Original Research Article

Antioxidantpotential and antibacterialactivity of stem barkextracts of *Kaya Senegalensis* (Desv) A.Juss., on in vitro growth of germsresponsible for urinary infections in humans

Abstract

Urinary infections todayconstitute a public healthproblem and responsiblegermsincreasingly express strongresistance to commonantimicrobials. Use of plants in fightagainstthese pathologies presentsitself as an alternative. The objective of thisstudyis to determine in vitro antioxidant power and antibacterialactivity of aqueous and hydro-ethanolicextracts 70% of stem bark of *Kaya senegalensis* (Meliaceae) on clinicalstrains of *E.coli* and *Staphylococcus spp*. urinary infections and on tworeferencestrains. The antioxidantactivitywasdemonstrated by the ABTS radical cation trappingmethodusingtrolox as a reference. Sensitivity of strains to the twoextracts and antibacterialparameterswhich are minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) weredeterminedrespectively by punch wellmethod in Mueller Hinton agar and liquid dilution method. Bothextractsexpressed a strongantioxidant power around 50 µmol TE/L of extract. The MICsvariedfrom 6.25 mg/mL to 12.5 mg/mL for aqueousextract and from 6.25 mg/mL to 25 mg/mL for hydro-ethanolicextract. For all the strains MBCwasidentical to the MIC indicatingthat the twoextracts have bactericidal power on the strainsexcluding the referencestrain of *S.aureus*. Theseresultssuggestthat*K. senegalensis*couldbe an alternative in the fightagainsturinary infections.

Keywords: *K.senegalensis*, antioxidant, antibacterialparameters, urinary infections

1- INTRODUCTION

urinary tract infection is a pathology of the urinary tract, thatis to say the kidneys, ureters, bladder and urethra. There are different types of urinary infections, namelylowerurinary tract infections and upperurinary infections (located in the kidneys). In lowerurinary tract infections located in the bladder, wedistinguishbetween non-

infectiouscystitis and especiallymicrobialcystitis. The latter can affect everyone, but they are more common in women of childbearingage and in men of advancedage[1,2].Approximately 50% of womendevelop a symptomaticurinary tract infection at least once in their life [3]. Treatment of these pathologies generally uses antibiotics. But, increasingly, strongresistance to thesemoleculesisbeingrecorded, leading populations to resort to medicinal plants. Indeed, manymedicinal plants presentthemselvestoday as a therapeutic alternative, especiallysincetheyconstitute the most important and inexhaustible source of bioactive compounds such as antioxidants, antimicrobials, antiparasitics and anti-inflammatories capable of prevent and treatdiseases[4]. Amongthesemedicinal plants are *Kaya senegalensis* (Méliaceae). This plant isverywidespread in the forests and savannahs of West Africa[5]. It has been the subject of severalscientificstudieswhich have revealeditsantimalarial, antioxidant, anti-inflammatory, antiproliferative, antimicrobial, anthelmintic, antidiarrheal and otherproperties[6,7]. Despitethisbroadspectrum of activity of the plant, there are no pharmacological data relating to urinary infections in Côte d'Ivoire. It iswithinthisframeworkthat the presentworkfalls, whichaims to show antioxidant and antibacterialpotential of aqueous and hydroalcoholicextracts of *K. Senegalensis* (Desv) A.Juss., on the in vitro growth of germsresponsible for urinary infections in humans.

2-MATERIAL AND METHODS

2.1-Material

2.1.1-Plant material

The plant materialconsists of stem bark of *Khaya senegalensis*, collected in Korhogo (Côte d'Ivoire) in July 2023. Subsequently, authentication of the plant wascarried out under the identifier UCJ012302, at the National FloristicsCenter (CNF) of the Félix HOUPHOUËT-BOIGNY University of Cocody (Abidjan, Côte d'Ivoire). Afterharvest, the sampleswerecarefullycleanedwithdistilled water thencutintosmallpieces and driedawayfrom the sun, at room temperature for twomonths. Once dried, barkswerepulverizedusing an electric grinder (RETSCH, Type AS 200) to obtain a fine powder. This plant powderwasstored in sterile jars and used to prepare the extracts.

2.1.2. Bacterialstrains

Differentbacterialstrainswereused in thisstudy. These are clinicalstrainsprovided by the microbiologylaboratory of the Regional Hospital Center of Korhogo and referencestrainsprovided by the Pasteur Institute of Côte d'Ivoire in September2023 (Table 1). Afterreceipt, the strainswerestored at -20° at the bacteriology unit of the National Agricultural Development Support Laboratory in Korhogo for microbiological tests.

