RAMReS Sciences des Structures et de la Matière Vol. 9, N°2 (2025) 263 - 273
http://publication.lecames.org/index.php/mat *ISSN: 2630-1180*

Antioxidant profile and antimicrobial activity of ascorbic acid: an *in vitro* study

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Received: 28 September 2025 / Received in revised form: 08 November 2025 / Accepted: 13 December 2025

***Abstract*:**

This study evaluated the biological properties of ascorbic acid by assessing its antioxidant capacity using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method and its antimicrobial activity against a panel of bacterial and fungal strains. For the antioxidant assay, DPPH solutions (0.003 mg/mL) were incubated with ascorbic acid at various concentrations (0.0625 – 2 mg/mL). The results revealed excellent dose-dependent radical scavenging activity, with reduction percentages exceeding 94% for concentrations of 0.5 mg/mL and above. The median reduction concentration (RC_{50}) value, determined graphically, was 0.0066 mg/mL, confirming its potent antioxidant power. Microbial susceptibility tests demonstrated that ascorbic acid exerts significant antibacterial activity, with a bactericidal effect observed at a high concentration of 200 mg/mL. In contrast, its activity against yeasts of the genus *Candida* was weak. This study concludes that ascorbic acid is a highly effective antioxidant at low doses, but its use as an antimicrobial agent requires much higher concentrations for a bactericidal effect, and it exhibits no notable efficacy against fungi. These results highlight a duality of application that is entirely dependent on the concentration used.

***Keywords*:** Ascorbic acid; Antioxidant activity; RC_{50}; Antimicrobial activity; DPPH; Bactericidal.

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https://doi.org/10.70974/mat09225263

1. Introduction

Vitamin C, or ascorbic acid, is a water-soluble organic compound with a strong redox potential, which underlies its remarkable antioxidant properties [1]. Chemically, its lactone structure derived from glucose enables it to readily donate electrons and protons, thereby neutralizing free radicals and regenerating other endogenous antioxidants such as Vitamin E [2]. Beyond its well-established protective role, Vitamin C has gained increasing attention for its antimicrobial potential, particularly in light of the global rise in antibiotic resistance. Several studies have shown that at high concentrations, ascorbic acid may act as a pro-oxidant by promoting the formation of reactive oxygen species (ROS) capable of damaging microbial membranes and cellular components [3, 4]. However, this dual antioxidant–antimicrobial behavior remains poorly documented in African contexts, and particularly in Côte d'Ivoire, where microbial strains often display atypical resistance profiles to conventional antibiotics. Investigating local clinical isolates thus represents a major scientific challenge, offering insight into the therapeutic relevance of alternative molecules and the influence of regional genetic and environmental factors on microbial sensitivity. The present study therefore aimed to evaluate both the antioxidant and antimicrobial properties of Vitamin C against bacterial and fungal strains isolated in Côte d'Ivoire.

2. Materials and methods

2.1. Study material

The study utilized pharmaceutical-grade Vitamin C and a panel of microorganisms implicated in cutaneous and mucosal infections.

The bacterial panel included six strains: three reference strains (*Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923) and three multidrug-resistant Ivorian clinical isolates (*S. aureus* 976/24, *Klebsiella pneumoniae* 2055/24, *E. coli* 988/24).

The fungal panel comprised five *Candida* species: *C. albicans* MY24264, *C. tropicalis* MY24203, *C. glabrata* MY24216, and two reference strains (*C. parapsilosis* ATCC 22019, *C. albicans* NR-50363).

All microbial strains were provided by the Pasteur Institute of Côte d'Ivoire.

2.2. Methods

2.2.1. Determination of antioxidant activity

A DPPH (2,2-diphenyl-1-picrylhydrazyl) solution was prepared in absolute ethanol to achieve a final concentration of 0.003 mg/mL. In parallel, Vitamin C solutions were prepared at different concentrations: 2, 1, 0.5, 0.25, 0.125, and 0.0625 mg/mL, also in absolute ethanol. For each assay, 2.5 mL of the Vitamin C solution were placed into a test tube, followed by the addition of 1 mL of the DPPH solution. The mixtures were incubated in the dark for 30 minutes to prevent photochemical degradation of DPPH. Absorbance was measured at 517 nm, corresponding to the maximum absorption peak of DPPH, using a spectrophotometer (Lovibond).

