



Phytochimic Study, Antioxidant Activity and Nutritional Interest of Extracts from Leaves of *Khaya senegalensis* (Desr) A. Juss (*Meliaceae*) Collected in the Northern Cote d'Ivoire

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Authors' contributions

This work was carried out in collaboration among all authors. Author KM designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors TYZ and KKF managed the analyses of the study. Author TA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Medicinal and food plants contain a large number of metabolites that have multiple interests in pharmacology, cosmetology and the food industry. The aim of this study was to determine the content of phenolic and flavonoid compounds, to evaluate the antioxidant activity and to raise the

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nutritional interest of extracts from leaves of *Khaya senegalensis*. The methodological approach consisted of carrying out extractions with distilled water, ethanol, methanol and butanol. The extracts thus obtained were subjected to phytochemical analysis by spectrophotometric assay to determine the content of minerals, total phenols and flavonoids and to evaluate the antioxidant activity. The investigations revealed that *K. senegalensis* leaves extract have a high calcium content (948.38 ± 11.57 mg / 100 g), magnesium (188.24 ± 0.97 mg / 100 g), phosphorus (304.98 ± 2.6 mg / 100 g) and iron (41.50 ± 1.57 mg / 100 g). A high content of total phenols and flavonoids was observed with butanol extract (91.53 ± 0.04 mg EAG / g extract) and (75.58 ± 0.05 mg EQ / g extract) respectively. The ethanol and methanol extracts recorded the best performances by reducing the residual iron while the aqueous and butanol extracts obtained the IC₅₀ values closest to those of the reference viz vitamin C and BHT. This study showed that *K. senegalensis* leaves are a source of active substances known for their therapeutic and nutritional properties. The leaves of *K. senegalensis* could be used to treat various pathologies and also as an alternative in cattle feeding.

Keywords: *Phytochemical study; antioxidant activity; minerals; Khaya senegalensis; nutritional interest.*

1. INTRODUCTION

Medicinal plants are widely used, in rural areas, to solve human and animal health problems. These plants contain a large number of metabolites that have multiple interests in pharmacology, the food industry and cosmetology [1]. The active substances have been phenolic compounds, coumarins, alkaloids, saponins, mucilages, volatile compounds, sterols and terpenes. Phenolic compounds or polyphenols are characterized by the presence of an aromatic ring bearing free hydroxyl groups or groups engaged with a carbohydrate [2]. They are present in all parts of higher plants (roots, stems, leaves, flowers, pollen, fruits, seeds and wood) and are involved in many physiological processes such as cell growth, rhizogenesis, seed germination or ripening [2]. They are powerful antiallergic, anti-inflammatory, hepatoprotective, antimicrobial, antiviral, anticarcinogenic, cardioprotective [3]. They occupy a prominent place in the prevention of various neurodegenerative diseases related to age and certain cancers [4]. Polyphenols also prevent lipid peroxidation and the formation of free radicals that promote oxidative stress. The evaluation of their antioxidant phytopharmaceutical properties remains a very interesting and useful task [5]. The chemical structure of these substances gives them a highly developed ability to bind to all kinds of molecules, essentially proteins and to inhibit the oxidative process [6]. These substances are of great interest in number of areas. Their consumption makes it possible to prevent various diseases, in particular those mentioned above [7]. According to same authors, they are involved

in the conservation of food products in agro-food industries. Recent publications have mentioned the possible toxic properties of synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) on human health [8]. Polyphenols could therefore be an alternative to the use of synthetic food additives. Moreover, several scientific studies have been developed for the extraction, identification and quantification of polyphenols from different sources such as agricultural and horticultural crops or medicinal plants [9,10]. This research is of particular interest. A contribution through the study of leaves of *K. senegalensis*, a plant with multiple virtues used by rural populations, not only to treat ulcers and dermatoses but also as an alternative for feeding cattle during the dry season. The recommended use of *K. senegalensis* leaves in traditional environment has motivated this scientific study. Our purpose was to determine the phytochemical composition and evaluate the antioxidant activity of the leaves of this plant. This study will justify the use of *K. senegalensis* and consider the formulation of a recipe for livestock nutrition during drought.

