

Antioxidant and antibacterial activity of aqueous and alcoholic stem bark extracts of *Kaya Senegalensis* (Desv) A. Juss., in human urinary infections

Abstract

Urinary infections constitute a public health problem today and the responsible germs increasingly express their strong resistance to common antimicrobials. The use of plants or plant products in fight against these pathologies are considered to be a good alternative. The objective of this study is to determine in vitro antioxidant power and antibacterial activity of aqueous and hydro-ethanolic extracts 70% of stem bark of *Kaya senegalensis* (Meliaceae) on clinical strains of *E.coli* and *Staphylococcus spp.* urinary infections and on two reference strains. The antioxidant activity was demonstrated by the ABTS radical cation trapping method using trolox as a reference. Sensitivity of strains to the two extracts and antibacterial parameters which are minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined respectively by punch well method in Mueller Hinton agar and liquid dilution method. Both extracts expressed a strong antioxidant power around 50 µmol TE/L of extract. The MICs varied from 6.25 mg/mL to 12.5 mg/mL for aqueous extract and from 6.25 mg/mL to 25 mg/mL for hydro-ethanolic extract. For all the strains MBC was identical to the MIC indicating that the two extracts have bactericidal power on the strains excluding the reference strain of *S.aureus*. These results suggest that *K. senegalensis* could be an alternative in the fight against urinary infections.

Keywords: *K.senegalensis*, antioxidant, antibacterial parameters, urinary infections

1- INTRODUCTION

Urinary tract infection is a pathology of the urinary tract, including the kidneys, ureters, bladder and urethra. There are different types of urinary infections, namely lower urinary tract infections and upper urinary infections (located in the kidneys). In lower urinary tract infections located in the bladder, we distinguish between non-infectious cystitis especially microbial cystitis [21-22]. The latter can affect everyone, but they are more common in women of childbearing age and in men of advanced age [1,2]. Approximately 50% of women develop a symptomatic urinary tract infection at least once in their life [3]. Since, the pathologies develop strong resistance to these molecules, the populations to

resort to medicinal plants. Medicinal plants are a therapeutic alternative, since they constitute the most important and inexhaustible source of bioactive compounds capable to prevent and treat diseases [4]. Among these medicinal plants are *Kaya senegalensis* (Méliaceae) is one among such medicinal plants. This plant is widespread in the forests and savannahs of West Africa [5]. The scientific studies undergone has revealed its potentiality as antimalarial, antioxidant, anti-inflammatory, antiproliferative, antimicrobial, anthelmintic, antidiarrheal agent [6,7]. Despite this broad spectrum of activity, there are no pharmacological data relating to urinary infections in Côte d'Ivoire. It is within this framework that the present work falls, which aims to show antioxidant and antibacterial potential of aqueous and hydroalcoholic extracts of *K. Senegalensis* (Desv) A.Juss., on the in vitro growth of germs responsible for urinary infections in humans.

2-MATERIAL AND METHODS

2.1-Material

2.1.1-Plant material

The plant material selected was the stem bark of *Khaya senegalensis*, collected in Korhogo (Côte d'Ivoire) in July 2023 and was authenticated under the identifier UCJ012302, at the National Floristics Center (CNF) of the Félix HOUPHOUËT-BOIGNY University of Cocody (Abidjan, Côte d'Ivoire). The samples were carefully cleaned with distilled water and cut into small pieces. These were then shaded dried, at room temperature for two months. The dried barks were pulverized using an electric grinder (RETSCH, Type AS 200) and the powdered samples were stored in sterile jars till it was further used.

2.1.2. Bacterial strains

The different clinical bacterial strains collected from the Microbiology Laboratory of the Regional Hospital Center of Korhogo and the reference strains from the Pasteur Institute of Côte d'Ivoire (Table 1). The strains were then stored at -20° at the bacteriology Laboratory of the National Agricultural Development Support Laboratory in Korhogo.

Table 1: Bacterial strains used for extract susceptibility testing

Types of strains		Clinics			Reference	
Strains	<i>E.coli</i>	<i>Staphylococcus spp</i>		<i>E.coli</i>	<i>S.aureus</i>	
Codes	8039	8133	9044	9109	ATCC 25922	ATCC 19213

2.2. Preparation of plant extracts

Aqueous and hydroethanolic 70% extracts were prepared according to method described by Zirihi *et al*[8]. One hundred (100) g of *K. senegalensis* bark powder were macerated in 1 L distilled water or ethanol diluted to 70% (70/30; V/V) using a magnetic stirrer (Froidlabo®) at room temperature for 24 hours. The solution was first drained through a square of white cloth, then doubly filtered through hydrophilic cotton and once through Whatman paper (3 mm). The filtrate obtained was concentrated in an oven until the solvent completely evaporated. The extracts were then stored in a sterile bottle for antioxidant and antibacterial tests.

