

Antioxidant Potential and Antiradical Activity of Two Bean Varieties (*Phaseolus vulgaris* L.) Cultivated in Congo

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Abstract

Plants are reservoirs of molecules of great interest in medicine, including degenerative diseases mainly due to oxidative stress and those caused by deficiencies in certain micronutrients. Since ancient times, in the Congo and elsewhere, humans have used natural resources to treat certain ailments. Among the plants used in traditional medicine is *Phaseolus vulgaris*. The methodology used here, based on qualitative research of chemical families, allowed us to identify terpenoids. Extracts of *P. vulgaris* revealed the presence of flavonoids as well as polyphenols. Quantitatively, the determination of total polyphenols yielded the following results: *P. vulgaris* var. Kitaka: 266.04 mgGAE/100g DM; *P. vulgaris* var Lékana: 602.91 mgGAE/100g dry matter. Regarding total flavonoids, we obtained: *P. vulgaris* var Kitaka: 120.86 mgEC/100g dry matter; *P. vulgaris* var Lékana: 289.98 mgEC/100g dry matter. TLC for the detection of free radical scavenging activity yielded a positive result for each sample. The free radical scavenging activity assay provided the following results regarding IC 50: *P. vulgaris* var Kitaka: 241.756 mg/ml; *P. vulgaris* var Lékana: 148.124 mg/ml. The bean seeds of the studied varieties have significant phytotherapeutic properties.

Keywords

Phytochemical Study, Seeds, Bean, Inhibitory Concentrations, Congo

1. Introduction

Phaseolus vulgaris L. is a legume consumed worldwide for its seeds and pods [1]. It belongs to the *Fabaceae* family, whose morphological characteristics are well known. The bean fruit is a two-valved pod, 4 to 25 cm long, typically containing 4 to 10 seeds [2].

Beans are produced in subtropical and tropical regions, most often by small-holder farmers, and constitute a major staple crop in both developing and developed countries.

In Congo, beans are consumed in most departments as a supplementary food; the seeds consumed include both locally produced and imported varieties. In the departments of Niari, Bouenza, and Pool, this plant is listed among the main crops contributing to household energy intake, alongside cassava, peanuts, and maize. These three southern departments (Niari, Bouenza and Pool) and the Plateaux department in the center (Lékana District) are the main centers of bean production in Congo.

Local bean production in Congo is based on several cultivars, but there is currently no exhaustive inventory or characterization of existing varieties. Among the bean cultivars inventoried by the National Institute of Agronomic Research (IRA) are the “White Kikata” variety, grown mainly in the south in the departments of Niari, Pool and Bouenza, and the “Lékana” variety produced in the center of the country in the Plateaux department [3]. These two cultivars produce white seeds, but they are particularly distinguished by their growth habit: “White Kikata” is a dwarf variety with determinate growth, while “Lékana” is a twining variety with indeterminate growth, the cultivation of which requires the use of stakes or poles [3]. The seeds from these two varieties are among the most marketed and consumed in our country. However, to date, the biochemical characterization of these seeds has not yet been done. Most of the work on beans in Congo-Brazzaville focuses more on the study of the potential of this crop, on increasing production yields, improving productivity, resistance to diseases and pests. The characteristics supporting consumption, including the chemical composition of seeds commonly used in meals, are not often taken into account by research. Thus, this present study on the antioxidant potential of “White Kikata” and “Lékana” seeds is a contribution to the knowledge of other needs sought by consumers of these bean varieties.

The scientific objective of this study is to determine the antioxidant potential of the seeds of these local varieties: “White Kikata” and “Lékana”. The goal here is to investigate the total polyphenols, total flavonoids, and their anti-free radical properties in these seeds.

2. Materials and Methods

2.1. Plant Material

The plant material used for this study consists of the seeds of two bean varieties: the “White Kikata” variety, grown primarily in southern Congo in the Niari, Pool,

and Bouenza Departments, and the “Lékana” variety, grown in the center of the country in the Plateaux Department (Lékana and Djambala).