2. MATERIALS AND METHODS

The plant material used in this study was leaves of *K. senegalensis*. They were harvested in the kouto department of Northern Côte d'Ivoire in March 2019. This plant was identified by Professor AKE Assi Laurent of the National Floristic Center of the University Felix Houphouët Boigny in 2010 during an ethnobotanical study carried out on the medicinal plants used in the treatment of abdominal infections [11].

2.1 Methods

2.1.1 Prepration of total extracts

The leaves were thoroughly washed with running water, dried at room temperature away from light, and then reduced to a fine powder using a blinder. The extracts were prepared from 50 g of this fine powder. The powder was thoroughly homogenized in 1.5 L of solvent using a magnetic stirrer. The homogenate obtained was first spun in a square of white fabric, then filtered successively twice on hydrophilic cotton and once on Whatman No. 3 filter paper. The filtrate obtained was then concentrated in a Med Center Ventical type oven at 50°C. for three days [12]. Several powders of varying colors were obtained.

2.1.2 Dosage of the minerals of *Khaya senegalensis* leaves

2.1.2.1 Total ash

In a clean and dry capsule (platinum or porcelain) of known mass (m_0), five grans (5g) of the sample were oven dried (me). The capsule containing the sample (total mass m_1) was placed in a muffle furnace at $550 \pm 15^\circ\text{C}$ for 12 hours. After incineration, the capsule was removed from the muffle furnace and cooled in a desiccator. After cooling, the whole (sample + capsule) was weighed. Let m_2 be the sample + capsule mass.

2.1.2.2 Preparation of ashes

The ash preparation consisted of dissolving 0.25 g of total ash in 10 mL of 1% nitric acid. The mixture was filtered and the filtrate was made up with demineralized water to 100 mL. This solution was used for the determination of the different minerals of interest.

2.2 Determination of Calcium by Complementometry

In an Erlenmeyer flask were placed 20 mL of total ash solution filtrate, 5 mL of 2 M NaOH and then a pinch of Patton and Reeder. The mixture was then titrated with 0.01 M of EDTA until bluish turn.

2.3 Determination of Iron by the Orthophenanthroline Method

In a test tube was introduced, 1 mL of total ash solution filtrate and 4.5 mL of boiled distilled

water. The whole was cooled away from the air. After cooling, 2 mL of 20% sodium acetate, 2 mL of 1% hydroxylamine hydrochloride and 0.5 mL of 0.5% orthophenanthroline were added. The whole was left to rest for 1 hour. The Optical Density (OD) was read at 490 nm against the white. The iron content of the sample was determined from a standard straight line established from a standard iron solution at 10 μg iron / mL.

2.4 Determination of Magnesium

In an Erlenmeyer flask, were successively introduced 20 mL of total ash solution filtrate, 10 mL of Ph 10 buffer and a pinch of NET (Tio black). The mixture was titrated with 0.01M of EDTA until sharp blue.

2.5 Determination of Phosphorus by the Briggs Method

In a test tube, 1 mL of total ash solution filtrate was placed. Then, 6 mL of distilled water, 1 mL of molybdc reagent, 1 mL of 1% hydroquinone and 1 mL of 20% sodium sulfite were added. The whole was left standing for 30 minutes in the dark. The OD was read at 700 nm against the blank. The phosphorus content of the sample was determined by means of a standard straight line established from a standard phosphorus solution at 10 μg of phosphorus / mL.