The percentage of DPPH reduction (PR) was calculated using the following equation:

$$\text{PR } (\%) = \left[\frac{A_b - A_e}{A_b}\right] \times 100$$

Where:

PR (%): percentage of DPPH reduction

A_b: absorbance of the blank (ethanol + DPPH, no Vitamin C)

A_e: absorbance of the sample (Vitamin C + DPPH)

All measurements were performed in triplicate to ensure reproducibility.

2.2.2. Determination of antibacterial activity

A sterility check of the extract was carried out prior to the various biological assays in order to confirm the absence of microbial contamination in the samples. The antibacterial activity of the extract was determined using both the solid medium diffusion

method and the broth double dilution technique. The activity of the extract was first assessed by the agar well diffusion method described by N'guessan [5]. To this end, different clinical bacterial strains of Ivorian hospital origin were reactivated and subcultured by streaking, followed by incubation at 37 °C for 18 to 24 hours. An isolated colony was then collected to prepare the bacterial inoculum according to a modified version of the method described in the EUCAST-CASFM reference protocol [6]. The bacterial suspension was streaked evenly over the surface of Mueller-Hinton (MH) agar plates. Three concentrations of Vitamin C, namely 200, 100, and 50 mg/mL, were prepared in sterile distilled water. Subsequently, an aliquot of 80 µL of each solution was dispensed into wells bored in the agar. A control well received 80 µL of sterile distilled water. Antibiotic discs, including cefoxitin (FOX), ceftazidime-avibactam (CZD), and cefepime (FEP), were also placed on the agar for comparison. After a pre-diffusion phase of 45 minutes at room temperature (16 °C), the plates were incubated at 37 °C for 18 to 24 hours. Following incubation, the antibacterial activity of Vitamin C was evaluated by measuring the diameter of the inhibition zones around the wells using a caliper, as described by Ouattara *et al.* [7]. The diameters of inhibition zones produced by the extract were compared with those observed around the control well in order to rule out any solvent-related interference. The interpretation of results, based on the presence or absence of an inhibition zone, followed the criteria established by Ponce *et al.* [8]. According to these authors, a bacterium is considered resistant to an extract if the diameter of the inhibition zone is less than 9 mm; for diameters equal to or greater than 9 mm, the bacterium is classified as sensitive according to various categories. Positive controls (antibiotics) were assessed for each bacterial strain in accordance with EUCAST-CASFM guidelines [6].

The antibacterial parameters of the extract, namely the Minimum Inhibitory Concentration (MIC) and the Minimum Bactericidal Concentration (MBC), were determined for the susceptible bacterial strains using the broth double dilution method. According to Fauchère [9], the mode of action of a substance is assessed based on the ratio between its MIC and MBC. A substance is considered bactericidal when the MBC/MIC ratio is ≤ 2, and bacteriostatic when the ratio is strictly greater than 2.

2.2.3. Determination of antifungal activity

Antifungal activity was evaluated using broth dilution according to CLSI (M27, M60) and EUCAST guidelines. A standardized inoculum suspension of initial density (DI) was prepared and serially diluted in liquid medium. Increasing volumes of the extract were added to 10 mL of each dilution, followed by incubation for 24 h at 35 °C [10]. Final densities were then determined.

The MIC was defined as the lowest concentration of extract for which no visible growth was observed. MIC interpretation followed Aligiannis *et al.* [11]:

- Strong activity: MIC $\leq$ 2 0.5 mg/mL
- Moderate activity: 0.6 – 1.5 mg/mL
- Weak activity: MIC $\geq$ 2 1.6 mg/mL

For reference antifungal drugs, clinical or epidemiological MIC breakpoints established by CLSI and EUCAST were used [12].

2.2.4. Statistical analysis

The diameter of the inhibition zones around the different Vitamin C concentrations was measured in millimeters. The data obtained were subjected to a one-way analysis of variance (ANOVA) using IBM SPSS Statistics version 25 software to assess the differences in antibacterial efficacy between concentrations. Differences between means were considered significant at the 5% level ($p < 0.05$). Each trial was performed in triplicate, and the results are presented as means ± standard deviations (mean ± SD).

3. Results

3.1. Antioxidant profile of Vitamin C

The antioxidant activity of Vitamin C was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay, according to the protocol established by Brand-Williams *et al.* [1].

