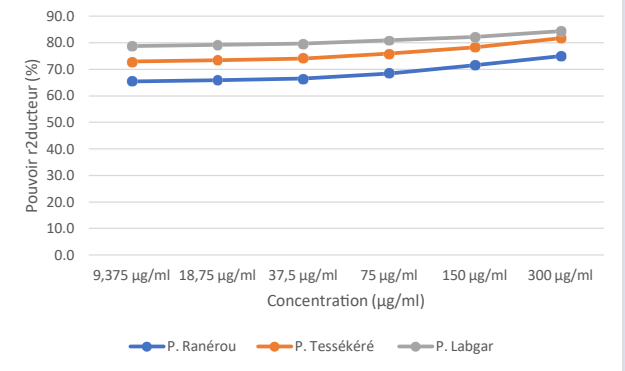

Figure 9: Reducing activity of pulp extracts: Variability between sites.

Sakine[11] in Chad, but flavonoids, triterpenes and sterols were not present in their extracts. Saponosides have a wide range of pharmacological properties such as analgesic and antidepressant effects[19], hemolytic properties[20,21], as well as antimicrobial and molluscicidal activities.[21] Alkaloids are known for their stimulating, sympathomimetic and anticholinergic properties. Some play the role of local anaesthetics, antimalarial drugs (quinine).[22] In addition, triterpenes are reported to have the following biological activities: antioxidant, hypoglycemic,[23] hypocholesterolemic,[24,25] immunomodulator,[26] antibacterial,[27] antiproliferative.[28] However, to our knowledge, there are no reports on the phytochemical composition of the fruit pulp of *B. senegalensis*.

The results of the assay revealed a high concentration of phenolic compounds especially in the pulp. These bioactive molecules play a very important role in the fight against reactive oxygen species (ROS), thus minimizing molecular damage. It should be noted that the differences in phenolic compound contents between localities could be explained according to Merouane[29] by geo-climatic factors. These results are very significant compared to those obtained at seed level by Deli et *al.*[30] on a hydro-methanolic extract from seeds at d'Oum - Madjer (Batha State, Chad) (1.60 ± 0.04 mg GAE/ g dry extract).

On the other hand, the dosage of flavonoids, one of the most important polyphenol groups in plants, revealed a small amount in the seeds and a significant amount in the pulp. Flavonoids generally protect plant tissue from the harmful effects of sunlight. This could explain their unequal distribution in the two parts of the fruit of *B. senegalensis*, since the pulp is more exposed to sunlight than the seeds and has the highest content. More important results were evaluated by Deli et *al.* [30] on *B. senegalensis* seed (0.24 ± 0.01 mg RE / g).

Studies have already shown that flavonoids are powerful antioxidants and have been shown to have beneficial properties for health, including antimicrobial, antibacterial, anti-inflammatory, antiviral, anti-tumor, vasculo-protective, anti-hepatotoxic, anti-allergic, anti-ulcer, etc.[31,32]

This suggests that these groups of compounds may be involved in the mechanisms of recovery of human or animal health. Indeed, in many African countries, *B. senegalensis* is used for the treatment of animal diseases, notably foot and mouth disease in the far north of Cameroon.[33]

The evaluation of antioxidant activity is becoming increasingly relevant in the field of nutrition in that it provides useful information on the quality of the raw material.[34] The parameter takes into account the presence of effective oxygen radical scavengers, such as vitamin C and phenolic compounds, as well as their synergistic and/or antagonistic effects.

Indeed, it has been established that antioxidants can prevent the oxidative stress involved in the genesis of several cardiovascular diseases.[35] This oxidative stress, a consequence of an imbalance between pro-oxidants and antioxidants in the body, is now recognized as a key phenomenon in the occurrence of chronic diseases.

The hydro-ethanol extracts of pulp and seeds showed weak antiradical activity. This low activity could be explained by their low flavonoid content. In addition, it was shown by several authors including[36] that the antioxidant activity of plant products is generally attributed to the radical scavenging activity of phenolic compounds such as flavonoids, polyphenols and tannins. Compared to ascorbic acid used as an antioxidant reference, hydro-ethanol extracts of *Boscia senegalensis* were relatively less active against DPPH radicals. Evaluation of the antioxidant activity of *B. senegalensis* seeds by the DPPH method was reported by Deli et *al.*[30] at an IC_{50} value of 86.160 µg/ml. However, our values are relatively lower than those reported by these authors.

On the other hand, our results showed a very interesting antioxidant activity of the extracts with regard to the reduction of ferric iron to ferrous iron. This reducing power is probably linked to the presence of hydroxyl groups in phenolic compounds that can serve as electron donors. Consequently, our extracts contain phenolic compounds and flavonoids that may be responsible for their reducing activity. In addition to the presence of phenolic compounds (flavonoids in particular), the fruits of *B. senegalensis* also contain other well-established antioxidants such as ascorbic acid.[37,38] This last compound has a powerful power to neutralize oxidizing agents generated during normal human metabolism. The differences in results observed within localities can be explained by the differences in polyphenol and flavonoid contents evaluated previously. No work on the antioxidant activity of pulp extracts has been dealt with by researchers in this field.