Table 1: Bacterialstrainsused for extractsusceptibilitytesting

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

2.2. Preparation of plant extracts

Aqueous and hydroethanolic70%extracts werepreparedaccording to methoddescribed by [8].. Indeed, one hundred (100) g of *K. senegalensis*barkpowderweremacerated in one liter (1 L) of distilled water or ethanoldiluted to 70% (70/30; V/V) at l using a magneticstirrer at room temperature for 24 hours. Aftermaceration, the mixture obtainedwas first drainedthrough a square of white cloth, thendoublyfilteredthroughhydrophiliccotton and once throughWhatmanpaper (3 mm). The filtrateobtainedwasconcentrated in an ovenuntil the solvent hadcompletelyevaporated to obtain the aqueous and hydro-ethanolicextracts, stored in a sterilebottle for antioxidant and antibacterial tests.

2.3. Evaluation of the antioxidantactivity of the extracts

Antioxidantactivitywascarried out according to methoddescribed by [9]. It isbased on the ability of compounds to reducecationic radical of 2,2-azino-bis-(3-ethylbenzothiazoline-6-sulfonic) or $ABTS^{+\bullet}$. The solution of the cationic radical $ABTS^{+\bullet}$wasobtained 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 wasthenincubated in dark at room temperature (28±2°C) for 16 hours. Then, the resulting $ABTS^{+\bullet}$ solution wasdiluted in absolutemethanol to obtain a solution with an absorbance of 0.7±0.02 at 734 nm. Furthermore, 3.9 mL of the diluted $ABTS^{+\bullet}$ solution wasadded to 100 µL of the extract to betested. Aftershaking, resulting mixture wasincubated for 6 min in the dark. The residual absorbance of the $ABTS^{+\bullet}$ radical ismeasured at 734 nm using a UV-visible spectrophotometer and must representbetween 20 and 80% of the absorbance of the white. The tests werecarried out in triplicate and resultswereexpressed as µmol of Troloxequivalent per liter of extract (µmol TE/L of extract).

Calibration of the spectrophotometerwascarried 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 $ABTS^{+\bullet}$wasdetermined by the relationship:

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

PI: Inhibition percentage (%); A: Absorbance of diluted ABTS containing the samples to betested; A_0: Absorbance of diluted ABTS (control absorbance);

Activity of the extractswasexpressed by Trolox Equivalent AntioxidantCapacity (TEAC) which corresponds to the concentration of Trolox (referenceantioxidant) inducing the sameantioxidantcapacity as a concentration of 1 mM of the extracttested. It isdetermined by the following formula:

$$C=(PI\times D)/(4.99X\ 10)$$

C: antioxidantcapacity (µmol TE/L of extract); D: dilution factor; PI: Inhibition percentage

2.4. Evaluation of the antibacterialactivity of the extracts

2.4.1. Preparation of bacterial inoculum

The inoculum of eachbacterialstrainwasprepared by homogenizingtwoyoung colonies aged 18 to 24 hoursin 10 mL of Mueller-Hinton broththenincubated for 3 hours at 37°C. Following incubation, 1 mL of brothwasadded to 9 mL of Mueller-Hinton broth to obtain inoculum estimated at 10^6bacteria/mLwith a turbidity of 0.5 Mac Farland.

2.4.2. Determination of strainsensitivity to plant extracts

Agar punch wellmethod in Petri disheswasused to test sensitivity of the strains to the extracts. Indeed, the Mueller-Hinton agar was first inoculated by floodingwith the previouslyprepared inoculum. After aspiration of the excessliquidusing a sterile Pasteur pipette and drying in an oven for 15 to 30 min at 37°C, wells of 6 mm in diameterwere made in the agar. Thesewellsseparated by at least 20 mm werefilledwith 80 µL of aqueousextract or hydro-ethanolicextract at 100 mg/mL[10]. At the same time, a control wellwaspreparedwith 80 mL of a mixture of DMSO/steriledistilled water (v/v) and gentamycin (30 µg) wasused as a standard positive control antibiotic. After 45 min of pre-diffusion, the wholewasincubated in an oven at 37°C for 18 hours.The effect of eachextract on the strainstudiedwasassessed by measuringdiameter of the growth inhibition zone around the well[11]. This test wascarried out in triplicate for eachextract.