2.6 Dosage of Phenolic Compounds

2.6.1 Dosage of total phenols

The total phenol content was determined by the method described by Li et al. [13]. Each volume of extract (0.5 mL) concentration 100 μg / mL are added respectively 5 mL of Folin-ciocolteu diluted 1/10 in distilled water and 4 mL of sodium carbonate (1M). The whole is incubated at room temperature for 15 minutes. The optical density is read on a spectrophotometer at 765 nm against a blank. A gallic acid solution is prepared in the same conditions as the extract. The total phenol contents are expressed in milligram equivalent of gallic acid per gram of extract (mg GAE / g extract).

2.6.2 Dosage of flavonoids

The total flavonoid assay was performed according to the method described by Huang and Prior [10]. A volume of 0.5 mL of methanolic extract is introduced into a test tube. 0.5 mL of

distilled water, 0.5 mL of aluminum chloride, 0.5 mL of potassium acetate and 2 mL of distilled water are successively added to the contents of the tube. The tube is left standing for 20 min in the dark and the optical density is read at 415 nm on a spectrophotometer against a blank. A stock solution of quercetin is prepared in the same conditions as the test. The flavonoid contents of the extracts are expressed in milligram equivalent of quercetin per gram of extract (mg EQ / g of extract).

2.7 Antioxidant Activity of the Extracts

2.7.1 Measurement of the reducing power

The measurement of the reducing power was carried out according to the method described by Topçu et al. [14]. One mL of plant extract or vitamin C at different concentrations is mixed separately with 2.5 mL of phosphate buffer (0.2 mM, pH 6.6) and 2.5 mL of 1% potassium ferricyanide. The mixture is incubated in a water bath at 50°C. for 30 minutes. After the addition of 2.5 mL of 10% trichloroacetic acid, the reaction medium is centrifuged at 3000 rpm for 10 minutes. To 2.5 mL of the supernatant is added 0.5 mL of 0.1% iron III chloride. After 10 minutes of incubation at room temperature, the absorbance is measured at 700 nm on a spectrophotometer.

2.7.2 Measurement of anti-radical power

The measurement of the anti-radical power was carried out according to the method used by Parejo et al. [15]. From a stock solution of 0.1 mg / mL plant extract, a concentration range is prepared by successive double dilution. Then at each concentration of extract, the same volume of methanol solution of DPPH is added. After 30 minutes of incubation at room temperature (37°C) and protected from light, the absorbance is read on a spectrophotometer at 517 nm against a blank. A vitamin C solution is prepared in the same conditions. The percentages of inhibition of the DPPH radicals are calculated by the following formula:

$$\% \text{ Inhibition} = \left[\frac{\text{white ABS} - \text{ABS sample}}{\text{white ABS}} \right] \times 100$$

2.8 Statistical Analysis

The data was processed using the Graph Pad Prism 5.0 software (Microsoft, USA). The statistical analysis of the results was carried out using the Anova One-Way variance analysis followed by Dunett tests for the comparison between the activity of the extracts and that of the reference molecules. The value of the averages is accompanied by the standard error on the mean (mean ± SEM). Probability values P≤0.05 were considered significant.

3. RESULTS

3.1 Extractions Yields

The yields (%), 15.42, 17, 21.54, 27.63 obtained with the solvents butanol, methanol, aqueous and ethanol respectively as well as the appearance and color of the extracts are shown in Table 1. The lowest value (15.42%) was obtained with butanol extract while the highest (27.63%) was obtained with the ethanol extract. The methanol and butanol extracts were sticky with a black color. As for the ethanol and aqueous extracts, they presented a pellet brown appearance and a black granulated appearance respectively.

3.2 Mineral Content

The average levels of minerals (major and trace elements) are presented in Table 2. The highest content of major minerals was recorded with calcium (948.38 ± 11.57 mg / 100 g) and the lowest with magnesium (188.24 ± 0.97). As for the trace element, only the iron content was evaluated. This content is (41.50 ± 1.57 mg / 100 g). These results indicated that extracts from *K. senegalensis* leaves are rich in calcium, phosphorus, magnesium and iron. The observed difference in mineral content is statistically significant at p≤0.05.

