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Evaluation of The Antimicrobial Activity of *Tetracarpidium conophorum* Seed Oil, Collected in Lékana, on Zoonotic Pathogens

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A B S T R A C T

The emergence of antimicrobial resistance necessitates the search for new bioactive molecules, particularly those derived from local plant resources. This study, conducted at the Veterinary Diagnostic Laboratory of Brazzaville (LDVB), evaluates the antimicrobial activity of *Tetracarpidium conophorum* seed oil collected in Lékana, Republic of Congo, against three bacterial strains (*Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae*) and one fungal strain (*Candida albicans*). The protocol relied on agar well diffusion using blank discs impregnated with oil and antibiotic controls, as well as the determination of minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) in 96-well microplates using p-iodonitrotetrazolium chloride as a colorimetric indicator. The results highlight a targeted and statistically significant inhibitory activity ($p < 0.05$): the oil proved particularly effective against Gram-negative pathogens (*K. pneumoniae* and *E. coli*), while no inhibition was observed for *S. aureus* and *C. albicans*. *Klebsiella pneumoniae* was more sensitive than *E. coli*, with increased sensitivity for the LDVB isolate. These data suggest that *T. conophorum* oil holds promising potential as a natural antibacterial agent, offering interesting prospects for biological risk management and the health safety of foods of animal origin.

Introduction

The management of food health safety and the fight against microbial infections remain major challenges in human and veterinary public health. Currently, the emergence of antimicrobial resistance (AMR) constitutes one of the most critical global threats. Sub-Saharan Africa bears the heaviest burden of this phenomenon, with 23,7 deaths per 100 000 inhabitants, compared to 5 per 100 000 in North America (Essack and Lenglet, 2024). It is also observed that AMR-related deaths continue to rise in sub-Saharan Africa, whereas those linked to HIV/AIDS and malaria are decreasing. For instance, recent studies in the African region reported four main pathogens responsible for over 100 000 AMR-associated deaths in 2019: *Streptococcus pneumoniae* (195 000 deaths), *Klebsiella pneumoniae* (184 000 deaths), *Escherichia coli* (147 000 deaths) and *Staphylococcus aureus* (136 000 deaths). It is estimated that each of these four pathogens was directly responsible for over 25 000 deaths in 2019, with respectively 50 000 deaths for *K. pneumoniae*, 39 000 for *S. pneumoniae*, 37 000 for *E. coli* and 30 000 for *S. aureus* (Antimicrobial Resistance Collaborators, 2024). In the Congo, a recent report by the National Institute of Biology and Health Surveillance (INBVS) presented the genera *Klebsiella spp.*, *Staphylococcus spp*., and the species *E. coli* as the primary agents responsible for infections among the Congolese, with resistance rates of 84.6%, 50.0% and 63.6% respectively, based on the reported tests (Institut National de Biologie et de Veille Sanitaire, 2026).

This phenomenon, unfortunately poorly documented in the Congo, also has a significant impact on animal health. Bacterial infections not only cause high mortality in livestock but also lead to the massive use of conventional antibiotics. This intensive use promotes the selection of multiresistant pathogenic strains, posing a direct risk of transmission to humans via the food chain and antibiotic residues in foods of animal origin (Quenum, Adenile and Anagonou, 2026).

Faced with this alarming impact on public and animal health, it has become urgent to develop ecological, sustainable, and environmentally friendly alternatives. Phytobiotics, derived from plant resources, represent a promising future path (Quenum, Adenile and Anagonou, 2026). The activity of these plant extracts relies largely on the presence of secondary metabolites such as phenolic compounds, terpenes, and flavonoids, which are widely recognized for their powerful antimicrobial and antioxidant properties (Cowan, 1999; Bakkali *et al.,* 2008; Kumar and Pandey, 2013). These secondary metabolites act in concert with other biomolecules, similar to certain specific fatty acids, to destabilize bacterial cell walls and generate an antimicrobial effect.

Among the forestry and agronomic resources of the Congo Basin, *Tetracarpidium conophorum (Müll.Arg.) Hutch. & Dalziel*, of the *Euphorbiaceae* family, draws attention for its numerous phytochemical and nutritional properties. Previous analyses, notably by gas chromatography (GC), have demonstrated the richness of *T. conophorum* oil in polyunsaturated fatty acids, such as linolenic acid and linoleic acid, as well as various organic compounds (Djikeng, 2024; Erukainure and Chukwuma, 2024). These elements confer a recognized antimicrobial potential to this plant, although the specificity of its spectrum of action depends heavily on the extraction methods and the exact lipid profile of the sample.