The seeds studied were purchased from bean producers in Boko-Songho (Bouenza Department) for the “White Kikata” variety and in Lékana (Plateaus Department) for the “Lékana” variety (**Figure 1**, **Figure 2**).



Figure 1. Seeds of the “White Kikata” bean.



Figure 2. Seeds of the “Lékana” bean.

Agriculture in the Congo is not mechanized; it rarely uses chemical fertilizers and pesticides. Bean cultivation in all departments of the Republic of Congo is done naturally without the use of chemical fertilizers or pesticides. The resulting end product is completely natural.

2.2. Methods

2.2.1. Determination of Polyphenols and Flavonoids and Evaluation of Antioxidant Potential

1) Preparation of Extracts

To obtain the seed extracts for analysis, we proceeded as follows:

- Maceration: 30 g of each crushed and defatted sample was macerated in 300

ml of hydroethanolic solvent (50% v/v). The resulting solution was kept at room temperature for 48 hours.

- Filtration and evaporation of the macerated solution: After 48 hours, the macerated solution was filtered and then concentrated by evaporation in a rotary evaporator.

2) Total Polyphenol Determination

The determination of total polyphenols was performed using a spectrophotometer. We determined the optical densities of the extracts and then compared the results to those obtained using a gallic acid standard. The determination was performed as follows: 0.9 ml of distilled water, 0.9 ml of Folin-Ciocalteu reagent (1N), and then 0.2 ml of a 20% Na₂CO solution were added to 0.1 ml of the plant extract placed in a test tube. The resulting mixture was incubated at room temperature for 40 minutes, protected from light. Absorbance was then measured using a spectrophotometer at 725 nm against a methanol solution used as a blank. The results obtained were expressed in mg gallic acid equivalent per 100 grams of dry matter (mgGAE/100 gMs).

2.2.2. Total Flavonoid Determination

Total flavonoids were also determined using a spectrophotometer as follows: 250 µl of the extract and 1 ml of distilled water were successively added to a test tube. At the initial time (0 minutes), 75 µl of a NaNO₂ solution (5%) were added, followed by 75 µl of AlCl₃ (10%) 5 minutes later. After 6 minutes, 500 µl of NaOH (1N) and 2.5 ml of distilled water were successively added to the mixture. The absorbance of the resulting mixture was directly measured using a UV-visible spectrophotometer at 510 nm, and the results were expressed as mg catechin equivalent per 100 grams of dry matter (mgEC/100 gMs). A calibration curve was developed with standard solutions of Catechin prepared at different concentrations.

2.2.3. Evaluation of the Antiradical Activity: Measurement of the Inhibitory Activity by UV-Visible Spectrometry

The antiradical activity was quantitatively evaluated by the DPPH test according to the protocol described by Hennebelle [4]. The solution test was carried out by mixing 10 ml of a 10 mg/250ml DPPH solution with 100 µl of the extract to be tested at different concentrations (60 mg/ml; 30 mg/ml; 15 mg/ml; 7.5 mg/ml; 3.75 mg/ml).

The activity was then measured at 517 nm using a spectrophotometer after 40 minutes of incubation away from light. The percentage inhibition was calculated as follows:

$$I\% = (\text{Blank Absorbance} - \text{Test Absorbance}) / (\text{Blank Absorbance}) \times 100$$

Abs0: Blank absorbance against a methanolic DPPH solution

Abst: Test absorbance

DPPH (2,2-Diphenyl-1-Picryl-Hydrazyl) is a stable radical and exhibits a characteristic absorption at 510 nm, which gives it a violet color. This color disappears

quickly when DPPH is reduced by a free radical scavenger (phenolic compounds).

The main mechanism of action is the trapping of free radicals by the transfer of the H atom to DPPH, which then transforms into a stable molecule, DPPHH.

