Phytochemical screening and in vitro antioxidant activity of the stem bark of *Khaya senegalensis*, a medicinal plant from northern Ivory Coast

ABSTRACT

Aim: to highlight the phytochemical constituents and evaluate the antioxidant activity of the aqueous (KsA) and 70% hydroethanolic (KsE) extracts of the bark of *Khaya senegalensis* (a medicinal plant used in the north of Ivory Coast).

Study design: The search for antioxidant molecules by scientists remains today a new alternative to overcome several pathologies linked to oxidative stress. The present study is a part of this perspective.

Methodology: Phytochemical screening was carried out by precipitation and/or staining methods. As for the antioxidant power of the extracts, it was evaluated by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical reduction method.

Results: Phytochemical screening results showed that KsE contains the following groups of secondary metabolites: sterols and terpenes, total polyphenols, flavonoids, tannins and alkaloids. The KsA extract contains the saponins and the same chemical composition as KsE without the alkaloids and terpene compounds. The DPPH test revealed significant antioxidant activity of the two extracts close to that of gallic acid (IC_{50} =3.6 ± 0.02 µg/mL) with IC_{50} of 6.4 ± 0.02 µg/mL and 7.5 ± 0.01 µg/mL for KsE and KsA respectively.

Conclusion: Ultimately, the richness of *K. senegalensis* extracts in secondary metabolites with antioxidant effects could be at the origin of its traditional use in the treatment of diseases.

Keywords: Aqueous and Hydroalcoolique Extract, phytochemical screening, antioxidant power.

1. INTRODUCTION

The resurgence of infectious and metabolic diseases are two issues that are undermining the global health sector. Africa with respect to other continents is not spared and this is felt at the level of all countries, particularly Ivory Coast. The reasons for this observation are, among other things, the resistance of microbes to antibiotics; the change in eating and behavioral habits (consumption of excess salt, sugar and fat; sedentary lifestyle and lack of physical exercise) and the oxidative stress. The latter has been widely defined as a situation where the cell no longer controls the excessive presence of toxic oxygen radicals **[1]**. It also plays an important role in the development of degenerative diseases such as atherosclerosis, diabetes or neurodegenerative pathologies **[2]**.

Furthermore, conventional medicine today finds itself at an impasse when faced with the thorny issue of antibiotic resistance in infectious microorganisms. It is therefore imperative to find new active ingredients in the fight against infectious and metabolic diseases. As a result, these sought-after molecules must possess various other chemical properties and new mechanisms of action that can overcome the resistance of pathogenic microbes **[3]**. The use of medicinal plants from traditional pharmacopoeia appears as a therapeutic alternative. Indeed, according to a study conducted by **[4]**, medicinal plants constitute the most important and inexhaustible source of bioactive compounds such as antioxidants, antimicrobials, antiparasitics and anti-inflammatories which can help prevent and treat diseases. Moreover, Africa is full of diverse flora and precious medicinal plants with nearly 50,000 species of vascularized plants, recorded in the treatment of various conditions **[5]**. This source is insufficiently exploited to the extent that only a small part of these known plant species has been investigated and each species could contain thousands of different constituents **[6]**. This is the case in Ivory Coast where an ethnobotanical study carried out by **[7]** on the Abidjan market made it possible to inventory 58 species marketed to treat various common diseases. Using all these plants and recipes, many illnesses are treated at lower cost by populations seeking healing **[8, 5, 9]**. It is therefore right that we are interested in the medicinal plant called *Khaya senegalensis*. This plant is used by populations to treat various conditions including diarrhea, abdominal pain and others **[10, 11, 12]**. The present study therefore aims to highlight the phytochemical constituents and to evaluate the antioxidant activity of the aqueous and 70% Hydroethanolic extracts of the barks of *K. senegalensis* in order to contribute to the research of new therapeutic molecules with a broad spectrum of pharmacological activities (antimicrobial and physiological).

2. MATERIEL AND METHODS

2.1. Plant material

The plant material chosen consists of the stem bark of *Khaya senegalensis*. They were harvested in Korhogo (Ivory Coast) in September 2022. Subsequently, the authentication of the plant was carried out at the National Floristic Center of the Félix Houphouët-Boigny University of Cocody (Abidjan, Ivory Coast). After harvest, the samples were thoroughly cleaned with tap water to remove all impurities. These samples were cut into small pieces then dried away from light, at room temperature for two weeks. At the end of drying, the barks were pulverized using an electric grinder (Retsch, Type AS 200) to obtain a fine powder. This plant powder was stored in sterile jars and used to prepare the extracts.

