

Antibacterial parameters of infused extracts of *Mangifera indica* cultivars (Kent) leaves on the *in vitro* growth of urinary tract infections strains

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Abstract

Background: *Mangifera indica* is a plant belonging to the Anacardiaceae family. It is traditionally used to treat a variety of conditions including diabetes, bronchitis, diarrhea, asthma, scabies, respiratory problems and urinary tract infections. The aim of the present work is to understand the use of Kent leaves in the treatment of urinary tract infections in traditional medicine.

Methods: In this study, qualitative phytochemical tests based on detection by staining and tube precipitation of phenolic compounds were carried out. Bacterial susceptibility to infusions was determined by the agar diffusion method from wells. MICs and BMCs were determined by double dilution in liquid medium coupled with spreading on Mueller Hinton agar.

Results: This work shows a dominance of sterols, polyterpenes, polyphenols, flavonoids, catechic tannins, alkaloids and quinones in infusions extracts of kent cultivar. The most sensitive strain was the one with the lowest MIC (MIC = 3.125mg/mL) and the largest diameters (19.33 mm). This was *S. aureus* 9044. All the germs studied were resistant to at least two families of antibiotics. They are therefore multi-resistant. **Conclusion:** These results could stimulate the use of kent cultivar leaves in traditional environments for the treatment of urinary tract infections.

Keywords: *Mangifera Indica*; Phytochemical; Antibacterial; Multi-Resistant; Urinary Infection

1. Introduction

Health is of paramount importance to man. To maintain good health, man has always resorted to medicines. However in recent years, the emergence and spread of antibiotic resistance represents a real threat to global public health [1]. The situation is alarming in countries where infectious diseases, poverty and malnutrition are endemic. Moreover, the abusive use of antibiotics, contributes to the development of bacterial resistance [2]. The emergence of bacterial resistance is a complex process, often involving host and pathogen environmental factors [3]. Faced with the limitations of available antibiotics, it is essential to search for new bioactive substances with a broad spectrum of action. Medicinal plants are an important source of bioactive molecules that could be exploited in the therapy of infectious diseases such as urinary tract infections [4], as shown by several ethno pharmacological studies [5]. Urinary tract infections are more common in infants and pregnant women. According to the World Health Organization, urinary tract infections (UTIs) are a health security problem for countries, especially those in the developing world [6]. Urinary tract infections are implicated in the onset or complication of serious chronic diseases with a poor prognosis, such as prostatitis, diabetes,

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sickle cell disease, renal failure and HIV/AIDS [7-9]. Several bacteria are implicated in urinary tract infections: *Escherichia coli* (80%), *Proteus mirabilis*, *klebsiella* spp, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and many others [10]. Indeed, several plants are used in the treatment of infectious diseases. Among these is the mango tree (*Mangifera indica*) [11], a member of the Anacardiaceae family. *Mangifera indica* is widely distributed throughout the world and includes several cultivars (Amélie, Kent...). In Côte d'Ivoire, the dominant cultivar is Kent. It accounts for over 95% of export production [12]. In addition to its famous fruits, the mango tree has many medicinal properties. Traditionally, extracts from the stem barks, leaves are used to treat various ailments such as diabetes, bronchitis, diarrhea, asthma, scabies, respiratory problems [13]. Despite the many therapeutic virtues possessed by the mango tree, the scientific literature remains insufficiently informed about the use of Kent cultivar leaves in traditional medicine. The aim of the present work is to understand the use of Kent leaves in the treatment of urinary tract infections in traditional medicine.

2. Materials and methods

2.1. Materials

2.1.1. Biological material

2.1.2. Plant material

The plant material consists of leaves of *Mangifera indica* cultivar Kent. The leaves were harvested in August 2023 at Korhogo in the Poro region of northern Côte d'Ivoire.

2.1.3. Bacterial strains tested

Three bacterial strains of clinical origin including *E. coli* (8312), *S. aureus* (9044), *P. aeruginosa* (8613) supplied by the Laboratoire d'analyse du CHR de Korhogo and three reference strains *E. coli* ATCC25922, *S. aureus* ATCC 19213, *P. aeruginosa* ATCC27853 from IPCI.

2.1.4. Reagents and chemicals

Several reagents and chemicals were used. Muller-Hinton agar, nutrient agar, nutrient broth, distilled water and physiological water (NaCl 0.9%) were used for antibacterial tests. The main developers and reagents listed below were used. Dragendorff and Bouchardât reagents were used to reveal alkaloids. Iron III chloride (FeCl₃) 2% was used to reveal polyphenols, tannins and phenolic acids. Hydrochloric alcohol was used for flavonoids. Sterols and terpenes were revealed using acetic anhydride. Quinones were detected using ammonia.