In order to consolidate epidemiological data and promote biosecurity measures, the present work specifically aims to determine the antimicrobial action of this oil on pathogens of interest in public health.

Materials and Methods

Plant Material and Oil Extraction

The fruits of *Tetracarpidium conophorum* were harvested at maturity in Lékana, in the Plateaux Department, Republic of Congo, according to the following GPS coordinates: 2°19’31.9728”S, 14°34’59.2921”E. A reference sample was deposited at the National Herbarium of the Congo and registered under identification number MJM 7665. The fruits were transported to the Food Control and Quality Laboratory of the National Higher School of Agronomy and Forestry, where their shells were removed. The extracted kernels were oven-dried at 60°C for 96 hours until a stable mass was achieved. The dry kernels were then finely ground using a Cookwork electric grinder before oil extraction.

The fat extraction was carried out continuously over six trials, following the Soxhlet method (AOAC, 2005). 30 g of kernel powder was placed in a cellulose thimble and inserted into the extractor. The flask, containing 200 mL of hexane, was heated under reflux for 3 hours. After extraction, the solvent was evaporated, and the residual golden-yellow oil was recovered, weighed to calculate

the yield, and stored protected from light at the Veterinary Diagnostic Laboratory of Brazzaville, awaiting further analysis.

Biological Material

Microbial Strains Used

The study focused on three bacterial strains: *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Escherichia coli*, as well as one fungal species: *Candida albicans*. All strains used in this study were clinical isolates. The strains were obtained either from the Veterinary Diagnostic Laboratory of Brazzaville of the General Directorate of Livestock or from the Department of Medical Biology of the National Public Health Laboratory. Two strains of each species were used, and each was assigned a specific identifier. The isolates were confirmed to be involved in cutaneous and pulmonary infections (Table 1). No information is available regarding the specific characteristics that distinguish the strains.

Treatment of Microorganisms

All bacteria were aseptically cultured in 5 ml of nutrient broth for approximately 16H at 37°C before being subcultured onto Petri dishes containing MacConkey agar for Gram-negative strains and Mueller-Hinton Agar for *Staphylococcus aureus*. The plates were incubated at 37°C for 24 h to obtain fresh cultures.

The *Candida albicans* strains were cultured on Sabouraud Dextrose agar and incubated 25°C at 48 h for.

Microbiological Analysis

Preparation of Culture Media

The culture media, including nutrient agar (NA) and nutrient broth (NB), Mueller-Hinton agar (MHA), and Sabouraud-dextrose agar (SDA), were purchased from *Inqaba Biotec* in Nairobi, Kenya. Mueller-Hinton broth (MHB) and Resazurin (p-iodonitrotetrazolium chloride or p-INT) were obtained from The *Heavy Champion Limited* in Dar Es Salaam, Tanzania. All used media were prepared according to the manufacturer's instructions, in large volumes accounting for the number of trials and replicates. The media were systematically sterilized at 121°C for 15 minutes in an autoclave and then allowed to cool after sterilization to about 40°C before being poured into Petri dishes to solidify.

Inoculum preparation for antimicrobial activity testing

To effectively study the antibacterial activity of the African walnut oil, inocula for agar diffusion tests were prepared according to the guidelines of the Clinical Laboratory Standards Institute (CLSI, 2012) and Sen and Batra (2012). The bacterial strains were cultured separately in 10mL of nutrient broth and Mueller-Hinton broth overnight. The obtained cultures were spread onto their respective agars using the streak plate method, then incubated at 37°C for 24 hours. From the incubated plates, inocula were prepared by direct suspension of two to three well-isolated colonies of *Staphylococcus aureus*, *Escherichia coli*, and *Klebsiella pneumoniae* in a tube of 0.9% NaCl physiological saline. The bacterial suspensions were then adjusted to an optical density of 0.5 McFarland (equivalent to). The exact density of the suspensions was verified using a BIOMÉRIEUX VITEK DENSICHEK densitometer equipped with a touchscreen displaying the experimental values for each sample. Reference tubes or McFarland standards provided by the kit were used to calibrate the densitometer prior to sample measurement. To ensure the turbidity of the suspension conformed to the McFarland standard, the suspensions and the McFarland standard were also compared visually. Additionally, the inoculum suspensions were used within minutes of their standardization to prevent any changes in inoculum size or loss of viability.