2.2. Extraction of plant material

2.2.1 Preparation of aqueous extract

The aqueous extract was prepared according to the method described by Zirihi *et al*. **[6]**. One hundred (100) g of *K. senegalensis* bark powder that was macerated in one liter (1 L) of distilled water using a magnetic stirrer at room temperature for 24 hours. After maceration, the mixture obtained was first drained through a square of white cloth, then doubly filtered through hydrophilic cotton and once through Whatman paper (3 mm). Then, the filtrate then obtained was concentrated in the oven until the water had completely evaporated. The extract obtained was weighed and stored in a sterile bottle (coded KsA). The extraction yield (*R*E) was determined according to the following formula:

$$RE\ (\%) = (m / M) \times 100$$

With M: mass of the vegetable powder (g); m: mass of the crude extract (g).

2.2.2 Preparation of 70 % hydroethanolic extract

The hydroethanolic (hydroalcoholic) extract was prepared by maceration of 100 g of *K. senegalensis* bark powder in one liter (1 L) of ethanol diluted to 70% (70/30; V/V) with a magnetic stirrer. at room temperature for 24 hours **[6]**. The macerate obtained was successively filtered twice through hydrophilic cotton then once through Whatman paper (3

mm). Then, the solvent was evaporated in an oven to concentrate the filtrate. The extract obtained was weighed and stored in a sterile bottle (coded KsE). The extraction yield was determined as above.

2.3 Phytochemical study of extracts

2.3.1 Screening of chemical groups

The method used is that described by **[4]** based on precipitation and coloring tests in tubes. The test mainly targeted alkaloids, total polyphenols, flavonoids, saponins, tannins, sterols and polyterpenes because of their great importance in human therapeutics.

2.3.2. Dosage of phenolic compounds

Total polyphenols were measured according to the method described by **[12]**. To 30 µL of extract were added 2.5 mL of Folin-Ciocalteu reagent diluted 1/10. The mixture obtained was kept for 2 minutes in the dark at room temperature (28 ± 2°C) then 2 mL of sodium carbonate solution at 75 g/L were added. The solution obtained was then incubated at 50°C for 15 minutes. Then, the absorbance was read using a UV-visible spectrophotometer at a wavelength of 760 nm against a blank. Gallic acid was used as a reference standard for the quantification of the total polyphenol content expressed in microgram of gallic acid equivalent per gram of dry extract (µg EAG/g of dry extract). The tests were carried out in triplicate.

The total content of total flavonoids was determined according to the method described by **[13]**. 0.75 mL of 5 % (w/v) sodium nitrite solution and 0.75 mL of 10 % (w/v) aluminum chloride solution were added to 2.5 mL extract ratio 1/500 (m/v). After 5 minutes of incubation, the mixture was brought into contact with 5 mL of a 1 M sodium hydroxide solution. The volume obtained was adjusted to 25 mL with distilled water then stirred vigorously. The absorbance was measured at the wave length of 510 nm. Quercetin was used as a reference standard for the quantification of total flavonoid contents expressed in micrograms of quercetin equivalent per gram of dry extract (µg EQ/g of dry extract). The tests were carried out in triplicate.

The condensed tannin content was determined by the vanillin method described by **[14]** with a slight modification. Indeed, 50 µL of the extract was added to 3000 µL of the vanillin solution in 4 % methanol, then the whole was vigorously shaken. Then, 1500 µL of concentrated hydrochloric acid (HCl) was added. The mixture obtained is left to incubate at room temperature in the dark for 20 min. Then, the absorbance is measured at 550 nm against a blank. Catechin was used as a standard and the calibration curve was constructed from concentrations between 0 and 1000 µg. mL^{-1}. The results were expressed as mg catechin equivalent (EC) per gram of dry extract (mg EC/g of dry extract). The experiment is also repeated three times.

2.3.3 Antioxidant activity of extracts

In this study, the antioxidant potential of the hydroalcoholic and aqueous extracts was evaluated by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) reduction method.

This method is based on the ability of antioxidants to trap the DPPH radical. The latter is reduced to the hydrazine form (non-radical) by accepting a hydrogen atom. The effect of each extract on DPPH was measured according to the protocol described by **[15]**. Gallic acid was used as a reference standard antioxidant. Volumes of 5 mL at different concentrations (5, 10, 25, 50, 100 and 200 µg/mL) of each extract were prepared and 50 µL of each concentration was added to 1,950 mL of the freshly prepared methanol solution prepared from DPPH (0.025 mg/mL). After 30 minutes of incubation, the absorbances were measured at 517 nm using a spectrophotometer and the inhibition powers were calculated from the formula:

$$PI\ (\%) = \left[(A_0 - A)/A_0\right] \times 100$$

PI: Power of inhibition;

A: Absorbance of diluted DPPH containing the samples to be tested;

A_0: Absorbance of diluted DPPH (control absorbance).

The 50 % inhibition concentration (IC_{50}) was determined by projecting 50 % inhibition as a function of the concentrations of the extracts and gallic acid. Thus, a lower IC_{50} value indicates greater antioxidant activity of the extract **[16]**. The tests were carried out in triplicate.