2.3. Evaluation of the antioxidant activity of the extracts

Antioxidant activity was carried out according to prescribed method[9]. It is based on the ability of compounds to reduce cationic radical of 2,2-azino-bis-(3-ethylbenzothiazoline-6-sulfonic) or ABTS⁺. The solution of the cationic radical ABTS⁺ was obtained by mixing an 8 mM ABTS solution (87.7 mg in 20 mL of distilled water) and a 3 M potassium persulfate solution (0.0162 g in 20 mL of distilled water) in a ratio 1:1 (v/v). The reaction mixture was then incubated in dark at room temperature (28±2°C) for 16 hours. Then, the resulting ABTS⁺ solution was diluted in absolute methanol to obtain a solution with an absorbance of 0.7±0.02 at 734 nm. Furthermore, 3.9 mL of the diluted ABTS⁺ solution was added to 100 µL of the extract to be tested. After shaking, resulting mixture was incubated for 6 min in the dark. The residual absorbance of the ABTS⁺ radical is measured at 734 nm using a UV-visible spectrophotometer (Infitek SP-LUV752) and must represent between 20

and 80% of the absorbance of the white. The tests were carried out in triplicate and results were expressed as μmol of Trolox equivalent per liter of extract ($\mu\text{mol TE/L}$ of extract). Calibration of the spectrophotometer was carried out by reading absorbance of different concentrations of Trolox (3.75; 5; 6.25; 10; 11.25, 13.75 and 15.10-4 mM). The percentage of inhibition (PI) of ABTS⁺⁺ was determined by the relationship:

$$\text{PI} = ((A_0 - A) / A_0) \times 100$$

PI: Inhibition percentage (%); A: Absorbance of diluted ABTS containing the samples to be tested; A₀: Absorbance of diluted ABTS (control absorbance);

Activity of the extracts was expressed by Trolox Equivalent Antioxidant Capacity (TEAC) which corresponds to the concentration of Trolox (reference antioxidant) inducing the same antioxidant capacity as a concentration of 1 mM of the extract tested. It is determined by the following formula:

$$C = (\text{PI} \times D) / (4.99 \times 10)$$

C: antioxidant capacity ($\mu\text{mol TE/L}$ of extract); D: dilution factor; PI: Inhibition percentage

2.4. Evaluation of the antibacterial activity of the extracts

2.4.1. Preparation of bacterial inoculum

The inoculum of each bacterial strain was prepared by homogenizing two young colonies aged 18 to 24 hours in 10 mL of Mueller-Hinton broth then incubated for 3 hours at 37°C. Following incubation, 1 mL of broth was added to 9 mL of Mueller-Hinton broth to obtain inoculum estimated at 10⁶ bacteria/mL with a turbidity of 0.5 Mac Farland.

2.4.2. Determination of strain sensitivity to plant extracts

Agar punch well method in Petri dishes was used to test sensitivity of the strains to the extracts. The Mueller-Hinton agar was first inoculated by flooding with the previously prepared inoculum. After aspiration of the excess liquid using a sterile Pasteur pipette and drying in an oven for 15 to 30 min at 37°C, wells of 6 mm in diameter were made in the agar. These wells separated by at least 20 mm were filled with 80 μL of aqueous extract or hydro-ethanolic extract at 100 mg/mL [10]. At the same time, a control well was prepared with 80 mL of a mixture of DMSO/sterile distilled water (v/v) and gentamycin (30 μg) was used as a

standard positive control antibiotic. After 45 min of pre-diffusion, the whole was incubated in an oven at 37°C for 18 hours. The effect of each extract on the strain studied was assessed by measuring diameter of the growth inhibition zone around the well [11]. This test was carried out in triplicate for each extract.

2.4.3. Determination of antibacterial parameters of plant extracts

2.4.3.1. Preparation of the concentration range

A range of concentrations of each extract, ranging from 100 to 0.8 mg/mL was prepared by the double dilution method in test tubes [12]. Thus, 1000 mg of fine powder of the extracts were mixed with 10 mL of distilled water to make the initial concentration $C_1 = 100$ mg/mL. Then, 5 mL of this solution was added to 5 mL of distilled water to obtain the concentration $C_2 = 50$ mg/mL. Using this technique, the other concentrations were prepared: $C_3 = 25$ mg/mL; $C_4 = 12.5$ mg/mL; $C_5 = 6.25$ mg/mL; $C_6 = 3.125$ mg/mL; $C_7 = 1.56$ mg/mL; $C_8 = 0.80$ mg/mL. The contents of the tubes thus prepared were sterilized at 121°C for 15 min in the autoclave.