2.4.3. Determination of antibacterialparameters of plant extracts

2.4.3.1. Preparation of the concentration range

A range of concentrations of eachextract, rangingfrom 100 to 0.8 mg/mLwasprepared by the double dilution method in test tubes [12].. Thus, 1000 mg of fine powder of the extractswere mixed with 10 mL of distilled water to constitute the initial concentration C_1 = 100 mg/mL. Then, 5 mL of this solution wasadded to 5 mL of distilled water to obtain the concentration C_2 = 50 mg/mL. Usingthis technique, the other concentrations wereprepared: 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 thuspreparedweresterilized at 121°C for 15 min in the autoclave.

2.4.3.2. Determination of the Minimum Inhibitory Concentration

The minimum inhibitory concentration (MIC) wasdetermined by adding 1 mL of the inoculum of eachstrain to 1 mL of the concentration of each plant extract in hemolysis tubes. Afterhomogenization of the mixture, the tubes wereincubated 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 whichdoes not leaveanygrowth visible to the nakedeye of the bacteriatested.

2.4.3.3. Determination of the minimum bactericidal concentration

To determine the minimum bactericidal concentration (MBC), two Petri dishes A and B eachcontaining a Mueller-Hinton agar wereused. Box A wasinoculated in parallelstreaks of 5 cm, with 0.1 mL of the contents of each of the tubes with a concentration greaterthan or equal to MIC using a sterilecalibratedloop. 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 werealsoinoculated by parallelstreaks in box B. Then, the two boxes wereincubated at 37°C for 18 to 24 hours. The MBC wasobtained 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 extractpresenting a number of colonies in box A. lessthan or equal to that of the 10^{-4} dilution of box B. This MBCis the smallest concentration whichallows at most 0.01% of the germs in the starting suspension to survive for 24 hours. Finally, MBC/MIC ratio wascalculated to determine the antibacterial power of eachextract[13].

2.5. Statisticalanalysis

Values werepresented as mean ± standard deviation. The graphs were made using Excel software and the data wereanalyzedwith Graph Pad Prism 8.0 software (Microsoft, USA) for multiple variances (ANOVA). The differencebetween the meanswasdeterminedusing the DUNCAN test with a threshold of 5% ($P < 0.05$ for significantdifferences).

3. RESULTS AND DISCUSSION

3.1. Antioxidantactivity of extracts

Determination of antioxidantactivity of hydroalcoholic and aqueousextracts by the ABTS+• radical cation trapping test wascarried out using the Trolox calibration line (Figure 1). The resultsobtained are presented in Figure 2. The analysis of theseresultsreveals a strongantioxidant power of the twoextracts. Indeed, the Troloxequivalentantioxidantcapacity

(TEAC) of the hydroalcoholicextractis 54.83 ± 0.02 µmol TE/L of extract. This value ishigherthanthat of the aqueousextractwhichis 47.58 ± 0.1 µmol TE/L of extract. However, thereis no significantdifferencebetweenthese values. Theseresults are in agreement withthoseobtainedfrom the DPPH radical trapping test alreadycarried out by [14]. on the sameextracts. Indeed, theseauthorsshowed by this technique that the twoextractshad a strong anti-radical power compared to gallicacidwith $IC_{s_{50}}$of 6.4 ± 0.02 µg/mL and 7.5 ± 0.01 µg/mLrespectively. Antioxidantactivity of the extractscouldbelinked to the chemicalconstituentspresent in the bark of this plant. A qualitative and quantitative phytochemicalstudycarried out by the sameauthors on the extracts of this plant revealed the presence of high quantities of total polyphenolswith a predominance for flavonoids and tannins. Thesesecondarymetabolites are known in the literature as antioxidantmolecules[15,16]..