2.4 Statistical analysis of results

Results were analyzed using Graph Pad Prism 8.0 software (Microsoft U.S.A) for multiple variances (ANOVA). The differences between the means were determined using the Duncan test at the 5 % threshold ($P < 0.05$ is considered significant). The graphical representation of the data was carried out using Excel software. The results were expressed as means accompanied by the standard error of the mean.

3. RESULTS AND DISCUSSION

3.1. Extraction yield

The yields obtained after extractions appear in **Table 1**. The hydroalcoholic extract gave the best yield (23.30 %) compared to the aqueous extract (12.70 %).

Table 1: Yield of *K. senegalensis* extractions

Extraits de *K. senegalensis*	**Extrait aqueux**	**Extrait éthanolique 70 %**
Rendement (%)	12.70	23.30

3.2. Phytochemical tests

The results of the phytochemical tests carried out on the extracts (KsE) and (KsA) of the bark of *Khaya senegalensis* are presented in **Table 2**.

Table 2: Phytochemical groups of the two extracts of plant (stem bark)

Groupes chimiques recherchés	Test de détection	**KsE**	**KsA**
Stérols et terpènes	Lieberman	+	-
Polyphénols	Perchlorure ferrique	+	+
Flavonoïdes	Cyanidine	+	+
Tanins catéchiques	Stiasny	+	+
Tanins galliques	Stiasny	+	+
Alcaloïdes	Dragendorf	+	-

Saponines	Mousse	-	+
Quinones	Borntrager	-	-

+ : Présence - : Absence

Phytochemical screening showed that the KsE and KsA extracts contain almost all of the secondary metabolites tested, with the exception of quinones. However, the aqueous extract has an absence of alkaloids and terpene compounds while the hydroalcoholic extract has no saponins. The absence of compounds could be explained by the fact that only polar compounds are better extracted by the water solvent, hence their absence in the aqueous extract **[17]**. The aqueous fraction containing a large number of secondary metabolites could justify the use of the aqueous decoction in traditional medicine **[18]**. The hydroalcoholic extract (KsE) contains the majority of secondary metabolite families present in crushed leaves. This result confirms the ability of the hydroalcoholic solvent to extract almost all secondary metabolites present in plant organs **[19]**. The richness of *Khaya senegalensis* extracts in secondary metabolites could be the origin of its numerous biological, pharmacological and therapeutic properties reported in the literature **[20]**.

3.3 Determination of total polyphenols and total flavonoids

The calibration line **(Figure 1)** drawn using gallic acid as a standard, made it possible to carry out quantitative analyzes of total polyphenols.

The total polyphenol contents of the KsE and KsA extracts were translated as a histogram in **Figure 2.**