The antiradical effect of the different extracts will be expressed as percentage inhibition of the DPPH radical. This antioxidant effect varies depending on the different concentrations of the extracts and their optical density, as defined by the spectrophotometer. Studying the variation in antiradical activity allows us to determine the concentration corresponding to 50% inhibition (IC₅₀). To determine the IC₅₀, we first found the equation of the regression line defined by the variation in extract concentrations; this line is of the form $Y = aX + b$. The IC₅₀ is equal to the value of x corresponding to $Y = 50\%$.

A low IC₅₀ value corresponds to a high antioxidant efficacy of the extract.

2.2.4. Statistical Analyses

All measurements were performed six times, depending on reagent availability. Statistical data processing was performed using MINITAB 17 software. Results are expressed as mean \pm standard deviation. Comparisons between multiple means were performed using analysis of variance (ANOVA). ANOVA was performed to determine significance at the 5% level for physicochemical parameters.

3. Results and Discussion

3.1. Total Polyphenol (TP) and Total Flavonoid (TF) Determination

Polyphenol levels were calculated from the linear regression equation of the calibration curve, plotted using gallic acid as the standard (Figure 3). The linear regression formula for this curve is: $Y = 1.8826X - 0.0095$ with a correlation coefficient R_2 equal to 0.9974. The results are expressed as mg gallic acid equivalent per gram of dry matter (mgGAE/g Dm).

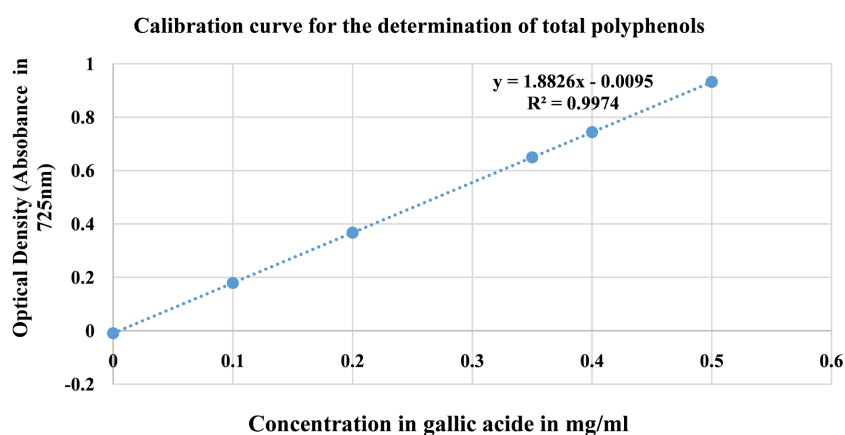


Figure 3. Calibration curve for the determination of total polyphenols.

3.2. Calibration Curve for Total Flavonoid (TFF) Determination

Total flavonoid levels were calculated from the linear regression equation of the

calibration curve plotted using catechin as the standard (Figure 4). The linear regression formula for this curve is: $Y = 2.4057X + 0.0317$ with a correlation coefficient R^2 equal to 0.9931. The results obtained are expressed as mg catechin equivalent per gram of dry matter (mgEC/g Dm).