Table 1. Extraction yield

Extracts	Yield (%)	Appearance	Color
Aqueous	21.54	Granulated	Black
Ethanol	27.63	Sticker	Brown
Methanol	17.00	Tights	Black
Butanol	15.42	Tights	Black

Table 2. Chemical content of *Khaya senegalensis* leaves

	Total Ash (%)	Calcium mg/100 g	Iron mg/100 g	Magnesium mg/100 g	Phosphorus mg/100 g
Levels	9.50±0.06*	948.38±11.57*	41.50±1.57*	188.24±0.97*	304.98±2.6*

(*): There is a significant difference between column values at $P \leq 0.05$

Table 3. Phenolic content of leaf extracts of *K. senegalensis*

	<i>E. aqueous</i>	<i>E. ethanol</i>	<i>E. methanol</i>	<i>E. butanol</i>
Total phenols	56,94±0,02 ^{ns}	48,27±0,02 ^{ns}	51,9±0,02 ^{ns}	91,53±0,04*
Flavonoids	34,29±0,03 ^{ns}	37,51±0,02 ^{ns}	60,74±0,01*	75,58±0,05*

Multiple comparison between column values at $P \leq 0.05$. (*): significant and (ns): not significant E.: Extract

3.3 Content of Phenolic Compounds

3.3.1 Total phenol content

The results of the determination of the total phenols revealed that a variability of contents between the different extracts. Butanol extract contains more total phenols with a content of 91.53 ± 0.04 mg GAE / g extract, followed by aqueous extract (56.94 ± 0.02 mg GAE / g extract) and methanol extract (51.9 ± 0.02 mg GAE / g extract). The lowest value was recorded at the ethanol level (48.27 ± 0.02 mg GAE / g extract). The variance analysis of the total phenol content of the extracts indicated that there is a significant difference between the values at $p \leq 0.05$ (Table 3).

3.3.2 Content of flavonoids

The results of the quantitative flavonoid analysis showed that butanol extract recorded the highest value (75.58 ± 0.05 mg EQ / g extract). The methanol and ethanol extracts obtained 60.74 ± 0.01 mg EQ / g extract and 37.51 ± 0.02 mg EQ / g extract, respectively. These values, lower than that of the butanol extract, are greater than that of the aqueous extract (34.29 ± 0.03 mg EQ / g of extract). The observed difference in extract levels is statistically significant at $p \leq 0.05$ (Table 3).

3.4 Antioxidant Activity of Leaf Extracts

3.4.1 Iron power reducing

The reducing power of an extract is associated with its antioxidant power. The curves of the Fig. 1, illustrate the evolution of the absorbance at 700 nm as a function of the concentration of the extracts. These plots allowed to determine the EC_{50} parameter which corresponds to the concentration which induces an absorbance of

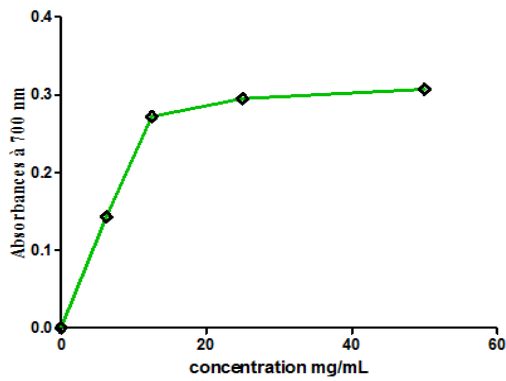
50%. The EC_{50} values vary according to the extract. The ethanol extract has the lowest EC_{50} value (1.03 ± 0.41 mg / mL). The highest value is obtained with the aqueous extract (2.28 ± 0.53 mg / mL). It can be seen that when the curve moves away from the y- axis, the value of EC_{50} is large. These values are higher than that of the reference molecule Vitamin C (0.91 ± 0.40). There is a statistically significant difference between the EC_{50} values of the aqueous extract and that of vitamin C at $p < 0.05$ (Table 4).