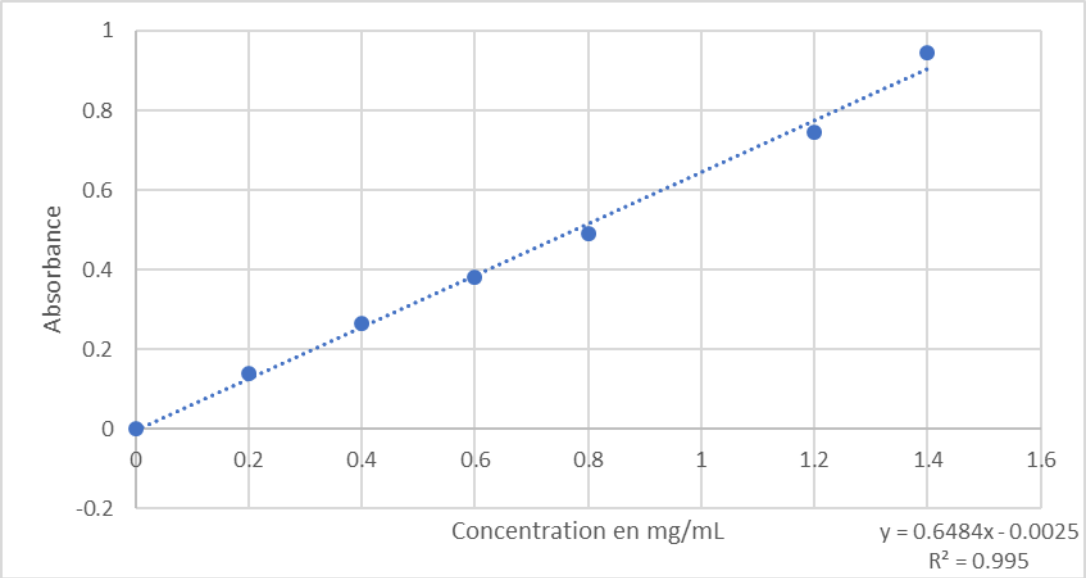

Figure 1: Gallic acid calibration line

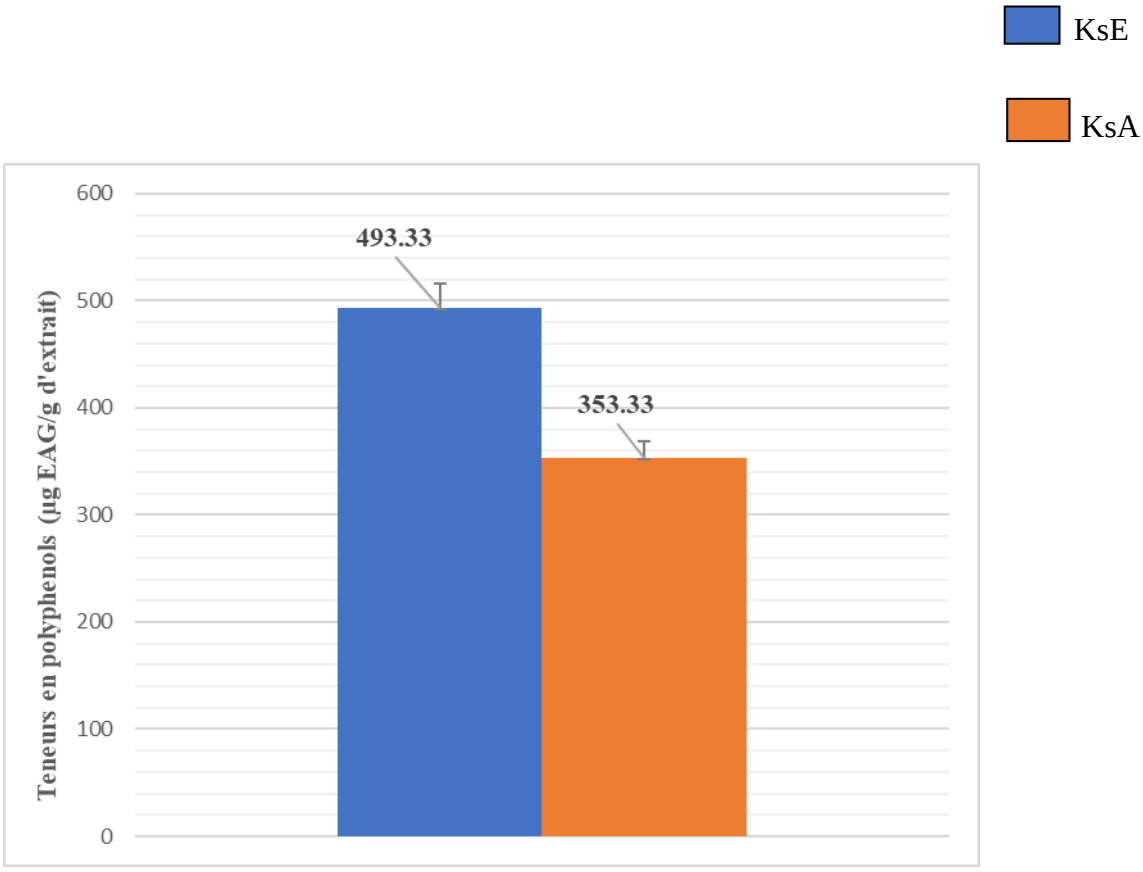

Figure 2: Total polyphenol content of the differents extracts

KsA: aqueous extract; KsE: hydroalcoolic extract

The hydroalcoholic KsE and aqueous KsA extracts respectively have total polyphenol contents of 493.33 ± 22.22 µg EAG/g and 353.33 ± 15.55 µg EAG/g of dry extract. The significantly high concentration of polyphenols in KsE would be linked to the high solubility of phenolic compounds in polar solvents **[21]**. This notion of polarity seems to be confirmed by the phytochemical screening which reveals the presence of total polyphenols, flavonoids and tannins both at the level of KsE and KsA. Thus, the phenolic compounds in the KsE and KsA extracts could correspond to flavonoids, tannins in condensed form and certain phenolic acids. Due to the sensitivity of the Folin-Ciocalteu method, this reagent can still react with the

aromatic amino acids of proteins (especially with tryptophan), reducing carbohydrates such as glucose and fructose and vitamin C **[22, 23]**. There is therefore the probability of the presence of these compounds in both extracts. The presence of a high level of polyphenols in KsE extracts could justify its use in traditional medicine.

The work carried out is in agreement with that of **[24]** and **[25]** which showed that ethanol in combination with water allows better extraction of total polyphenols. Indeed, the addition of water to organic solvents increases the solubility of polyphenols **[26]** by modulating the polarity of the organic solvent **[27]**.

Quantitative analyzes of total flavonoids were carried out from the calibration line **(Figure 3)**, plotted using quercetin as a standard. The results obtained are presented in **Figure 4**.