2.2. Methods

2.2.1. Leaf processing

Leaves were harvested in August 2023 in Korhogo and dried at room temperature for 3 days. The dried leaves were ground using an electric grinder, and the fine powder obtained was stored in jars in a dry place protected from light and moisture until use.

2.2.2. Preparation of total extracts

Total extracts were prepared using the infusion method described by Nogaret and Ehrhart, (2011)14, with slight modifications. This involves soaking the drug, which has been wrapped in blotting paper, in boiling water. The container is covered and the drug is left to infuse for 5 minutes before being removed. After cooling and filtration, the various filtrates are placed in an oven at 50°C for 3 days. In this study, different extracts were prepared by varying the mass. Thus, for the infused extract (1%), 1 g of plant powder is soaked in 100 mL of boiling water.

2.2.3. Phytochemical screening

Phytochemical screening tests were based in part on qualitative analysis, using tube staining and precipitation reactions [15].

2.2.4. Evaluation of antibacterial activity

Checking the purity of bacterial species

To check the purity of bacterial species, strains were streaked on specific culture media TBX (for *E. coli*); Chapman agar (for *S. aureus*) and King A (for *P. aeruginosa*). Plates were incubated at 37°C (for *S. aureus* and *P. aeruginosa*) and 44°C (for *E. coli*) for 24 h. The presence of blue-green colonies on TBX was indicative of *E. coli*; yellow colonies on Chapman indicated *S. aureus* and green colonies on King A were indicative of *P. aeruginosa*.

Inoculum preparation

Using a pasteur pipette, an 18 h colony is picked and placed in a test tube containing 10 mL of sterile Muller-Hinton broth. The mixture is incubated at 37°C for 3 hours. After opalescence, a 0.1 mL suspension of this pre-culture is taken and diluted in 10 mL sterile Muller-Hinton broth, then homogenized. This solution constitutes the stock solution at 10⁰ containing 10⁶ cells/mL [16].

Inoculum counting

Inoculum counting is carried out by diluting pure inoculum to the 10th. The various dilutions obtained, together with the pure inoculum, are inoculated onto Mueller Hinton agar without antimicrobial using a calibrated loop of 2 µL per 5 cm long streak. This plate is called plate A or bactericidal control plate. It is incubated at 37°C for 24 h [17].

Preparation of the extract concentration range

The plant extract concentration range from 200 mg/mL to 1.56 mg/mL was prepared in test tubes using the double dilution method. The prepared range was autoclaved at 121°C for 15 min [18].

Seeding of the concentration range of extracts

Seeding of the concentration range is carried out by adding 1 mL of the contents of each concentration range tube to 1 mL of inoculum at dilution 10⁰ containing 10⁶ cells/mL, around the flame of the Bunsen burner. The Growth Control (TC) tube contains 2 mL of inoculum. The Sterility Control (SC) tube contains 2 mL of sterile culture medium.

Susceptibility test

Sensitivity of strains to plant extracts was carried out using the agar diffusion technique. Mueller Hinton medium was inoculated by swabbing. Using a sterile punch, wells approximately 6 mm in diameter were made in the agar. Each well received 80 µL of the test substance. After 15 min diffusion at laboratory temperature, the Petri dishes were incubated at 37°C for 24 h. The presence of a zone of inhibition was observed and interpreted according to Ponce *et al.* (2003)¹⁹.

2.2.5. Determination of antibacterial parameters

Minimum Inhibitory Concentration (MIC)

The MIC is determined from the seeded concentration range by observation of the test tubes with the naked eye. A cloudy medium means that the culture is growing, and a clear medium means that growth has been inhibited. The MIC corresponds to the lowest inoculated concentration without growth visible to the naked eye [16].

Minimum Bactericidal Concentration (MBC)

The MBC is determined by subculturing all the experimental tubes in the seeded concentration range without growth visible to the naked eye. This subculture constitutes box B. The BMC is defined by comparing Box A and Box B. It corresponds to the smallest concentration in Box B whose colony count is less than or equal to the colony count of the 10⁻⁴ dilution of Box A [16].