2.4.3.2. Determination of the Minimum Inhibitory Concentration

The minimum inhibitory concentration (MIC) was determined by adding 1 mL of the inoculum of each strain to 1 mL of the concentration of each plant extract in hemolysis tubes. After homogenization of the mixture, the tubes were incubated at 37°C for 18 to 24 hours. After incubation, observation of the tubes made it possible to obtain the MIC corresponding to the lowest concentration which does not leave any growth visible to the naked eye of the bacteria tested.

2.4.3.3. Determination of the minimum bactericidal concentration

To determine the minimum bactericidal concentration (MBC), two Petri dishes A and B containing a Mueller-Hinton agar were used. Box A was inoculated in parallel streaks of 5 cm, with 0.1 mL of the contents of each of the tubes with a concentration greater than or equal to MIC using a sterile calibrated loop. At the same time, dilutions from the mother suspension (10^0) were made up to the dilution 10^{-4} . Then, these dilutions and the mother suspension were also inoculated by parallel streaks in box B. Then, the two boxes were incubated at 37°C for 18 to 24 hours. The MBC was obtained by comparing the different colonies in box A to those in the 10^{-4} dilution of Petri dish B. The MBC corresponds to the concentration of plant extract presenting a number of colonies in box A. less than or equal to

that of the 10^{-4} dilution of box B. This MBC is the smallest concentration which allows at most 0.01% of the germs in the starting suspension to survive for 24 hours. Finally, MBC/MIC ratio was calculated to determine the antibacterial power of each extract [13].

2.5. Statistical analysis

Values were presented as mean \pm standard deviation. The graphs were made using Excel software and the data were analyzed with Graph Pad Prism 8.0 software (Microsoft, USA) for multiple variances (ANOVA). The difference between the means was determined using the DUNCAN test with a threshold of 5% ($P < 0.05$ for significant differences).

3. RESULTS AND DISCUSSION

3.1. Antioxidant activity of extracts

Determination of antioxidant activity of hydroalcoholic and aqueous extracts by the ABTS+• radical cation trapping test was carried out using the Trolox calibration line (Figure 1). The results obtained are presented in Figure 2. The analysis of these results reveals a strong antioxidant power of the two extracts. Trolox equivalent antioxidant capacity (TEAC) of the hydroalcoholic extract is 54.83 ± 0.02 $\mu\text{mol TE/L}$ of extract. This value is higher than that of the aqueous extract which is 47.58 ± 0.1 $\mu\text{mol TE/L}$ of extract. However, there is no significant difference between these values. These results are in agreement with those obtained from the DPPH radical trapping test already carried out by [14]. on the same extracts. Indeed, these authors showed by this technique that the two extracts had a strong anti-radical power compared to gallic acid with IC_{50} of 6.4 ± 0.02 $\mu\text{g/mL}$ and 7.5 ± 0.01 $\mu\text{g/mL}$ respectively. Antioxidant activity of the extracts could be linked to the chemical constituents present in the bark of this plant. A qualitative and quantitative phytochemical study carried out by the same authors on the extracts of this plant revealed the presence of high quantities of total polyphenols with a predominance for flavonoids and tannins. These secondary metabolites are known in the literature as antioxidant molecules [15,16].