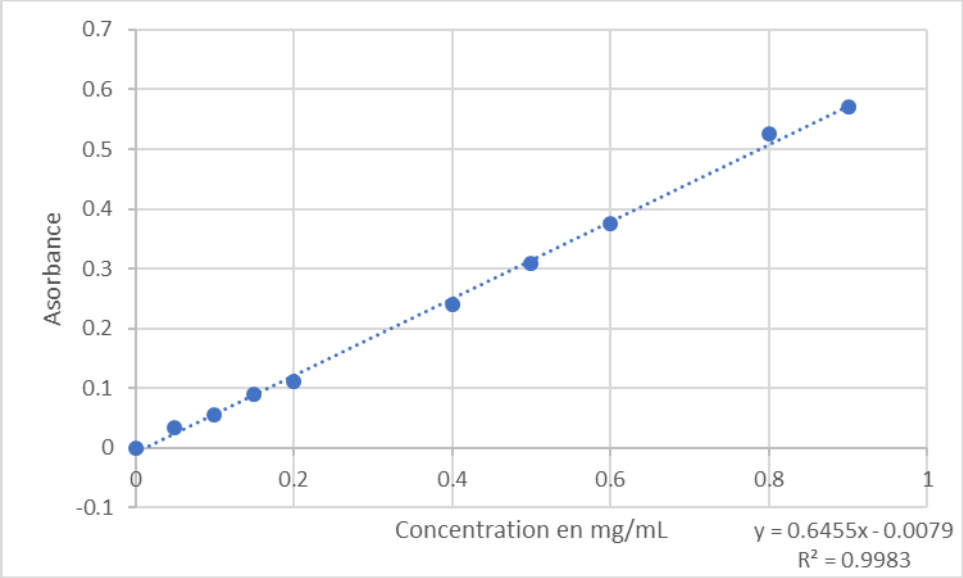

Figure 3: Quercetin calibration line

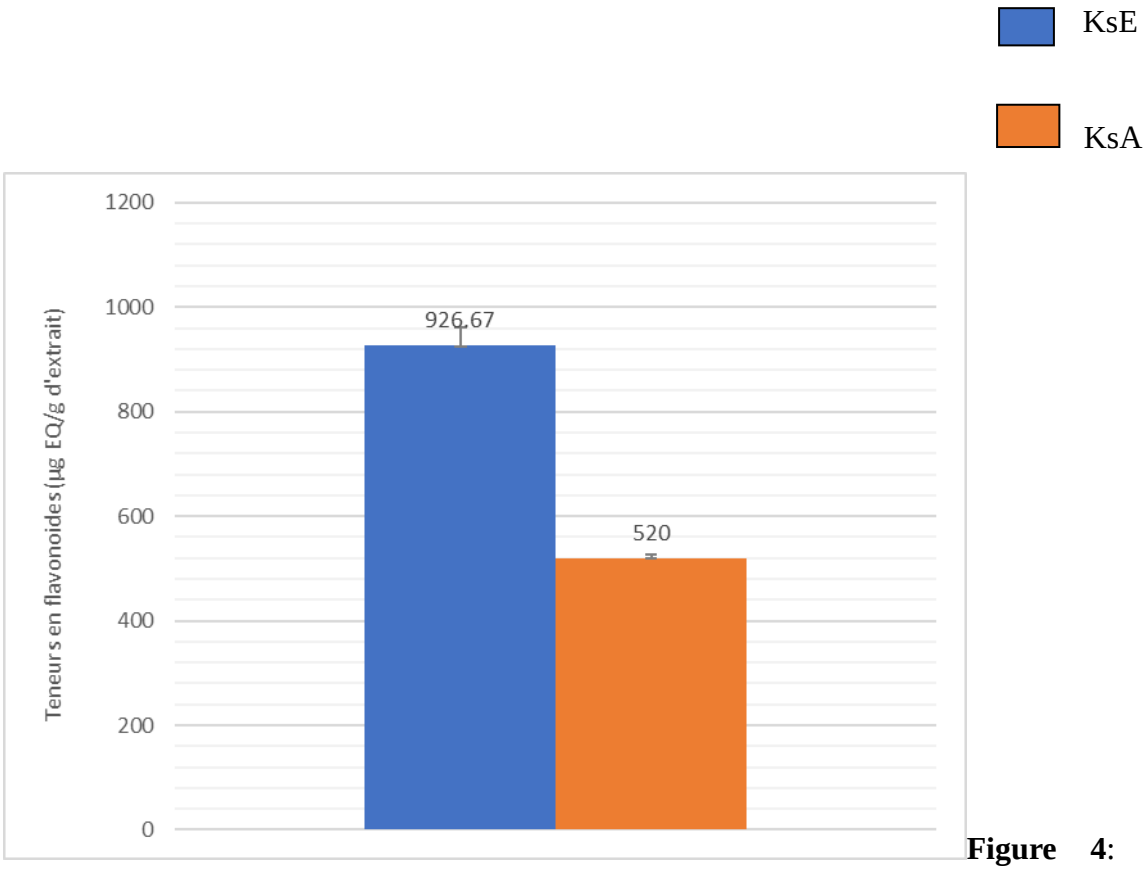

Figure 4: Total flavonoid content of the different extracts

KsA: aqueous extract; KsE: hydroalcoolic extract

It appears that the flavonoid content varies depending on the solvent used. Indeed, the KsE and KsA extracts significantly ($P < 0.05$) have high flavonoid contents of 926.67 ± 35.55 and 520 ± 6.67 mg EQ/g of dry extract, respectively. However, the proportion of flavonoids in the KsE extract is practically the double of that of the KsA extract. This could be explained by the fact that the ethanol-water mixture (70 %) is the best solvent for extracting flavonoids **[28]**.