3.5 DPPH radical Scavenging Activity

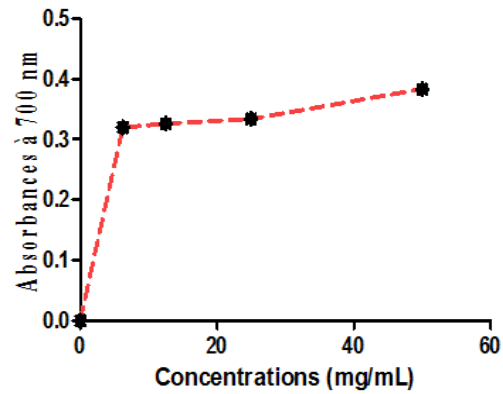
The trapping power of the DPPH radical has been evaluated through the determination of the IC_{50} . Among the extracts studied, the aqueous extract has the lowest IC_{50} value (3.37 ± 0.61 mg / mL). This result is close to that of butanol extract (4.97 ± 1.10) but lower than that obtained from the ethanol (9.900 ± 1.2 mg / mL) and methanol ($8,10 \pm 1,8$ mg/mL) extracts. The reference molecules (vitamin C and BHT) had IC_{50} values of 1.03 ± 0.41 mg / mL and 1.83 ± 0.50 mg / mL, respectively. Ethanol extract obtained the highest IC_{50} value of $9,900 \pm 1.2$ mg / mL, which is 9 times higher than that of vitamin C and BHT. A statistical analysis of the antiradical activity of extracts and reference molecules (vitamin C and BHT) indicates that there is no significant difference between IC_{50} of the extracts (aqueous and butanol) with those of the reference molecules at $p < 0.05$ (Fig. 2).

4. DISCUSSION

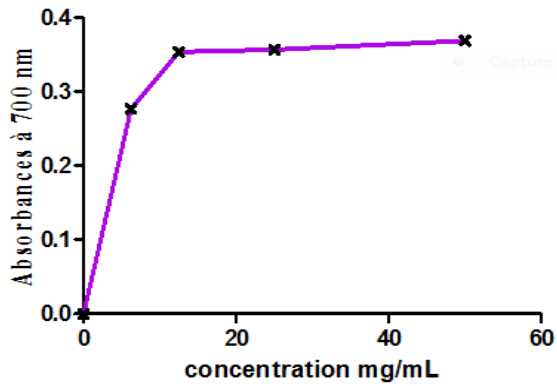
In this study, distilled water, 70% ethanol, methanol and butanol were used as extraction solvents for the secondary metabolites contained in the studied plant. Among these solvents, ethanol 70% obtained the highest extraction yield with an average of 27.63% against 15.42% for butanol. These results are similar to those