For the antifungal activity of the African walnut oil, the fungal suspensions used were prepared from two to three morphologically similar colonies of Candida albicans from a -hour culture on Sabouraud dextrose agar. The turbidity of the suspensions was adjusted to McFarland (equivalent to 1.5 x 10^8 CFU/mL) with a sterile normal saline solution (0.9% NaCl w/v). All experiments were performed in duplicate and repeated three times.

Determination of Antimicrobial Activity

Agar Diffusion Method

The antimicrobial activity of different oil concentrations was determined by a modified agar diffusion method, described by Perez-Eid, Pauli and Bazerque (1990) and

Adeniyi, Odelola and Oso (1996). Mueller-Hinton agar (MHA) plates were inoculated with 18 h broth cultures of each bacterial isolate. For fungal isolates, the agar was inoculated with 24 h broth cultures of Candida albicans. The plates were inoculated separately by spreading a specific amount of liquid inoculum (0.5 McFarland for bacteria and 1.0 McFarland for *Candida albicans*) using sterile swabs to cover the agar surface with the tested microorganisms. All tested microorganisms were inoculated separately onto different plates. Sterile blank discs of 6 mm in diameter were impregnated with different concentrations of *T. conophorum* oil before being gently placed on the plates, maintaining a minimum spacing of 25 mm between them and 15 mm from the edges. Discs of streptomycin (for bacteria) and Fluconazole (for yeast) served as positive antibiotic controls. A disc impregnated with solvent (Tween 80) constituted the negative control on each plate. After incubation at 37°C for 24 h for bacteria and 25°C pendant 48 h for yeast, the diameters of the inhibition zones were measured using a caliper. All experiments were performed in duplicate and repeated three times. Concentrations (v/v) of 80, 40 and 20 % of oil in the solvent (Tween 80) were used.

Determination of Minimum Inhibitory (MIC) and Bactericidal (MBC) Concentrations

The quantitative antibacterial activity of the oil, reflected by the Minimum Inhibitory Concentration (MIC) and the Minimum Bactericidal Concentration (MBC), was evaluated by the liquid microdilution method in 96-well microplates. Initially, 100 µL of Mueller-Hinton broth (MHB) was distributed into the wells of columns 2 to 12 of the microplate. A volume of 100 µL of a stock solution of the oil (100mg/mL, emulsified using Tween 80) was introduced into the wells of the first column. Two-fold serial dilutions were then performed by transferring 100 µL from the first well to the adjacent well, successively up to column 10, from which 100 µL was finally discarded to maintain a constant volume. Columns 11 and 12 were used respectively as a positive growth control (culture medium and inoculum, without oil) and a negative sterility control (culture medium only).

Subsequently, 100 µL of standardized inoculum was added to each well in columns 1 to 11, bringing the final volume of each well to 200 µL. After incubation at the appropriate temperature for 18 to 24 h depending on the microorganism, 40 µL of an aqueous solution of p-iodonitrotetrazolium chloride (p-INT) was added to all wells to overcome the turbidity of the oil. The microplates were subjected to reincubation for 30 minutes. The MIC was defined as the lowest concentration of oil inducing a colorimetric shift of the indicator from pink to purple, indicating total inhibition of bacterial respiratory metabolism.

To determine the MBC, a volume of 10 µL was taken from each of the inhibited wells (purple color) from the previous step and plated onto blank nutrient agar plates. After another 24H incubation, growth observation was performed. The presence of colonies indicated a bacteriostatic effect, while the total absence of growth confirmed a bactericidal effect. The MBC was defined as the lowest concentration of oil for which no colonies were observed on the agar. All experiments were performed in duplicate and repeated independently at least three times.

Analyse statistique

The experimental data were expressed as mean standard deviation (mean ± SD) derived from three independent repetitions executed in duplicate (n=6). One-way analysis of variance (ANOVA1) followed by Tukey's multiple comparison test was performed using SAS software (version 9.4). A value of $p<0.05$ was considered the threshold for statistical significance.