3-4-Dosage of condensed tannins

Quantitative analyzes of condensed tannins were carried out from the calibration line, plotted using catechin as standard **(Figure 5)**.

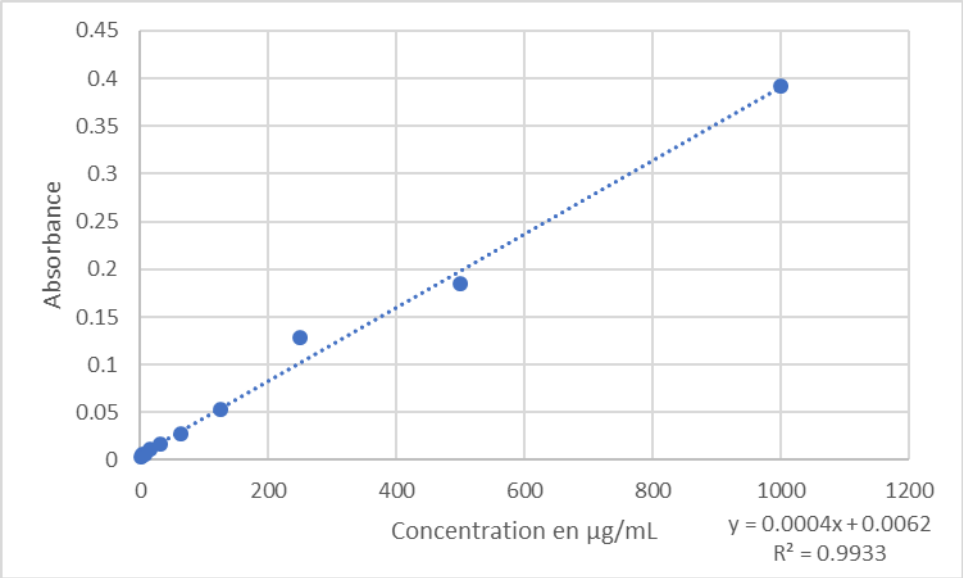

Figure 5: Calibration line for catechin

The dosage of condensed tannins revealed variable quantities depending on the different extraction solvents **(Figure 6)**. Indeed, KsE gave the best content of tannic compounds (148.74 ± 9.76 mgEC/g of dry extract) compared to the aqueous extract (68.85 ± 4.62 mgEC/g of dry extract). The presence of condensed tannins in the extracts was well predicted by the phytochemical screening **(Table 2)**. The high content of the two extracts in condensed tannins could justify the use of this plant to treat certain pathologies such as varicose veins and hemorrhoids thanks to their astringent, anti-inflammatory, hemostatic and vasoconstrictor effects **[29]**