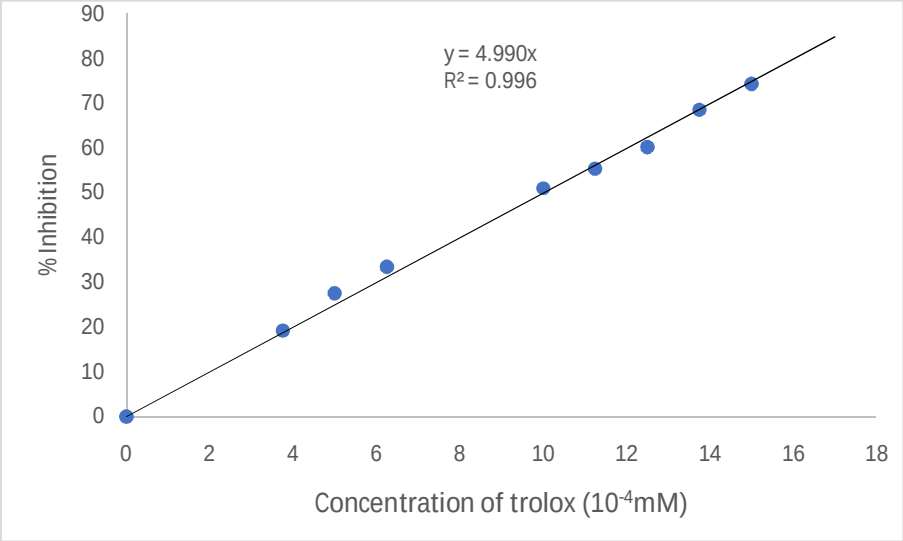

Figure 1: Trolox calibration curve for antioxidantactivityassay

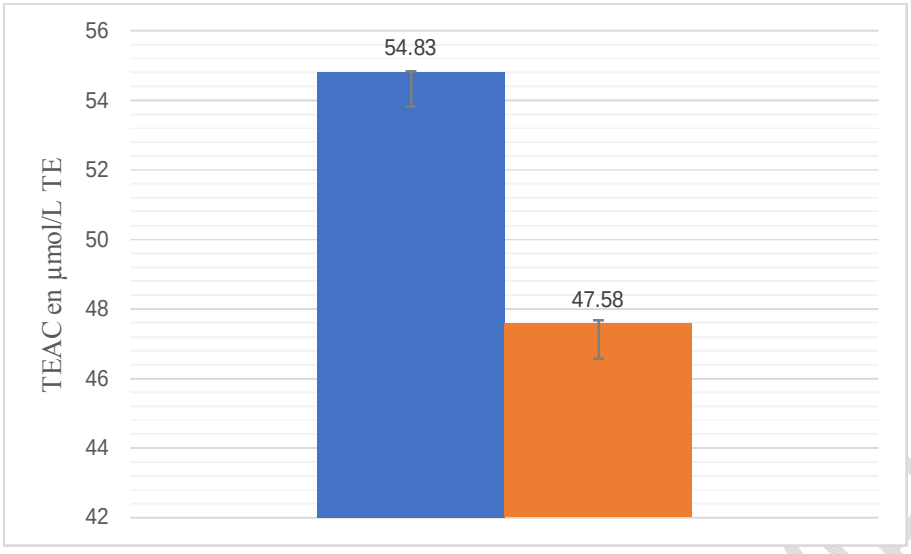

Figure 2: ABTS+• antioxidantcapacity of aqueous and hydroethanolic extract

: :Hydro-ethan xtract : Aqueousextra

3.2. Antibacterialactivity of K. senegalensis extracts

3.2.1. Sensitivity of strains to extracts

Table 2 indicatessensitivity of differentstrains to the two plant extracts and gentamicinacross the diameters of inhibition zones. It appearsfromthis test that for the aqueousextract, the diameters of the inhibition zones of the strainsvariedfrom 10 ± 0.2 mm to 21.6 ± 1.5 mm whilethose of hydro-ethanolicextractrangedfrom 10 ± 0.2 mm to 21.6 ± 1.5 mm.from 12± 0.3 mm to 22± 1.0 mm.Referring[11], all strainswere sensitive to bothextracts. Indeed, according to theseauthors, a bacterialstrainis sensitive to a plant extractwhen the diameter of the inhibition zone thatitinducesisgreaterthan or equal to 10 mm.However, strains of the *Staphylococcus*genuswere more sensitive than*E.coli*strainswith inhibition diameters of 18±02 mm to 22±1.0 mm compared to 10±0.2 mm to 12.00±1 .00 mm for *E.coli*. There is no significantdifferencebetween the diameters of the inhibition zones of species of the samegenusunlikethoseobservedbetweenstrains of *E. coli* and *Staphylococcus*.

As for the referenceantibiotic (gentamycin), the inhibition diametersobservedshowedsensitivity of all the strainswithdiametersvaryingfrom 11 ± 0.1 mm to 26 ± 0.4 mm excluding the strain. *E. coli* 8133 (8 ± 1.2 mm). This antibioticwas more active on the tworeferencestrainstested.