Results and Discussion

Oil Extraction Yield

The oil extraction was carried out in six trials to ensure the reliability of the results. The average yield obtained was 49.91 ± 0.49 %. This high yield confirms that *T. conophorum* kernels are particularly oleaginous.

Antimicrobial activity by agar diffusion

The qualitative antimicrobial activity of *T. conophorum* oil was evaluated considering the diameters of the inhibition zones recorded in Table 3 below.

Measurable inhibition zones were observed only on Gram-negative bacterial strains, namely *Klebsiella pneumoniae* and *Escherichia coli*. Conversely, no inhibition zone was detected (0.0 ± 0.0mm) for *Staphylococcus aureus* (SA1V and SA1SP) as well as for the fungal isolates of *Candida albicans* (CA1SP and

CA2SP), translating to a total resistance of these species at the tested concentrations.

A detailed analysis of the diameters shows a significant sensitivity difference ($p<0.05$) based on the oil concentration and strain origin. In *Klebsiella pneumoniae*, for example, the observed inhibition diameters are generally higher than those measured for *E. coli*. Moreover, the veterinary origin isolate (KP1V) proved to be significantly more sensitive to the oil than the human origin isolate (KP1SP). At the concentration of 80%, KP1V presented an inhibition zone of 16.8 ± 0.42 mm compared to 14.9 ± 0.38 mm for KP1SP.

An opposite trend is observed between the *Escherichia coli* isolates depending on the laboratories of origin. The inhibition diameters are slightly lower for the LDVB strain (EC1V: 11.4 ± 0.31 mm at 80%) compared to the LNSP strain (EC1SP: 12.8 ± 0.35 mm at 80%).

Determination of Minimum Inhibitory (MIC) and Bactericidal (MBC) Concentrations

The quantitative evaluation of antimicrobial activity on microplates confirms the trends observed during the agar diffusion antibiogram. The p-INT (p-iodonitrotetrazolium chloride) detection made it possible to evaluate the potential of our oil (Table 4 and Table 5).

In accordance with macroscopic observations, the color change of p-INT (indicating bacterial respiration) is observed starting from column 6 (1.563 mg/mL) for the E. coli EC1SP strain, which sets its MIC at the previous dilution, i.e., 3.125 mg/mL (column 5). For the EC1V (LDVB) strain, which is slightly less sensitive, the shift is observed starting from column 5, setting its MIC at 6.25 mg/mL (column 4).

In Klebsiella pneumoniae, inhibition persists further into the dilution range. For strain KP1V, the MIC is reached at column 7 (0.781 mg/mL). For KP1SP (LNSP), the MIC is located at well 6 (1.563 mg/mL).

Bactericidal / Bacteriostatic Character

Subculturing on blank agar from samples taken from the inhibited wells revealed that bacterial regeneration (bacteriostatic effect) ceases completely starting from the 2nd to the 3rd column ascending towards the higher concentrations.

As summarized in Table 4 and illustrated in Table 5, for all sensitive strains (K. pneumoniae and E. coli), the Minimum Bactericidal Concentration (MBC) is obtained precisely at a concentration equal to 4 times the MIC (MBC/MIC = 4).

According to medical and veterinary microbiology standards, an MBC/MIC ratio ≤ 4 formally defines a bactericidal effect (Marmonier, 1990; French, 2006). Beyond the MIC threshold, with the greatest dilutions, bacteria retain their cellular viability, characterizing a state of bacteriostasis.

The evaluation of the antimicrobial activity of *Tetracarpidium conophorum* oil originating from Lékana revealed a remarkably selective spectrum of action. The lack of inhibition observed on *Staphylococcus aureus* (Gram-positive) and *Candida albicans* (yeast), coupled with a marked bactericidal activity against *Klebsiella pneumoniae* and *Escherichia col*i (Gram-negative), constitutes an atypical finding of significant scientific interest.

Indeed, Gram-negative bacteria are conventionally considered more tolerant to lipophilic plant extracts due to the presence of their hydrophilic outer membrane, rich in lipopolysaccharides (LPS), which acts as an impermeability barrier (Cowan, 1999).