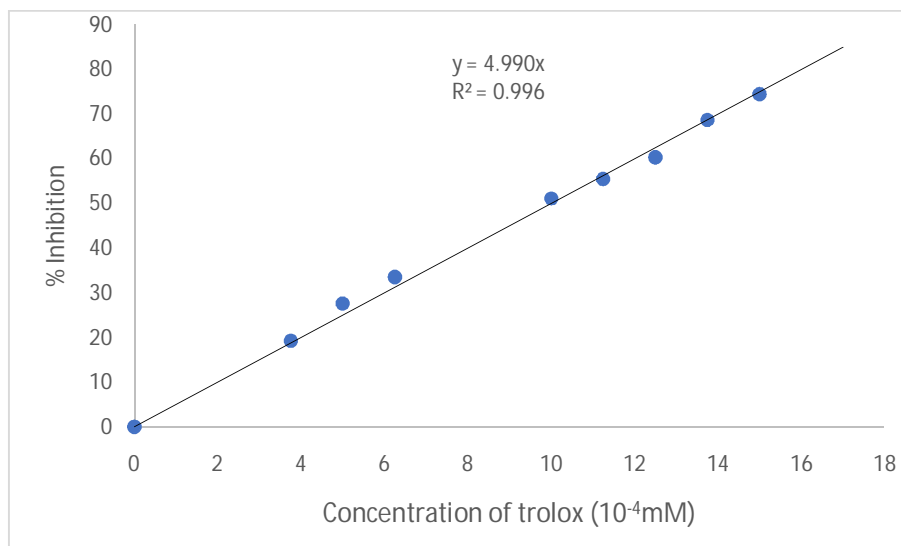


Figure 1: Trolox calibration curve for antioxidant activity assay

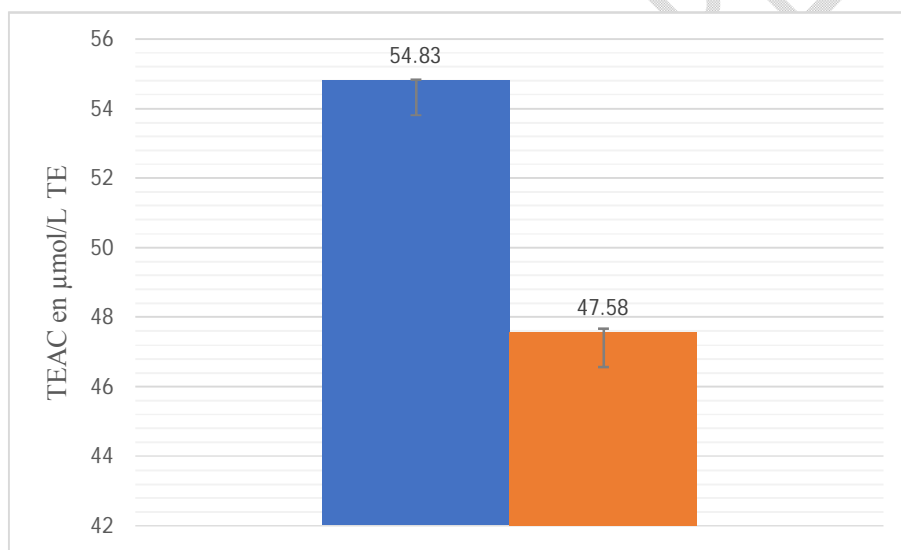


Figure 2: ABTS+• antioxidant capacity of aqueous and hydroethanolic extract

: Hydro-ethanolic extract: Aqueous extract

3.2. Antibacterial activity of *K. senegalensis* extracts

3.2.1. Sensitivity of strains to extracts

Table 2 indicates sensitivity of different strains to the two plant extracts and gentamicin across the diameters of inhibition zones. It appears from this test that for the aqueous extract, the diameters of the inhibition zones of the strains varied from 10 ± 0.2 mm to 21.6 ± 1.5 mm while those of hydro-ethanolic extract ranged from 10 ± 0.2 mm to 21.6 ± 1.5 mm. Referring [11], all strains were sensitive to both extracts. A bacterial strain is sensitive to a plant extract when the diameter of the inhibition zone that it induces is greater than or equal to 10 mm. However, strains of the *Staphylococcus* genus were more sensitive than *E.coli* strains with inhibition diameters of 18 ± 0.2 mm to 22 ± 1.0 mm compared to 10 ± 0.2 mm to 12.00 ± 1.00 mm for *E.coli*. There is no significant difference between the diameters of the inhibition zones of species of the same genus unlike those observed between strains of *E. coli* and *Staphylococcus*.

As for the reference antibiotic (gentamicin), the inhibition diameters observed showed sensitivity of all the strains with diameters varying from 11 ± 0.1 mm to 26 ± 0.4 mm excluding the strain. *E. coli* 8133 (8 ± 1.2 mm). This antibiotic was more active on the two reference strains tested.

Table 2: Diameters of zones of inhibition of bacterial strains by extracts

Strains	Aqueous extract	Hydro-ethanolic	Reference antibiotic
	(100 mg/mL)	extract (100 mg/mL)	(Gentamycin, 30µg)
Inhibition diameters (mm)			
<i>E.coli</i> 8039	10 ± 0,2	12 ± 0,3	11 ± 0,1
<i>E.coli</i> 8133	11 ± 0,3	13 ± 0,5	8 ± 1,2
<i>E.coli</i> ATCC 25922	12 ± 1,0	11 ± 1,5	26 ± 0,4
<i>Staphylococcus spp</i> 9044	18 ± 0,2	18 ± 0,3	16 ± 0,3
<i>Staphylococcus spp</i> 9109	15 ± 1,0	18 ± 0,2	16 ± 0,2
<i>S.aureus</i> ATCC 19213	21 ± 1,5	22 ± 1,0	25 ± 0,1

3.2.2. Determination of antibacterial parameters of plant extracts

Antibacterial parameters of the plant extracts on the different strains are summarized in Tables 3 and 4.