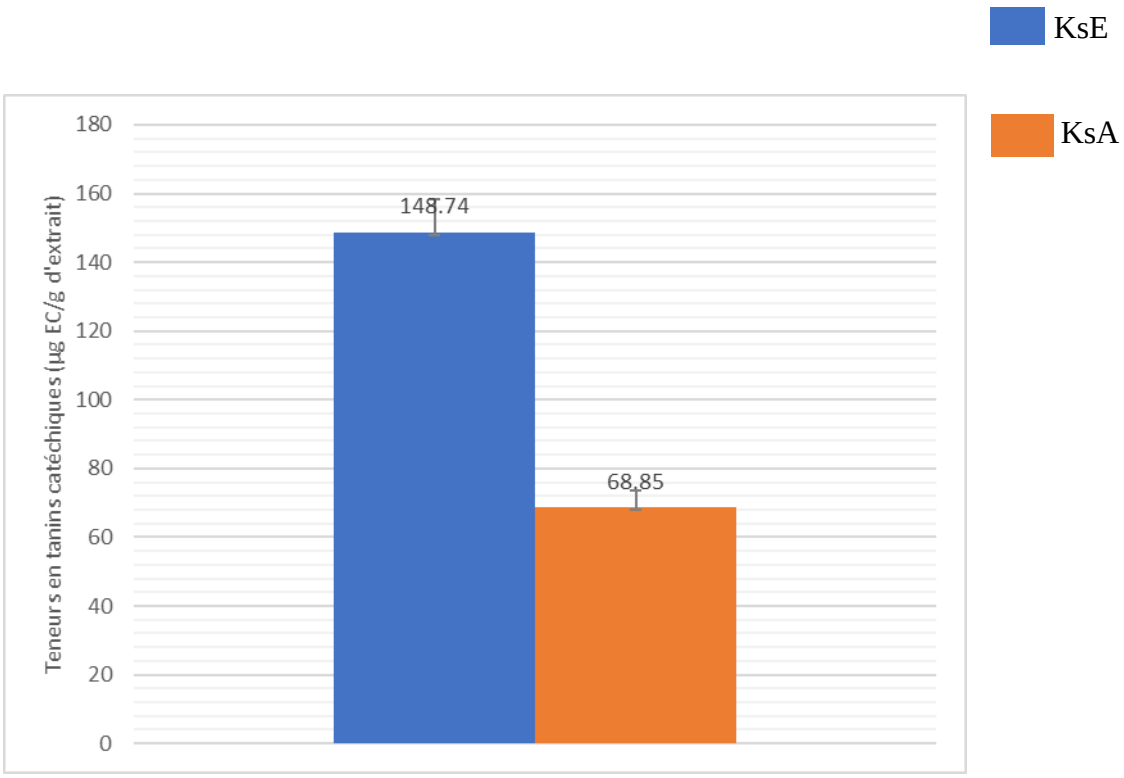

Figure 6: Condensed tannin content of the differents extracts

KsA: aqueous extract; KsE: hydroalcoolic extract

3.5. Antioxidant activity of extracts

Figures 7 and **8** respectively translate the inhibition power (PI) of the DPPH free radical at different concentrations and the 50 % inhibitory concentration (IC_{50}) of the DPPH radical.

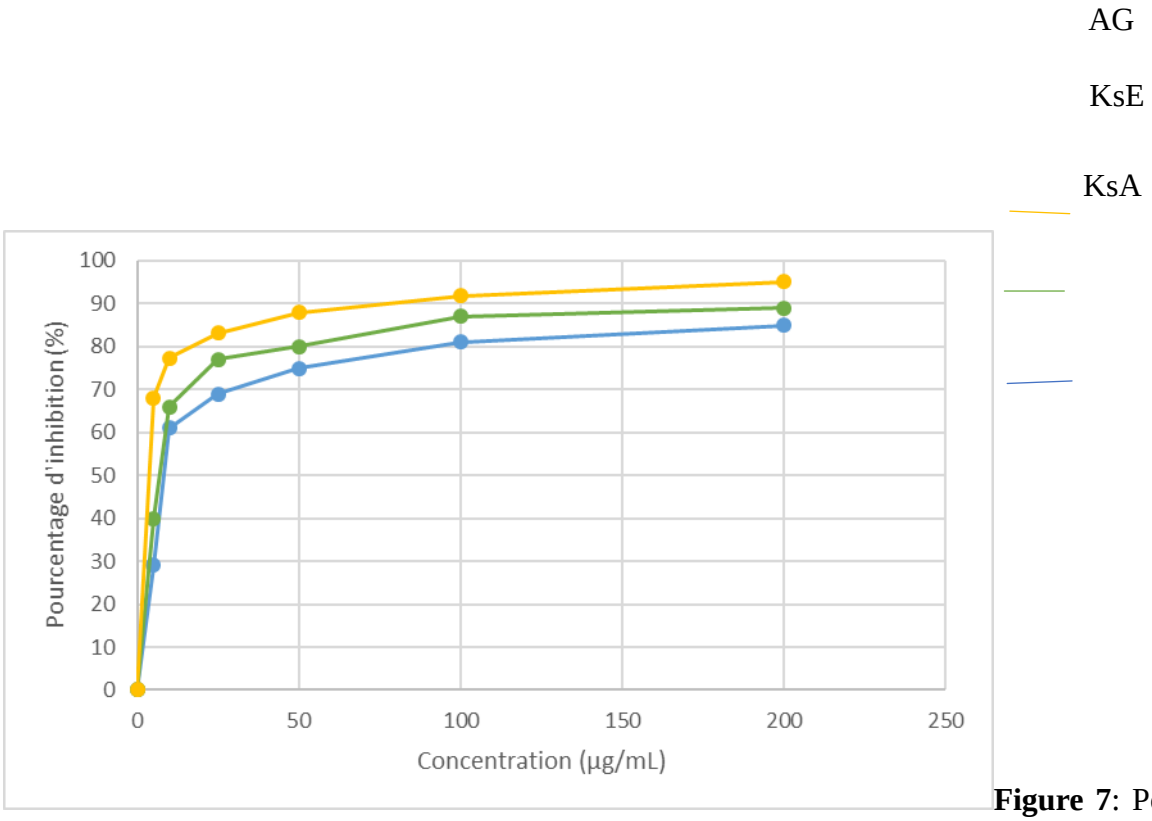

Figure 7: Power of inhibition of the DPPH radical by the differents extracts and gallic acid

KsA: aqueous extract; KsE: hydroalcoolic extract; AG: gallic acid

To better compare the activities of the different plant extracts tested, the IC_{50} were determined **(Figure 8)**. Thus, the KsE extract has an IC_{50} lower than that of the KsA extract with respective values of 6.4 ± 0.02 µg/mL and 7.5 ± 0.01 µg/mL. However, the two extracts have significantly lower inhibition powers compared to gallic acid, the IC_{50} of which was 3.6 ± 0.02 µg/mL.