The results obtained (Figure 1) confirm that Vitamin C exhibits a strong and dose-dependent antioxidant activity. At higher concentrations (0.020 to 0.333 mg/mL), a pronounced antioxidant effect was observed, with reduction percentages (RP) exceeding 94%. Conversely, a significant decrease in activity was recorded at concentrations below 0.010 mg/mL. In this range, the reduction percentage dropped from 78.9% to 0.6% at the lowest concentration (0.0013 mg/mL). The 50% DPPH reduction concentration (RC_{50}) determined in this study was 0.0066 mg/mL (6.6 µg/mL).

The results obtained confirm that Vitamin C exhibits a marked antioxidant activity, consistent with its well-established role as a free radical scavenger. The strong radical-scavenging capacity observed can be attributed to the presence of hydroxyl and enediol groups within the ascorbic nucleus, which are capable of donating an electron or a proton to neutralize radical species [1].

Studies conducted by Njus *et al.* [13] and Mavrić Scholze *et al.* [14] further support this observation, highlighting the role of Vitamin C as a hydrogen donor that stabilizes DPPH or ABTS radicals through the formation of semidehydroascorbate intermediates. Investigations on wines have emphasized that antioxidant efficiency is highly dependent on factors such as pH, temperature, and solvent matrix, parameters that may have influenced the RC_{50} value measured in the present study (6.6 µg/mL). The findings obtained are therefore consistent with those reported by Anu *et al.* [15], who documented RC_{50} values for standard Vitamin C ranging from 5 to 15 µg/mL, depending on the analytical method employed.

3.2. Antibacterial profile of Vitamin C

No microbial growth was observed on the agar surface (Figure 2), confirming the sterility of the tested extract.

Table 1 presents the sensitivity of Ivorian clinical and reference bacterial strains to Vitamin C, as well as to the negative and positive controls.

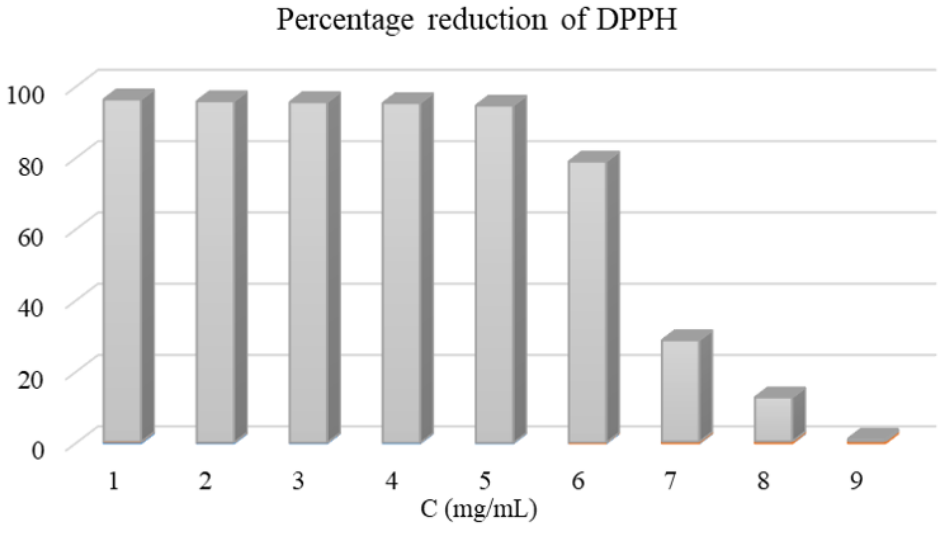


Fig. 1. Reducing power of Vitamin C.

The ANOVA statistical analysis yielded p-values of 0.000, which are below the significance threshold of 0.05, indicating a statistically significant difference in the inhibition zone diameters among the various bacterial strains tested.