Concerning the hydro-ethanolic extract, the MIC recorded on the two clinical strains of *E.coli* was identical at 6.25 mg/mL. This value is lower than that observed with the reference strain *E.coli* ATCC 25922 (25 mg/mL). For all of these strains, the MBC was identical to the MIC. The MBC/MIC ratio determines the antibacterial power. According to [13], when this ratio is less than 4 the extract is bactericidal, but the extract is said to be bacteriostatic if this ratio is greater than or equal to 4. For all the strains of *E.coli* tested, this ratio is 1 indicating that the hydro-ethanolic extract has a bactericidal effect. As for *Staphylococcus*, the highest MIC (12.5 mg/mL) was recorded with strain 9044 while clinical strain 9109 and reference strain ATCC 19213 indicated the lowest value (6.25 mg/mL). For the two clinical strains of *Staphylococcus spp*, the MBC was identical to the MIC indicating a bactericidal effect of the hydro-ethanol extract on these strains while for the reference strain *Staphylococcus* ATCC

19213, the MBC was 25 mg/mL, corresponding to a bacteriostatic effect of the extract on this strain (Table 3).

Table 3: Antibacterial parameters of the hydro-ethanolic extract

Strains	<i>E.coli</i>	<i>E.coli</i>	<i>E. coli</i>	<i>Staphylococcus</i> sp	<i>Staphylococcus</i> sp	<i>S.aureus</i>
Para-Meters	8039	8133	ATCC 25922	<i>p</i> 9044	<i>p</i> 9109	ATCC 19213
MIC (mg/mL)	6,25	6,25	25	12,5	6,25	6,25
MBC (mg/mL)	6,25	6,25	25	12,5	6,25	25
MBC/MIC	1	1	1	1	1	4

For the aqueous extract, apart from the reference strain *Staphylococcus* ATCC 19213 which showed the lowest MIC (6.25 mg/mL), all the other strains recorded the same MIC of 12.5 mg/mL. Furthermore, MBC was identical for all strains of *E.coli* (25 mg/mL) and for all strains of *Staphylococcus* (12.5 mg/mL). The MBC/MIC ratio varied from 1 to 2, allowing us to note that aqueous extract had a bactericidal effect on all the strains tested (Table 4).

These results confirm those of [17] and [18] with the roots and leaves of the same plant (*K.senegalensis*), respectively. A dose-dependent antibacterial activity on various strains including *E.coli*, *Staphylococcus spp*, *Salmonellaspp* with aqueous and hydro-ethanol extracts of the leaves were studied earlier. In the same vein, these authors obtained great effectiveness on the strains tested with the hydro-ethanolic extract. This is in accordance with the antibacterial parameters found in this study. The analysis of these parameters shows lower MICs and MBCs with the hydro-ethanolic extract compared to the aqueous extract. Ethanol therefore presents itself as the solvent which allows better extraction of the bioactive constituents from the root bark of this plant. The antibacterial activity exerted by these

extracts could be explained by the presence of these bioactive constituents. Indeed, according to the work of [14], the roots of this plant are very rich in total polyphenols, flavonoids and alkaloids. All of these biomolecules are recognized for their antioxidant and antibacterial activities [19,20]. This could also justify the traditional use of this plant against numerous pathologies.

Table 4: Antibacterial parameters of the aqueous extract

Strains	<i>E.coli</i> 80	<i>E.coli</i> 8	<i>E.coli</i> ATCC 25922	<i>Staphylococcus</i> <i>spp</i> 9044	<i>Staphylococcus</i> <i>spp</i> 9109	<i>S.aureus</i> ATCC 19213
Para-Meters	39	133				
MIC (mg/ml)	12,5	12,5	12,5	12,5	12,5	6,25
MBC (mg/ml)	25	25	25	12,5	12,5	12,5
MBC/MIC	2	1	2	1	1	2

4. CONCLUSION

This study aimed to highlight antioxidant and antibacterial potential of aqueous and hydro-ethanolic extracts of stem bark of *K. senegalensis*. At the end of the work, it appears that the two extracts have a strong antioxidant power compared to the reference antioxidant (trolox). Both extracts were also active on clinical strains involved in urinary infections and reference strains of *E.coli* and *Staphylococcus*. These extracts could therefore be a good basis for the implementation of traditionally improved drugs (TID) in the fight against urinary infections.

DISCLAIMER (Artificial intelligence)

Author(s) hereby declare that no generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

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