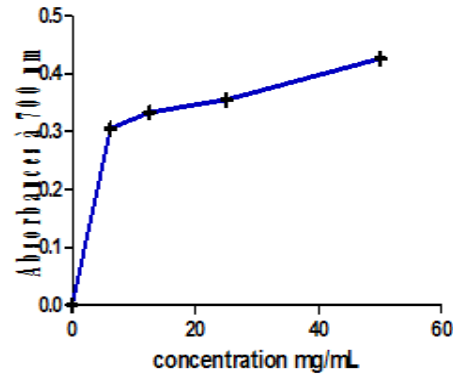
a- reducing power of aqueous extract



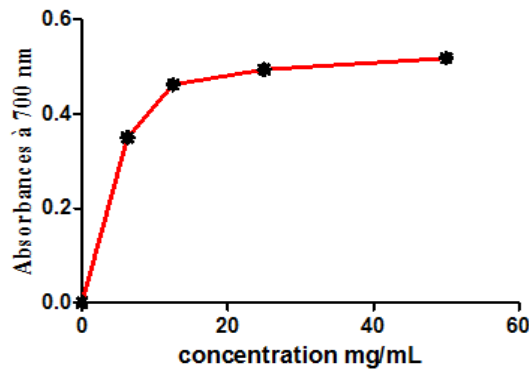
b- reducing power of ethanol extract



c- reducing power of methanol extract



d- reducing power of butanol extract



e- reducing power of the Vitamin C

Fig. 1. Reducing power of various extracts of leaves from *K. senegalensis*

obtained by Bouzid et al. [16]. These authors reported that hydroalcoholic solutions are the most used for the recovery of phytoconstituants. The results obtained suggest that 70% ethanol is a good solvent for extracting the active substances of medicinal plants. Regarding to the

polyphenol composition of the studied leaves, several studies have reported that the bark, fruits and leaves of *K. senegalensis* reveal the presence of a large quantity of catechin tannins, anthocyanins, leucoanthocyanins and flavonoids, coumarin, carbohydrates, reducing compounds

and saponins [17,18,19]. These results corroborate those of our test which showed a high content of total phenols and flavonoids of the extracts studied. The highest content was obtained with butanol extract while the flavonoids recorded the lowest value in the aqueous extract. These results revealed that there is a variability of content between the different extracts. This variability would be related to extraction solvents. Indeed, according to the work of Ko et al. [20], extraction yields of polyphenols are higher with solvents of lower polarity than water. Similar studies conducted by Koudoro et al. [21], on the bark of this same plant gave different results to those of our test. These authors reported levels of $(3,328 \pm 0,185)$ mg GAE / g MS and $(6,731 \pm 0,018)$ mg GAE / g MS as total phenols of hydroethanol and aqueous extracts respectively. This means that leaves of *K. senegalensis* are richer in total phenols than barks. This difference in content observed between the different organs of the same plant could be justified in part by the recognized influence of various factors related to the study area on the contents of phenolic compounds including exposure to light, high temperature, dryness and salinity. These factors would be stimulants for the biosynthesis of secondary metabolites [22]. With regard to the antioxidant activity of the extracts, the results obtained with the method of FRAP and DPPH show contrary results. Indeed, considering the EC_{50} and IC_{50} values of the extracts, it is found that by the DPPH method, the butanol and aqueous extracts had the best antioxidant activities because the EC_{50} and IC_{50} values are closer to those of the reference molecules. (Vitamin C and BHT). However, by the method of FRAP, the best antioxidant power has been recorded with the ethanol extract followed by the methanol extract. By this method, the EC_{50} value of the aqueous extract is three (3) times greater than that of vitamin C. This difference may be due to the sensitivity to the reagents used in each of the methods [23]. In view of all the results, butanol extract recorded the highest levels of polyphenols as well as the best antioxidant activities. It can thus be noted that the content of phenolic compounds is in line with

the anti-radical activity [24]. There is therefore a correlation between the antioxidant activity and the polyphenol content of the extracts studied. Similar results have been proven by Tapsoba [25] who showed through the ethno medicinal plant study of Burkina Faso that there is a good correlation between the total phenol profile and the antioxidant activity of plant extracts suggesting that phenolic compounds are responsible antioxidant activity of the extracts. As for the nutritional value of the leaves of *K. senegalensis*, several studies have examined the chemical composition of this plant. Thus, according to the work of Agassounon et al. [26], the digestibility of leaves of *K. senegalensis* by sheep is related to the content of dry matter, mineral matter, organic matter, total nitrogenous matter, hemicellulose, cellulose. This author in determining the mineral content of *Grewia flavescens*, *Commiphora africana*, *Acacia raddiana*, *Pterocarpus lucens*, *Maerua crassifolia* resulted in results (ranging from 91.4 to 111.1 g / kg DM) which are similar to those of our test. Similar results have also been reported by Oyaizu [27] through the phytochemical study of leaves of *rytigynia canthioides*, a medicinal and food plant used in Benin. Some authors have also noted the important role of minerals and especially iron in the human body. The adult male organism has 4 g of iron about 2/3 of which is in the form of hemoglobin, an additional 10% is in the form of myoglobin and a small amount in several enzymes such as cytochrome P450 monooxygenases. Our results indicated that leaves of *K. senegalensis* have a high iron, calcium, magnesium and phosphorus content. This high mineral content of *K. senegalensis* leaves could cover the nutritional requirements of cattle and play an important role in the functioning of the human organism. The high antioxidant activity, the high total phenol and flavonoid content of extracts of *K. senegalensis* associated with the high content of total ash and minerals (calcium, magnesium, phosphorus and iron) could justify the diversified use of this plant both medicinally and nutritionally among traditional breeders in the north of Ivory Coast.