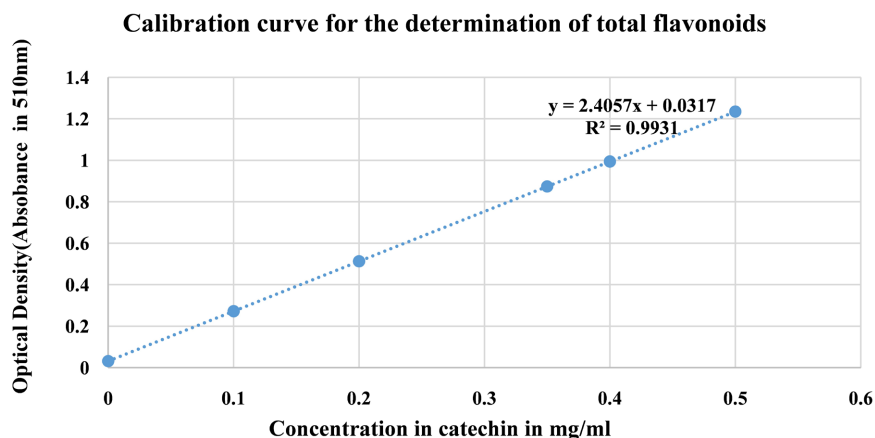


Figure 4. Calibration curve for the determination of total flavonoids.

3.3. Determination of Total Polyphenols and Total Flavonoids in Hydroethanolic Extracts

The results of quantitative analyses of hydroethanolic extracts using UV-visible spectrophotometers are presented below (Table 1):

Table 1. Polyphenol and flavonoid content of the analyzed extracts.

Polyphenols content		Flavonoids content	
Variety KB	Variety LKN	Variety KB	Variety LKN
266.04 mgEAG/ 100gMs	602.91 mgEAG/ 100g Ms	120.86 mgEC/ 100gMs	289.98 mgEC/ 100gMs

KB: White Kitaka; LKN: Lékana.

These results show that seeds of the LKN variety are quantitatively richer in total polyphenols and flavonoids than those of the KB variety. Total polyphenol levels are 602.916 mgGAE/100gMs for the LKN variety and 266.042 mgGAE/100gMs for the KB variety. Total flavonoid levels are 289.981 mgEC/100gMs and 120.861 mgEC/100gMs for the LKN and KB varieties, respectively.

Several factors could explain the significant difference observed between the levels expressed by the two varieties: plant genotypes, their growing environment (soil and climate), the treatments the plants received during the growing season, etc. The abundance of total polyphenols and flavonoids manifested by the LKN variety would make it an interesting cultivar in the prevention of pathologies due to free radical attacks (cancer, diabetes, cardiovascular diseases, etc.). The quantities of total polyphenols and flavonoids obtained on the LKN variety (602.916 mgGAE/100gMs and 289.981 mgEC/100gMs respectively) are close to those found

in a study conducted (6.37 mgGAE/g and 3.64 mgEC/g) on a collection of Vietnamese cultivars [5]. The values obtained on the KB variety: 2.66 mgGAE/g and 1.209 mg EC/g respectively for total polyphenols and total flavonoids, are close to those reported by a study conducted in Algeria: 1.36 mgGAE/g for total polyphenols and 1.125 mgEQ/g for total flavonoids [6].

On the other hand, the values we found are higher than those obtained on a batch of 26 Mexican bean varieties (maximum values recorded: 5.4 mgGAE/g for total polyphenols and 0.78 mg EC/g for total flavonoids) [7].

However, our results are weak compared to a study conducted in the United States of America and which evaluated a series of 29 bean cultivars including 7 with white seeds [8]. The maximum content of total polyphenols recorded on white seeds in their study is around 11.35 mgGAE/g Ms. This last value is almost 2 times higher than that which we obtained on the LKN variety (6.029 mgGAE/g Ms). Certainly factors related to the environment, experimental conditions and genotypes of the varieties could explain this excessively large gap. Regarding their genotypes, referring to the bibliography, several authors maintain that white bean seeds generally have low levels of polyphenolic compounds compared to colored seeds. This observation has been made by other researchers [9]-[11]. The latter emphasize that colored dried beans were found to be richer in polyphenols than white dried beans. The polyphenol values of 602.916 mgGAE/100gMs obtained on Lékana beans and 266.04 mgGAE/100gMs for Bouenza beans (Kitaka) show that these beans are richer in polyphenols than the seeds of *P. macrophylla* (160.73 mg GAE/gMS); *P. glabra* (8.42 mg EAG/gMS) and *T. conophorum*: 5.5 mgGAE/gMS studied in Congo Brazzaville [12]. These two varieties of beans (120.86 mgEC/100gMs for Kitaka and 289.98 mgEC/100gMs for Lékana) studied are also richer in total flavonoids than the seeds of *P. macrophylla* (0.48 mgEG/gMS); of *T. conophorum* (0.133 mg EG/g DM) and *P. glabra* (0.183 mg EG/g DM) studied in Congo Brazzaville [12].