This specific vulnerability of Gram-negative strains observed in our study suggests a targeted mechanism of action intimately linked to the chemical composition of the oil. Our previous investigations on *T. conophorum* from Lékana (Miakayizila *et al.,* 2024) highlighted the presence of three major secondary metabolites known for their antimicrobial activity: terpenoids, polyphenols (5.5 mg GAE/g DW), and the flavonoid subgroup (0133 mg QE/g DW). The result obtained could therefore be due to a synergistic action between the oil's secondary metabolites—namely terpenoids—and its widely recognized fatty acid potential. For example, the work of Tchiegang, Kapseu and Parmentier (2001) demonstrated that *T. conophorum* oil is exceptionally rich in long-chain polyunsaturated fatty acids (PUFAs), with linolenic acid (C18:3) levels reaching 70% and linoleic acid (C18:2) around 15%. In the literature, it is established that PUFAs can bypass the LPS barrier due to their amphiphilic structure, inserting themselves directly into the internal phospholipid bilayer of the bacteria.

Table.1 Sources of tested microorganisms.

Microorganism	Strain ID	Origin	Anatomical part from which strains were isolated
Bacterial Strains			
Escherichia coli	EC1V	LDVB	Isolated from an anal swab on a goat
	EC1SP	LNSP	Isolated from a urine sample
Klebsiella pneumoniae	KP1V	LDVB	Isolated from a nasal swab on a goat
	KP1SP	LNSP	Isolated from human sputum
Staphylococcus aureus	SA1V	LDVB	Isolated from a skin abscess on poultry
	SA1SP	LNSP	Isolated from a vaginal swab
Fungal Strains			
Candida albicans	CA1SP	LNSP	Isolated from a vaginal swab
	CA2SP	LNSP	Isolated from a vaginal swab

LDVB: *Veterinary Diagnostic Laboratory of Brazzaville;* ***LNSP:*** *National Public Health Laboratory.*

Table.2 96-well microplate dilution plan for MIC determination**.**

Column	Half-dilution concentration	Before inoculum Final concentration after adding 100 µL inoculum	Dilution nature
Col 1	100 mg/mL	50 mg/mL	Highest tested concentration
Col 2	50 mg/mL	25 mg/mL	Dilution 1/2
Col 3	25 mg/mL	12.5 mg/mL	Dilution 1/4
Col 4	12.5 mg/mL	6.25 mg/mL	Dilution 1/8
Col 5	6.25 mg/mL	3.125 mg/mL	Dilution 1/16
Col 6	3.125 mg/mL	1.5625 mg/mL	Dilution 1/32
Col 7	1.5625 mg/mL	0.78125 mg/mL	Dilution 1/64
Col 8	0.78125 mg/mL	0.3906 mg/mL	Dilution 1/128
Col 9	0.3906 mg/mL	0.1953 mg/mL	Dilution 1/256
Col 10	0.1953 mg/mL	0.0976 mg/mL	Lowest tested concentration
Col 11	0 mg/mL	0 mg/mL	Growth control (+) (Broth + Bacteria)
Col 12	0 mg/mL	0 mg/mL	Sterility control (-) (Broth only)

Col: *Column*

Table.3 Inhibition zone diameters (mm) of *Tetracarpidium conophorum* oil on tested microbial isolates.

Tested Micro organisms	Strain / Origin	Oil Concentration (% v/v)			Positive Control (Streptomycin/ Fluconazole)	Negative Control (Tween 80)
		20%	**40%**	**80%**		
Gram-negative						
K. pneumoniae	KP1V (LDVB)	9.8 ± 0.25^{d}	13.5 ± 0.31^{b}	16.8 ± 0.42^{a}	25.4 ± 0.38^{a}	0.0 ± 0.0^{e}
	KP1SP (LNSP)	8.2 ± 0.20^{de}	11.8 ± 0.25^{c}	14.9 ± 0.38^{b}	24.8 ± 0.41^{a}	0.0 ± 0.0^{e}
E.coli	EC1V (LDVB)	6.0 ± 0.0^{e}	8.5 ± 0.22^{de}	11.4 ± 0.31^{c}	22.6 ± 0.35^{a}	0.0 ± 0.0^{e}
	EC1SP (LNSP)	7.2 ± 0.18^{e}	9.7 ± 0.28^{d}	12.8 ± 0.35^{bc}	23.1 ± 0.29^{a}	0.0 ± 0.0^{e}
Gram-positive						
S. aureus	SA1V (LDVB)	0.0 ± 0.0^{e}	0.0 ± 0.0^{e}	0.0 ± 0.0^{e}	0.0 ± 0.0^{e}	0.0 ± 0.0^{e}
	SA1SP (LNSP)	0.0 ± 0.0^{e}	0.0 ± 0.0^{e}	0.0 ± 0.0^{e}	0.0 ± 0.0^{e}	0.0 ± 0.0^{e}
Yeast						
C. albicans	CA1SP (LNSP)	0.0 ± 0.0^{e}	0.0 ± 0.0^{e}	0.0 ± 0.0^{e}	0.0 ± 0.0^{e}	0.0 ± 0.0^{e}
	CA2SP (LNSP)	0.0 ± 0.0^{e}	0.0 ± 0.0^{e}	0.0 ± 0.0^{e}	0.0 ± 0.0^{e}	0.0 ± 0.0^{e}