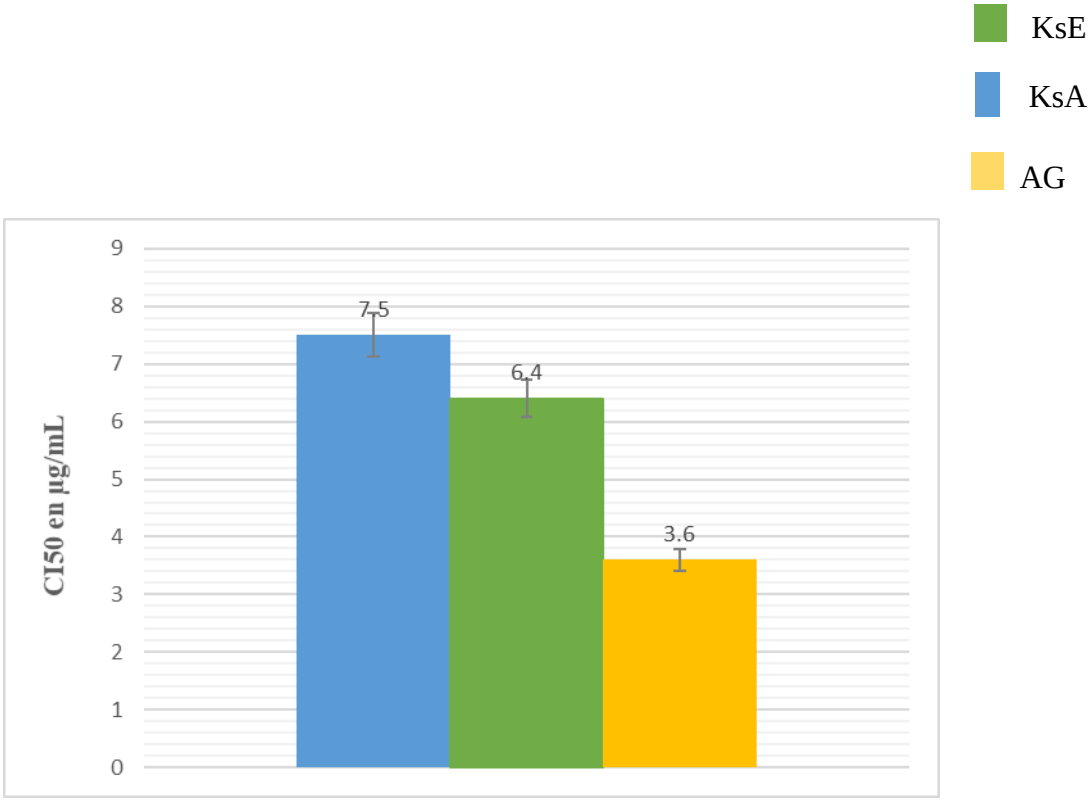

Figure 8: IC_{50} of the differents extracts and the gallic acid

KsA: aqueous extract; KsE: hydroalcoolic extract; AG: gallic acid

Thus, these results show that the antioxidant activity of the KsE extract is stronger than that of the KsA extract. This could be explained by the higher total polyphenol content of the KsE extract compared to that of the KsA extract. These results are consistent with those revealing the important role of polyphenols and flavonoids in the antioxidant activity of plant secondary metabolites. Indeed, several studies have found a good correlation between total phenol or flavonoid contents and antioxidant activities **[30, 31]**.

CONCLUSION

The objective of this study was to carry out the phytochemical screening of aqueous and hydroalcoholic extracts of *K. senegalensis* and to evaluate its antioxidant power. At the

end of this study, qualitative and quantitative phytochemical tests revealed that the plant extracts are rich in secondary metabolites with a significant proportion of phenolic compounds. As for the evaluation of antioxidant activity, the extracts also showed anti-radical properties in correlation with its many traditional uses. However, the hydroalcoholic extract gave the best antioxidant activity compared to the aqueous extract. This result could be enhanced by the development of a new Traditionally Improved Medicines (ATM) for the benefit of the well-being of populations.

AUTHOR'S CONTRIBUTIONS

This study was carried out in collaboration between three authors. Author Gboko Abiba O. designed the study, wrote the protocol, and wrote the first draft of the manuscript with approval of the other two. Author Kamagaté Tidiane performed the data collection, laboratory analyses, and author Kassim Dosso managed the literature searches and statistical data analysis Boni Ahoussi P. participated in the preparation of plant extracts. The final manuscript was read and corrected by all authors.

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COMPETING INTERESTS

Author's have declared that no competing interests exist.

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