Lékana beans are richer in total polyphenols than *I. batatas* and *C. pepo* studied in Congo Brazzaville and whose values are respectively 536.02 ± 0.01 and 533.60 ± 0.05 mg GAE/100g. However, the latter two are richer in total polyphenols than beans from southern Congo (Kitaka variety) [13].

3.4. Antiradical Activity: Quantitative Evaluation of the Antioxidant Effect Using the DPPH Test

3.4.1. Percentages of DPPH Radical Inhibition as a Function of Extract Concentrations

The antiradical effect of hydroethanolic extracts of bean seeds on the DPPH radical, assessed using a spectrophotometer, is accompanied by a change from its violet color (DPPH*) to a slight color change tending toward yellow (DPPH-H). The results of the quantitative evaluation of the antioxidant effect are expressed as % inhibition of the DPPH radical as a function of the different concentrations of the extracts. These results are presented in the table below (Table 2):

Table 2. Percentage of DPPH radical inhibition as a function of the different concentrations of the extracts.

Concentration of the extract in mg/ml	Variety LKN		Variety KB	
	Optical Density measured at 517 nm	Percentage of inhibition of the DPPH radical	Optical Density measured at 517 nm	Percentage of inhibition of the DPPH radical
60	0.866	17.0498	0.937	10.249
30	0.923	11.5900	0.985	5.651
15	1.036	0.7663	1.067	-2.203
7.5	1.051	-0.6705	1.06	-1.533
3.75	1.065	-2.0115	1.043	0.096

Note: White Optical Density = 1.044.

3.4.2. Measurement of the 50% Inhibitory Concentration (IC50)

The variation of the antiradical activity (expressed as % inhibition of the DPPH radical) makes it possible to determine the concentration corresponding to 50% inhibition (IC50). Using Microsoft Office Excel software, we defined the equations of the straight lines of the linear regression of the values of the percentages of inhibition and calculated the IC50 of each extract analyzed. The extract of the seeds of the variety “Lékana” presented a better potential of inhibition of the radical DPPH (IC50 = 148.124 mg/ml) than that of the seeds of the variety “White Kikata” (IC50 = 241.756 mg/ml). Indeed, as we have already emphasized in the chapter on materials and methods, a low value of IC50 corresponds to a high antioxidant efficiency of the extract. These values obtained are lower than those obtained on *T. conophorum* (178.09 mg/ml); *P. glabra* (45.82 mg/mL) and *P. macrophylla* (0.71 mg/mL) [12]. The seeds of the beans studied have a great phytotherapeutic advantage compared to the kernels of the seeds of *T. conophorum*; *P. glabra* and *P. macrophylla*.

This greater antiradical activity expressed by the extract of the seeds of the LKN variety compared to the extract of the seeds of the KB variety seems to agree with the results of the dosage of total polyphenols and flavonoids. Our results indeed reveal the superiority of the quantities of these metabolites in the seeds of the variety from the Plateaux. The abundance of these metabolites in the extract of the seeds of the LKN variety from the Plateaux could explain the superiority of the antiradical activity of these seeds. According to Pietta, polyphenols and flavonoids are excellent antioxidants whose redox property allows them to act as reducing agents, hydrogen donors and oxygen inhibitors [14]. This interpretation is consistent with that given by Tabart [15], who showed that the inhibition value of DPPH is proportional to the concentration of antioxidants.