Table 2: Diameters of zones of inhibition of bacterialstrains by extracts

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

3.2.2. Determination of antibacterialparameters of plant extracts

Antibacterialparameters of the plant extracts on the differentstrains are summarized in Tables 3 and 4.

Concerning the hydro-ethanolicextract, the MIC recorded on the twoclinicalstrains of *E.coli*wasidentical at 6.25 mg/mL. This value islowerthanthatobservedwith the referencestrain*E.coli* ATCC 25922 (25 mg/mL). For all of thesestrains, the MBCwasidentical to the MIC. The MBC/MIC ratio determines the antibacterial power. According to [13], whenthis ratio islessthan 4 the extractisbactericidal, but the extractissaid to bebacteriostatic if this ratio isgreaterthan or equal to 4. For all the strains of *E.coli*tested, this ratio is 1 indicatingthat the hydro-ethanolicextract has a bactericidaleffect. As for *Staphylococcus*, the highest MIC (12.5 mg/mL) wasrecordedwithstrain 9044 whileclinicalstrain 9109 and referencestrain ATCC 19213 indicated the lowest value (6.25 mg/mL). For the twoclinicalstrains of *Staphylococcus spp*, the MBC wasidentical to the MIC indicating a bactericidaleffect of the hydro-ethanolextract on thesestrainswhile for the referencestrain*Staphylococcus* ATCC 19213, the MBC was 25 mg/mL, corresponding to a bacteriostaticeffect of the extractonthisstrain (Table 3).

Table 3: Antibacterialparameters of the hydro-ethanolicextract

Strains / Para-Meters	*E.coli* 8039	*E.coli* 8133	*E. coli* ATCC 25922	*Staphylococcussp p* 9044	*Staphylococcussp p* 9109	*S.aureus* 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 aqueousextract, apartfrom the referencestrain*Staphylococcus* ATCC 19213 whichshowed the lowest MIC (6.25 mg/mL), all the otherstrainsrecorded the same MIC of 12.5 mg/mL. Furthermore, MBCwasidentical for all strains of *E.coli* (25 mg/mL) and for all strains of *Staphylococcus* (12.5 mg/mL). The MBC/MIC ratio variedfrom 1 to 2, allowing us to note thataqueousextracthad a bactericidaleffect on all the strainstested (Table 4).

Theseresultsconfirmthose of [17] and [18]with the roots and leaves of the same plant (*K.senegalensis*), respectively. Indeed, theseauthorsshoweda dose-dependentantibacterialactivity on variousstrainsincluding*E.coli*, *Staphylococcus spp*, *Salmonellaspp*withaqueous and hydro-ethanolextracts of the leaves. In the samevein, theseauthorsobtainedgreateffectiveness on the strainstestedwith the hydro-ethanolicextract. This is in accordance with the antibacterialparametersfound in thisstudy. Indeed, the analysis of theseparameters shows lowerMICs and MBCswith the hydro-ethanolicextractcompared to the aqueousextract. Ethanol thereforepresentsitself as the solvent whichallowsbetter extraction of the bioactive constituentsfrom the root bark of this plant. The antibacterialactivityexerted by theseextractscouldbeexplained by the presence of these bioactive constituents. Indeed, according to the work of [14], the roots of this plant are veryrich in total polyphenols, flavonoids and alkaloids. All of thesebiomolecules are recognized for theirantioxidant and antibacterialactivities[19,20]. This couldalsojustify the traditional use of this plant againstnumerous pathologies.

Table 4: Antibacterialparameters of the aqueous extract

Strains / Para-Meters	*E.coli*80 39	*E.coli*8 133	*E.coli* ATCC 25922	*Staphylococcus spp* 9044	*Staphylococcus spp* 9109	*S.aureus* ATCC 19213
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 studyaimed to highlight antioxidant and antibacterialpotential of aqueous and hydro-ethanolicextracts of stem bark of *K. senegalensis*. At the end of the work, itappearsthat the twoextracts have a strongantioxidant power compared to the referenceantioxidant (trolox). Bothextractswerealso active on clinicalstrainsinvolved in urinary infections and referencestrains of *E.coli* and *Staphylococcus.* Theseextractscouldthereforebe a good basis for the implementation oftraditionallyimproveddrugs (TID) in the fightagainsturinary infections.

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