** Streptomycin 10µL (for bacteria); Fluconazole 25µL (for yeast).*

Values represent the mean ± standard deviation of 3 replicates (n=6). Different superscript letters indicate statistically significant differences according to Tukey's test ($p < 0.05$).

Table.4 Microdilution inhibition profile and evaluation of MICs and MBCs of *T. conophorum* oil.

Microorganism	Strain / Origin	First well without p-INT shift (MIC)	MIC Concentration (mg/mL)	MBC Concentration (mg/mL)	Rapport CMB/CMI	Antimicrobial Effect
K. pneumoniae	KP1V (LDVB)	Colonne 7	0.781^{d}	3.125^{c}	4.0	Bactericidal
	KP1SP (LNSP)	Colonne 6	1.563^{c}	6.250^{c}	4.0	Bactericidal
E.coli	EC1V (LDVB)	Colonne 4	6.250^{b}	25.00^{a}	4.0	Bactericidal
	EC1SP (LNSP)	Colonne 5	3.1250^{c}	12.500^{ab}	4.0	Bactericidal
S. aureus	SA1V / SA1SP	No inhibition	> 50.000	> 50.000	N/D	Not determined
C. albicans	CA1SP CA2SP	No inhibition	> 50.000	> 50.000	N/D	Not determined

Table.5 Cell viability kinetics as a function of microplate concentrations.

Puits (Concentration mg/mL)	*K. pneumoniae* KP1V (LDVB)	*K. pneumoniae* KP1SP (LNSP)	*E. coli* EC1SP(LNSP)	*E. coli* EC1V (LDVB)
Col 1 (50.0 mg/mL)	Bactericidal (0 colony)	Bactericidal (0 colony)	Bactericidal (0 colony)	Bactericidal (0 colony)
Col 2 (25.0 mg/mL)	Bactericidal (0 colony)	Bactericidal (0 colony)	Bactericidal (0 colony)	MBC (25.0 mg/mL)
Col 3 (12.5 mg/mL)	Bactericidal (0 colony)	Bactericidal (0 colony)	MBC (12.5 mg/mL)	Inhibitory (Bacteriostatic)
Col 4 (6.25 mg/mL)	Bactericidal (0 colony)	MBC (6.25 mg/mL)	Inhibitory (Bacteriostatic)	Growth (+) / p-INT shift
Col 5 (3.125 mg/mL)	CMB (3.125 mg/mL)	Inhibitory (Bacteriostatic)	Growth (+) / p-INT shift	Growth (+) / p-INT shift
Col 6 (1.563 mg/mL)	Inhibitory (Bacteriostatic)	MBC (1.563 mg/mL)	Growth (+) / p-INT shift	Growth (+) / p-INT shift
Col 7 (0.781 mg/mL)	MBC (0.781 mg/mL)	Growth (+) / p-INT shift	Growth (+) / p-INT shift	Growth (+) / p-INT shift
Col 8 (0.391 mg/mL)	Growth (+) / p-INT shift	Growth (+) / p-INT shift	Growth (+) / p-INT shift	Growth (+) / p-INT shift

Col*: Column*

This insertion drastically alters membrane fluidity and permeability, thereby disrupting the respiratory chain—which justifies the relief of p-INT inhibition observed during our MIC tests—and causing a leakage of intracellular constituents leading to lysis and thus the bactericidal effect corroborated by our MBC/MIC ratio = 4. Furthermore, these PUFAs are highly susceptible to auto-oxidation, generating reactive oxygen species (ROS) that induce a fatal oxidative stress for Gram-negative bacteria, which are often less equipped with membrane antioxidant enzymes against this type of lipid aggression.