Table 4. Measurement of antioxidant activity of leaf extracts of *K. senegalensis*

	Vita C	BHT	E. Aque	E. Etha	E. Metha	E. Buta
FRAP	0,90±0,4	-	2,28±0,5*	1,03±0,4 ^{ns}	1,30±0,3 ^{ns}	1,5±0,4 ^{ns}
DPPH	1,03±0,4	1,83±0,5 ^{ns}	3,36±0,6 ^{ns}	9,90±1,2*	8,10±1,8*	4,96±1,1 ^{ns}

Multiple comparison between the reference molecule (vitamin C) and the extracts at $P \leq 0.05$. (*): significant, and (ns): not significant E. Extract, Aque: aqueous, Etha: Ethanol, Metha: Methanol, Buta: Butanol, Vita C: Vitamin C

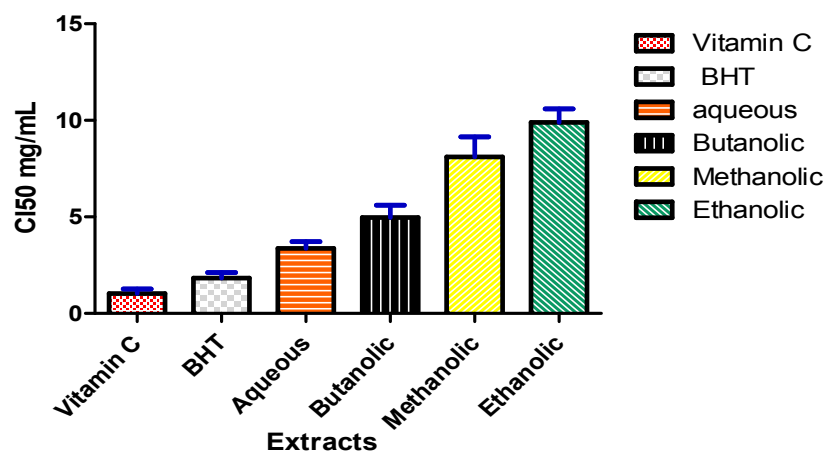


Fig. 2. Histogram of antiradical power

5. CONCLUSION

We have determined the polyphenol content, the total ash and the minerals (calcium, magnesium, phosphorus and iron) and evaluated the antioxidant activity of extracts from leaves of *K. senegalensis*. The ethanol extract obtained the highest extraction yield. All extracts from the leaves of this plant have the ability to trap the DPPH radical and reduce ferric iron to ferrous iron. However, the total butanol extract is characterized by a high residual iron reducing power and a strong inhibition of the DPPH radical. A correlation has been observed between the antioxidant activity and the contents of phenolic compounds. The extracts from the leaves of *K. senegalensis* could be used to treat various diseases because of their high content of phenolic compounds. Its high mineral content and high antioxidant power could justify the use of this plant by traditional farmers in northern Ivory Coast as an alternative in the diet of cattle during the dry season.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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