2.2.6. Preparing the antibiogram

Preparing the bacterial inoculum

The inoculum was prepared from a pure culture. One or two 18h bacterial colonies were picked with a pasteur pipette and emulsified in a tube containing 2mL of 0.85% NaCl physiological water (Bio Mérieux, reference 08026 E). This mixture was homogenized by vortexing and the suspension was calibrated to the 0.5 Mac Farland scale. The inoculum was obtained by adding 100 µL of the previous mixture to 10 mL of sterile physiological water in a screw-top tube [16].

Inoculation of Mueller-Hinton agar

Agar poured into 120 mm-diameter petri dishes was inoculated by swabbing. The plate was dried at 37°C for 5 minutes. Antibiotic discs were applied to the agar surface with forceps, spaced 2 cm apart. Once applied, the antibiotic disc is not moved. The plates are incubated for 24 hours at 37°C for all strains. After incubation, the different diameters of the zones of inhibition obtained around the antibiotic discs were measured and interpreted as Susceptible (S), Intermediate (I) or Resistant (R) according to the criteria defined by EUCAST/CA-SFM, 2023.

2.3. Statistical analysis

Statistical analyses of the results were carried out using Statistica software version 7.1. Fisher's minimum significant difference (LSD) test was used to determine significant differences between several means. Differences were considered significant at the 5% level.

3. Results

3.1. Extraction yields

Extraction yields are shown in Table 1. The highest yields were obtained with the 1% infusates (25.28 ± 1.42). The lowest yields were obtained with the 5% infusions (21.56 ± 0.83). The lowest infusion percentage (1%) recorded the highest extraction yields. Infused extracts from the Kent cultivar showed a sticky appearance and brown coloration, except for the 5% infused which showed a brown coloration. Statistical analysis indicated that there was a significant difference between extraction yields at $p \leq 0.05$.

3.2. Phytochemical screening

Phytochemical screening showed the presence of sterols, polyterpenes, polyphenols, flavonoids, catechic tannins, alkaloids and saponins in all three Kent extracts. The difference in phytochemical compounds between the three infused extracts is observed in quinones and gall tannins. The 1% and 2.5% extracts contain gall tannins but no quinones, while the 5% extract shows the presence of quinones but no gall tannins (Table 2).

Table 1 Extraction yields for the Kent variety

Infused	Yield (%)	Color	Appearance
1%	$25,28 \pm 1,42^a$	Brown	Tights
2,5%	$24,67 \pm 1,25^a$	Brown	Tights
5%	$21,56 \pm 0,83^b$	Brown	Tights

Data are expressed as mean \pm standard deviation (3 trials). Averages assigned the same letter in the same column are not statistically different at the 5% threshold

Table 2 Phytochemical screening

Extracts	Sterols and Polyterpenes	Polyphenols	Flavonoides	Tannins		Quinones	Alcaloides	Saponines
				Cat	Gal		B	D
K.1%	+	+	+	+	+	-	+	+
K.2,5%	+	+	+	+	+	-	+	+
K.5%	+	+	+	+	-	+	+	+

(-) : absence ; (+) : presence ; K : Kent ; Cat. : Catechique ; Gal. : Galliques ; B : Bouchardât ; D : Dragendorff

3.3. Abacterial activity

3.3.1. Germ Susceptibility

The results of bacterial sensitivity testing on extracts are shown in Table 3. The largest inhibition diameters were obtained at a concentration of 200 mg/mL. The K.2.5% infusion obtained the best inhibition diameters. The largest

inhibition diameter (19.33 ± 0.94 mm) was obtained with the *S. aureus* strain (9044) and the smallest with *E. coli* (8312) and *E. coli* ATCC 25922. For the 5% infusion, the largest inhibition diameter (16.66 ± 1.67 mm) was obtained with *P. aeruginosa* (8613). As for the 1% infusion, the largest inhibition diameter was obtained with *S. aureus* strain 9044 (14.67 ± 0.47 mm). All germs were sensitive to all three extracts except *E. coli* (8312) and *E. coli* ATCC25922, which showed no inhibition diameter. *S. aureus* was the most sensitive strain to the different extracts, and *E. coli* (8312) the least sensitive. Statistical analysis indicates that for each germ, there is no significant difference between the mean inhibition diameters obtained with K.1% and K.5% infusions at $p \leq 0.05$.

3.3.2. Determination of antibacterial parameters (MIC and MBC)

All extracts showed minimum inhibitory concentrations (MIC) ranging from 3.125 to 12.5 mg/mL. Minimum bactericidal concentrations (MBC) ranged from 0 to 50 mg/mL for the three (3) infused extracts (Table 4; 5 and 6). The 1% and 5% infusions exerted a bacteriostatic effect on *P. aeruginosa* and *S. aureus* strains, while the 2.5% infusion had a bactericidal effect on *S. aureus* strains and a bacteriostatic effect on *P. aeruginosa* strains.