Quantitatively, the MIC values obtained, ranging from 0.781 mg/mL (for *K. pneumoniae* KP1V) to 6.25 mg/mL, underscore notable biological efficacy. In pharmacognosy, an MIC below 1 mg/mL for a raw plant extract is generally considered indicative of strong antimicrobial activity (Rios and Recio, 2005). The increased sensitivity of the LDVB *K. pneumoniae* strain (KP1V), isolated from an animal sample, suggests a particular receptivity among isolates of veterinary origin. This efficacy aligns with the recent conclusions of Quenum, Adenile and Anagonou (2026) regarding the use of phytobiotics rich in bioactive metabolites as

viable alternatives in animal health. The present study demonstrates that *T. conophorum* oil possesses inhibitory capacities capable of competing with certain plant extracts already in use, such as ginger, in suppressing opportunistic and gastrointestinal pathogens.

However, while these results are quite promising for the "One Health" approach, offering an ecological alternative to conventional antibiotics in farming or human usage, this study has certain limitations.

The absence of specific GC-MS chemical characterization for the Lékana oil sample used in this trial restricts us to literature-based deductions regarding its exact lipid profile, which may vary depending on edapho-climatic factors. Moreover, extrapolating these in vitro data to clinical or zootechnical applications requires rigorous in vivo validation.

These perspectives pave the way for future studies aimed at elucidating the exact molecular interactions between *T. conophorum* PUFAs and the porins of Gram-negative bacteria, as well as evaluating the potential toxicity of this oil to formulate standardized veterinary bioproducts.

In conclusion, this study highlights the selective antimicrobial potential of *Tetracarpidium conophorum* seed oil originating from Lékana. The results demonstrate strong inhibitory and bactericidal activity specifically targeting Gram-negative pathogens, particularly *Klebsiella pneumoniae* and *Escherichia coli*, while remaining inactive against *Staphylococcus aureus* and *Candida albicans*. These remarkable biological properties could be explained by the synergy between the oil's high content of polyunsaturated fatty acids and the presence of bioactive secondary metabolites, thus corroborating previous phytochemical data.

In a global context marked by the critical emergence of antibiotic resistance, this local plant resource positions itself as a promising phytobiotic candidate. It offers interesting prospects for veterinary public health, notably as a natural alternative to conventional antibiotics, thereby contributing to limiting the dissemination of resistant animal-origin strains and improving biosecurity in livestock farming. Further studies, focusing on in vivo models, toxicity evaluation, and fine elucidation of molecular mechanisms of action, will be necessary to eventually envision the development of veterinary bioproducts or natural preservatives for foodstuffs.

Author Contributions

Blaise Divin Emmanuel MIAKAYIZILA: Conceived and designed the study, performed oil extraction and microbiological laboratory assays, analyzed data, and wrote the original manuscript draft. Snelle MIAKAYIZILA BAONDA: Participated in experimental design, biological material processing, and critical manuscript revision. Jean Paul Latran OSSOKO: Supervised laboratory extraction procedures, validated analytical techniques, reviewed the manuscript, and approved the final manuscript version. NKAYA-TOBI: Authorized access to and conduct of experiments at the veterinary laboratory, facilitated strain isolation at LDVB, assisted in microbiological testing and data curation, provided resources. Arnaud Wenceslas TAMBA SOMPILA: Performed statistical analysis (ANOVA SAS 9.4) and formatted tables. Etienne NGUIMBI: Contributed to theoretical interpretation of microbiological mechanisms and manuscript editing. Michel Didace MVOULA TSIERI: Supervised the overall research project, and approved the final manuscript version.

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Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

References

Adeniyi, B. A., Odelola, H. A., and Oso, B. A. (1996). Antimicrobial potentials of Diospyros mespiliformis (Ebenaceae). African Journal of Medicine and Medical Sciences, 25(3), 221-224.