On the other hand, some authors argue that trace elements (Cu, Zn, Se, Mn, Fe, etc.) act as cofactors of enzymes constituting the first line of defense against oxidative attacks [16]. They are catalysts of these enzymes. In this study, not all mineral substances contained in the analyzed seeds were highlighted. However, our analyses reveal the presence of a greater quantity of ash (mineral substances) in

the seeds of the LKN variety. The abundance of mineral substances in these seeds could also contribute to the expression of a strong antiradical activity.

The values of the antiradical potential obtained in this study are very low compared to the results of some works reported in the literature. A study conducted in Zimbabwe recorded inhibition percentages of 76.45% at 250 µg/mL (*i.e.* 76.45% at 0.250 mg/ml) for white seeds and 98.11% at 250 µg/mL (*i.e.* 98.11% at 0.250 mg/mL) for brown seeds [17]. These figures are very high compared to those we recorded (50% at 148.124 mg/mL for the seeds from the plateaus and 50% at 241.756 mg/mL for those from Bouenza). Some authors have shown that antioxidant activity varies significantly depending on the color of the seeds in the following order: Brown > black > white [17]. Similar work by other authors has shown that white seed coats do not exhibit any antioxidant activity [18], while red and black seed coats show significant antiradical activity [19] [20]. This implies that dark-hulled bean seeds have better antioxidant activity than white-hulled ones.

3.4.3. Varietal Difference and Seed Use

The results we obtained reveal a very clear difference between the two varieties, regarding the concentrations of organic molecules, mineral substances, total polyphenols, total flavonoids, and free radical scavenging potential. As shown in the previous graphs, organic molecules (carbohydrates, proteins, fiber) as well as parameters related to organic molecules such as energy value are higher in the KB variety grown in Bouenza than in the LKN variety from the Plateaux. Conversely, the levels of mineral matter, antioxidant molecules (total polyphenols and total flavonoids), as well as parameters related to antioxidant molecules such as Inhibitory Concentration 50, are higher in the LKN variety than in the KB variety.

This difference could be related to the genotypes of these two plants, their growing environment (climatic factors, soil texture, etc.), or cultivation techniques (fertilization, for example). Future research may provide a better understanding of the factors underlying this nuance. However, this difference could be exploited, for example, in the composition of food flours. A mixed use of flours from both plants should be considered. Flour from the KB variety would provide more organic molecules and energy, while that of the LKN variety would be a good source of mineral salts necessary for the body. This opinion may find application in the field of infant nutrition, where, after weaning, food flours must provide weaned children with the major nutrients: proteins, lipids, and carbohydrates in balanced proportions [21].

Furthermore, thanks to their superior anti-radical potential, the use of seeds from the “Lékana” variety in diets designed to prevent diseases related to free radicals, such as cancer, type 2 diabetes, cardiovascular and neurodegenerative diseases, may produce better results compared to those from the “White Kikata” variety.

4. Conclusion and Outlook

This study was conducted to evaluate the antioxidant potential of two bean varie-

ties: “White Kikata” (KB) grown in Bouenza, Niari, and Pool, and “Lékana” (LKN) grown in the Plateaux department. These two varieties are among the most widely marketed and consumed in our country, and the results of this study showed that their free radical scavenger compounds differ quantitatively.

Regarding polyphenol and flavonoid levels, and free radical scavenger activity, the “Lékana” variety yielded the highest values (602.91 mgGAE/100g DM, 289.98 mgEC/100g DM, IC₅₀ = 148.124 mg/ml for polyphenols, flavonoids, and free radical scavenger potential, respectively) compared to the “White Kikata” variety. Several factors could explain the observed differences in the results obtained: environmental factors, genetic factors, and factors related to cultivation.

The results of this study may help promote the use of these varieties in various fields, but more specifically in the health field.

To complement this study, it would be interesting to determine the different molecules that make up the polyphenolic and terpenoid fractions.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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