Table 3 Inhibition diameter of extracts

Inhibition diameter of the concentration of 200 mg/mL						
Origines	Germes	K.1%	K.2.5%	K.5%	Gen. 30.10 ³ mg	Cefo. 5.10 ³ mg
Souches cliniques	<i>E. coli</i> (8312)	0 ^a	0 ^a	0 ^a	9	0
	<i>S. aureus</i> (9044)	14.67 ± 0.58^b	19.33 ± 1.15^c	15.33 ± 0.58^b	14	12
	<i>P. aeruginosa</i> (8613)	14 ± 1.73^d	14.67 ± 2.51^d	16.66 ± 2.08^d	13	8
Souches de références	<i>E. coli</i> ATCC25922	0 ^e	0 ^e	0 ^e	21	13
	<i>S. aureus</i> ATCC19213	14.5 ± 0.50^f	15.5 ± 0.50^f	15 ± 0.00^e	20	11
	<i>P. aeruginosa</i> ATCC27853	14.5 ± 1.50^g	15 ± 0.00^g	16 ± 1.00^g	23	7

Data are expressed as mean \pm standard deviation (3 trials). Averages assigned the same letter in the same column are not statistically different at the 5% threshold

Table 4 Kent 1% antibacterial parameters

		Paramètres antibactériens de l'extrait			
Origines	Germes	CMI (mg/mL)	CMB (mg/mL)	CMB / CMI	Interprétation
Souches cliniques	<i>E. coli</i> 8312	Nd	Nd	Nd	Nd
	<i>S. aureus</i> 9044	3,125	50	16	Bactericidal
	<i>P. aeruginosa</i>	12,5	50	4	Bacteriostatic
Souches de références	<i>E. coli</i> ATCC25922	Nd	Nd	Nd	Nd
	<i>S. aureus</i> ATCC 19213	3,125	50	16	Bacteriostatic
	<i>P. aeruginosa</i> ATCC27853	12,5	50	4	Bacteriostatic

Nd : not determined

Table 5 Kent 2.5% antibacterial parameters

Origines	Germes	Paramètres antibactériens de l'extrait			
		CMI (mg/mL)	CMB (mg/mL)	CMB / CMI	Interprétation
Souches cliniques	<i>E. coli</i> 8312	Nd	Nd	Nd	Nd
	<i>S. aureus</i> 9044	3,125	3,125	1	Bactericidal
	<i>P. aeruginosa</i>	12,5	50	4	Bacteriostatic
Souches de références	<i>E. coli</i> ATCC25922	Nd	Nd	Nd	Nd
	<i>S. aureus</i> ATCC 19213	3,125	6,25	2	Bactericidal
	<i>P. aeruginosa</i> ATCC27853	12,5	50	4	Bacteriostatic

Nd : not determined

Table 6 Kent 5% antibacterial parameters

Origines	Germes	Paramètres antibactériens de l'extrait			
		CMI (mg/mL)	CMB (mg/mL)	CMB / CMI	Interprétation
Souches cliniques	<i>E. coli</i> 8312	Nd	Nd	Nd	Nd
	<i>S. aureus</i> 9044	3,125	12,5	4	Bacteriostatic
	<i>P. aeruginosa</i>	12,5	50	4	Bacteriostatic
Souches de références	<i>E. coli</i> ATCC25922	Nd	Nd	Nd	Nd
	<i>S. aureus</i> ATCC 19213	3,125	50	16	Bacteriostatic
	<i>P. aeruginosa</i> ATCC27853	12,5	50	4	Bacteriostatic

Nd : not determined

3.4. Resistance profile of the bacteria studied

The antibiotics studied have been grouped into 4 families (Aminosides, Penicillins, Cephalosporins and Beta-lactamins). Each antibiotic family contains two antibiotics tested, with the exception of the Beta-lactam family, which has a single antibiotic (Ceftazidime). Results were interpreted according to CASFM, 2023. All germs were resistant to gentamicin (CN-30mcg), amikacin (AK-30µg), amoxicillin (AX-30mcg), amoxicillin + Ac. clavulanique (AUG.30 µg), ceftriaxome (CRO-30 µg), cefotaxime (CTX 5µg), ceftazidime (CAZ 30µg) except *E. coli* (8312) which was sensitive to the antibiotic Amikacin (AK-30µg).