Antimicrobial Resistance Collaborators. (2024). The burden of bacterial antimicrobial resistance in the WHO African region in 2019: A cross-country systematic analysis. The Lancet Global Health, 12(2), e201–e216. https://doi.org/10.1016/S2214-109X(23)00539-9

AOAC. (2005). Official Methods of Analysis. 18th Edition, Association of Official Analytical Chemists, Gaithersburg, MD.

Bakkali, F., Averbeck, S., Averbeck, D., and Idaomar, M. (2008). Biological effects of essential oils—A review. Food and Chemical Toxicology, 46(2), 446–475. https://doi.org/10.1016/j.fct.2007.09.106

CLSI. (2012). Performance standards for antimicrobial susceptibility testing; approved standard M02-11. Clinical and Laboratory Standards Institute, Wayne, PA.

Cowan, M. M. (1999). Plant products as antimicrobial agents. Clinical Microbiology Reviews, 12(4), 564–582. https://doi.org/10.1128/CMR.12.4.56

Djikeng, F. T. (2024). Extending African walnut oil (Tetracarpidium conophorum) shelf-life by blending it with palm, groundnut and avocado oils and study of their oxidative stability during accelerated storage. Hybrid Advances, 7, 100331. https://doi.org/10.1016/j.hybadv.2024.100331

Erukainure, O. L., and Chukwuma, C. I. (2024). African walnut (Plukenetia conophora) oil promotes glucose uptake while improving energy metabolism and steroidogenesis and maintaining surface architecture in rat testes. Frontiers in Nutrition, 11, 1505453. https://doi.org/10.3389/fnut.2024.1505453

Essack, S. Y., and Lenglet, A. (2024). Bacterial antimicrobial resistance burden in Africa: accuracy, action, and alternatives. The Lancet Global Health, 12(2), e171–e172.https://doi.org/10.1016/S2214-109X(23)00587-9

French, G. L. (2006). Bactericidal agents in the treatment of MRSA infections—the potential role of daptomycin. Journal of Antimicrobial Chemotherapy, 58(6), 1107–1117. https://doi.org/10.1093/jac/dkl393

Institut National de Biologie et de Veille Sanitaire. (2026). Bulletin de surveillance microbiologique et de résistance aux antibiotiques. République du Congo. https://www.inbvs.cg

Kumar, S., and Pandey, A. K. (2013). Chemistry and biological activities of flavonoids: An overview. The Scientific World Journal, 2013, Article 162750. https://doi.org/10.1155/2013/162750

Marmonier, A. (1990). Introduction aux techniques d'étude des antibiotiques. In F. Denis, M.-C. Ploy, C. Martin, P. E. Bingen, & R. Quentin (Eds.), Bactériologie médicale: Techniques usuelles (pp. 227–236). Elsevier.

Miakayizila, B.D.E., Mondjo, R.D.E., Nguié, S.G., Miakayizila, S.B., Tsiba, G., and Ossoko, J.P.L. (2024). Phytochemical Study and Anti-Radical Power of Seeds of Tetracarpidium Conophorum, Pachira Glabra and Pentaclethra Macrophylla. International Journal of Development Research, 14, 66260-66265.

Perez-Eid, C., Pauli, W. M., and Bazerque, P. (1990). An antibiotic assay by agar-well diffusion method. Acta Biol. Med. Exp., 15, 113-115.

Quenum, C., Adenile, A., and Anagonou, S. I. N. (2026). Phytobiotiques comme alternatives aux antibiotiques en aviculture: revue de littérature et analyse du potentiel du gingembre (Zingiber officinale). Revue Africaine de Santé et de productions Animales, 3(2), 1-16. https://doi.org/10.46298/raspa.16938

Rios, J. L., and Recio, M. C. (2005). Medicinal plants and antimicrobial activity. Journal of Ethnopharmacology, 100(1-2), 80–84. https://doi.org/10.1016/j.jep.2005.04.025

Sen, A., and Batra, A. (2012). Evaluation of antimicrobial activity of different solvent extracts of medicinal plant: Melia azedarach L. Int J Curr Pharm Res, 4(2), 67-73.

Tchiegang, C., Kapseu, C., and Parmentier, M. (2001). Chemical composition of oil from Tetracarpidium conophorum ((Müll. Arg.) Hutch. and Dalz.) nuts. Journal of Food Lipids, 8(2), 95-102.

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