CONCLUSION

At the end of our study, it appears that the phytochemical tests carried out made it possible to highlight flavonoids, sterols and triterpenes, alkaloids, saponosides in both parts of the fruit (pulp and seed). In addition, the assay of total polyphenols and flavonoids revealed a differential distribution between the two parts of the fruit (pulp and seed). Indeed, the pulp is richer in polyphenols and flavonoids than the seeds.

These phenolic compounds have multifunctional properties and can act as singlet oxygen scavengers and trap free radicals. The hydro-ethanol extracts of fruits (pulp and seeds) have shown good reducing activity, especially the pulp extract, and a significant free radical scavenging capacity.

The fruit of this plant is a potential source of antioxidants of natural origin which justifies its traditional use in the treatment of many ailments.

The consumption of this fruit should be encouraged to prevent certain dietary imbalances or enhance with the aim of developing new bioactive compounds.

Current investigations are directed towards biochemical and nutritional characterization, the study of acute and sub-acute toxicity and the identification of specific antioxidants contained in the fruit, responsible for their antioxidant activity, particularly reducing.

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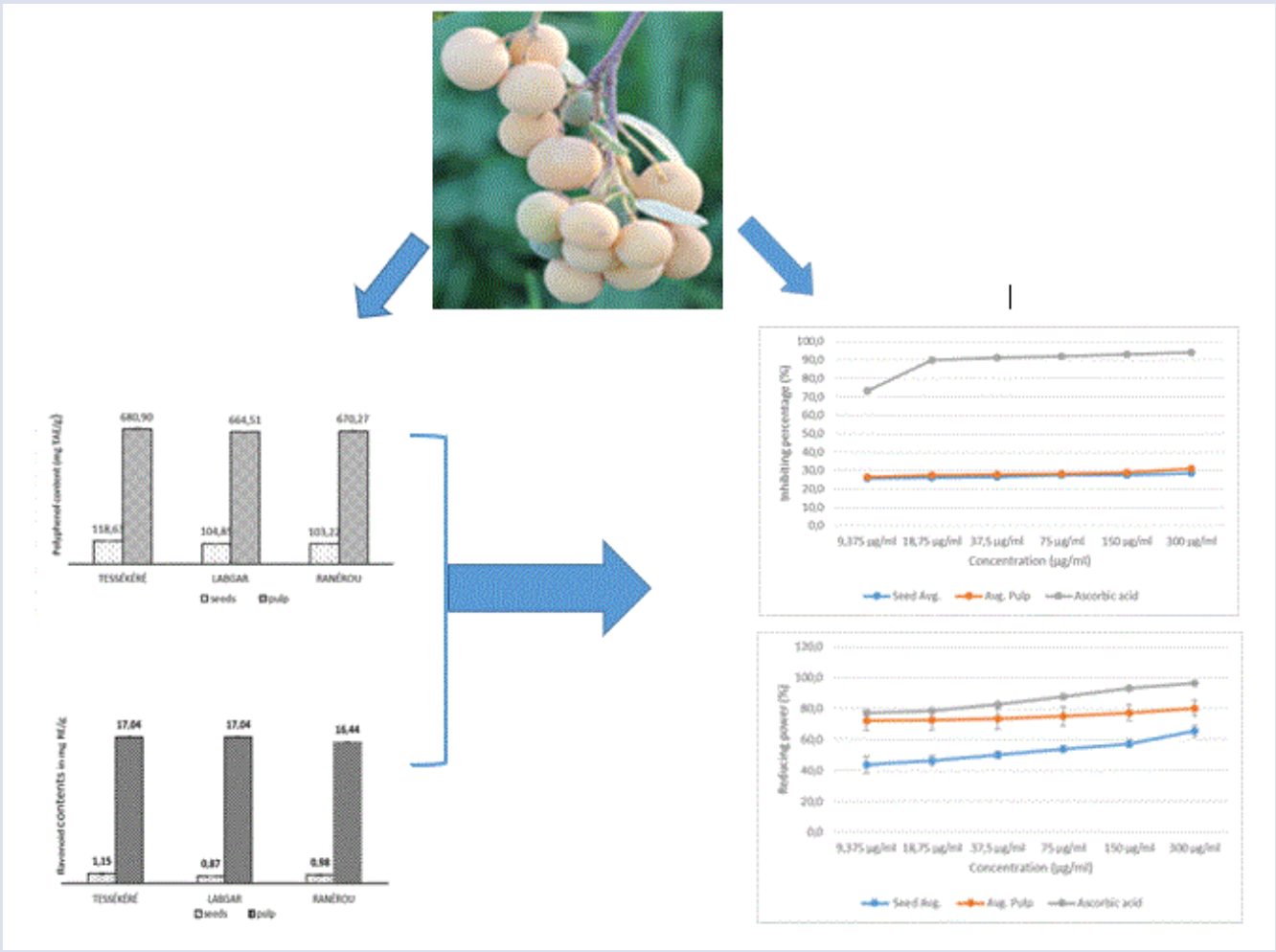
GRAPHICAL ABSTRACT



SUMMARY

Extracts of *Boscia senegalensis* seed and pulp powders contain high levels of polyphenols and significant levels of flavonoids. The hydro-ethanol extracts of these fruits have a very interesting antioxidant activity, particularly reducing at the pulp level. This activity could be partly explained by the presence of polyphenols and flavonoids.

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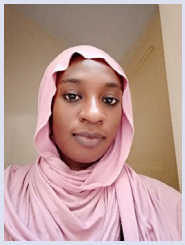

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