4. Discussion

Bioactive molecules present in plant species are very important in the treatment of certain pathologies. In this work, we aimed to contribute to an in-depth understanding of the therapeutic potential of mango leaves from the Kent cultivar. The highest extraction yields were obtained with 1% infusions and the lowest with 5% infusions. Statistical analysis shows a significant difference between extraction yields. This difference can be explained by the solid/liquid ratio. The higher the solvent volume, the greater the degree of contact between the drug and the extraction solvent. This increases the solvent's capacity to penetrate the drug, enabling the extraction solvent to come into contact with a large number of compounds. These results are similar to those of Koné *et al.* (2017)²⁰, who showed that yields are higher when the volume/mass ratio of the grind is high. Phytochemical screening showed the presence of sterols, polyterpenes, polyphenols, flavonoids, catechic tannins, alkaloids and saponins in infused extracts of the Kent cultivar. The presence or absence of quinones and gall tannins differentiates the composition of the extracts. The K.1% and K.2.5% infusions have the same phytochemical composition, but differ from that of K.5%. The absence of quinones in K.1% and K.2.5% infusions may be explained by less efficient extraction at lower concentrations. Conversely, the absence of gall tannins in the K.5% infusion may be due to complexation, precipitation or chemical interaction with other compounds at higher

concentrations, limiting their availability in the final extract. These observations are consistent with the work of **Sarker and Nahar (2012)**²¹, who studied the effects of extraction methods and chemical interactions on the phytochemical composition of plant extracts. Our results corroborate those of **Mustapha et al. (2014)**²². These authors found the same phytochemical compounds in mango leaves harvested in Nigeria. However, the literature has reported that *Mangifera indica* leaves from Burundi harvested at different times and sites contained no alkaloids [23]. These results show that the phytochemical composition of medicinal plant leaves can vary according to the time of harvest, the nature of the soil, the age of the plant and the drying conditions of the organ studied [24]. The active ingredient content of *Mangifera indica* leaves is comparable to that previously found for *Psidium guajava* leaves [25]. *Mangifera indica*'s richness in these major groups of active chemical compounds could then justify the traditional use of this plant to treat numerous illnesses such as diabetes, bronchitis, diarrhea, asthma, scabies and respiratory problems [26]. With regard to antimicrobial testing, the tests carried out highlighted the growth-inhibiting activity *in vitro* of *E. coli* (8312), *S. aureus* (9044) and *P. aeruginosa* (8613) by the infused extracts. The strains tested were resistant to at least two antibiotics from two different families. They are therefore described as multi-resistant. The sensitivity of the strains studied to the infused extracts varied from one bacterial strain to another. *S. aureus* 9044 was the most sensitive strain to Kent 1% and 2.5% infused extracts. As for the Kent 5% infusion, *P. aeruginosa* (8613) was the most sensitive. *E. coli* (8312) was the least sensitive strain to the plant extracts studied. Despite the multi-resistance of the strains studied, our extracts achieved interesting inhibition diameters, except on *E. coli*. Determination of antibacterial parameters reinforced this sensitivity. The 1% and 5% infusions of the Kent cultivar showed a bacteriostatic effect on *P. aeruginosa* and *S. aureus* strains, while the 2.5% infusion showed a bactericidal effect on *S. aureus* strains and a bacteriostatic effect on *P. aeruginosa* strains. The high presence of phytochemicals, notably tannins, could be at the root of this efficacy.

5. Conclusion

This work contributes to the valorization of the medicinal plant *Mangifera indica*. Extraction by infusion showed that 1% is the best percentage for extracting active ingredients from plant extracts. Phytochemical screening of infused extracts from the leaves of the kent cultivar revealed a richness in sterols, polyterpenes, polyphenols, flavonoids, tannins, catechins, alkaloids and saponins. The antibacterial activity of the infusions showed promising results despite the multiresistance of the bacteria tested. The 1% and 5% infused extracts demonstrated a bacteriostatic effect on *P. aeruginosa* and *S. aureus*, while the 2.5% infused extract exhibited a bactericidal effect on *S. aureus* and a bacteriostatic effect on *P. aeruginosa*. The richness of phytochemical compounds in the infusions could explain the observed biological activities. These results suggest that *Mangifera indica* could offer hope in the treatment of urinary tract infections, a major public health threat. It would be relevant to extend the study to other multi-resistant bacterial strains in order to broaden the spectrum of action of these infused extracts.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

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