Both Table 1 and Figure 3 highlight the antibacterial activity of Vitamin C against the tested strains. The inhibition zone diameters, which were dose-dependent, ranged from 10.50 to 22 mm at 200 mg/mL, from 8.67 ± 1.15 to 19 mm at 100 mg/mL, and from 6 to 12 mm at 50 mg/mL. All strains were classified as sensitive (S) at the highest concentration (200 mg/mL), confirming a clear dose–response relationship. The most pronounced activity was observed against *Staphylococcus aureus* 976/24 (22 mm at 200 mg/mL), a strain otherwise resistant to cefoxitin (FOX), the reference antibiotic. This contrasting sensitivity suggests that Vitamin C may act through a mechanism distinct from that of β-lactam antibiotics. Reference strains (*Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, and *Staphylococcus aureus* ATCC 25923) also exhibited marked sensitivity to Vitamin C. Conversely, the clinical strains *Klebsiella pneumoniae* 2055/24 and *Escherichia coli* 988/24 displayed lower sensitivity, becoming resistant (R) at lower concentrations (50 and 100 mg/mL). This pattern is consistent with their resistance phenotype to the reference antibiotics (FEP and FOX), confirming their status as multidrug-resistant strains. The absence of inhibition in the negative control (sterile distilled water, SDW) validates the specificity of the antibacterial effect observed for Vitamin C. The determination of antibacterial parameters, namely the Minimum Inhibitory Concentration (MIC) and the Minimum Bactericidal Concentration (MBC), enabled the quantification of Vitamin C efficacy and the characterization of its mode of action against previously sensitive strains. The results (Table 2 and Figure 4) demonstrate a clear bactericidal activity of Vitamin C across all tested strains, as evidenced by MBC/MIC ratios ≤ 2 for every isolate, and even a ratio equal to 1 (MIC = MBC) for *Pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922. These findings indicate that Vitamin C not only inhibits bacterial growth but effectively induces cell death at concentrations close to its inhibitory threshold. Analysis of the MIC values revealed a notable antibacterial potency, particularly against the ATCC reference strains (MIC = 3.125 mg/mL). This effect was markedly stronger than suggested by the agar diffusion assays, in which the initial concentration was 200 mg/mL. Clinical isolates (*Staphylococcus aureus* 976/24, *Klebsiella pneumoniae* 2055/24, and *Escherichia coli* 988/24) displayed higher MIC values (25 mg/mL), confirming their comparatively lower susceptibility observed earlier. Nevertheless, the MBC value (50 mg/mL) obtained for these multidrug-resistant strains remains clinically meaningful, reinforcing the potential of Vitamin C as a promising bactericidal agent. The results reveal a significant dose-dependent activity, with a confirmed bactericidal effect (MBC/MIC ≤ 2) against most of the tested strains. These findings are consistent with the work of Abdelraheem *et al.* [3], who demonstrated a dose-dependent inhibition of *Pseudomonas aeruginosa* by Vitamin C, as well as with the studies of AlSaleh *et al.* [4] and Dey & Bishayi [16], which highlighted a synergistic effect of ascorbic acid with rifampicin, vancomycin, and ciprofloxacin, respectively, against *Staphylococcus aureus*.

The proposed mechanism of action is based on the pro-oxidant properties of Vitamin C at high concentrations. In the presence of Fe^{2+} and Cu^{2+} ions, Vitamin C promotes the formation of reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2) and hydroxyl radicals ($^{\bullet}OH$), inducing lethal oxidative stress in bacterial cells [17]. This phenomenon likely explains the observed sensitivity of strains resistant to the tested antibiotics (Table 1), suggesting that Vitamin C acts through a mechanism distinct from that of λ-lactam antibiotics.

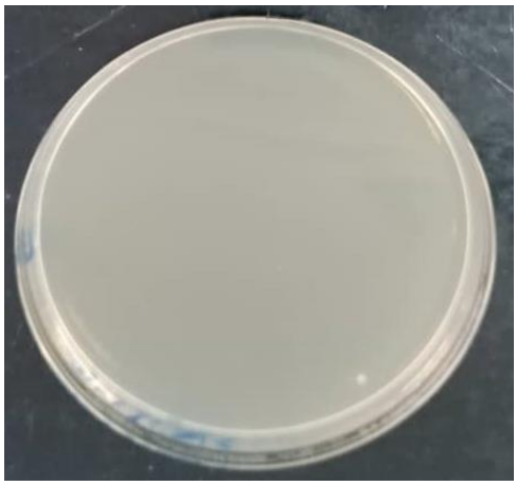


Fig. 2. Sterility test result of Vitamin C.

Table 1
Inhibition zone diameters produced by Vitamin C and controls against bacterial isolates.

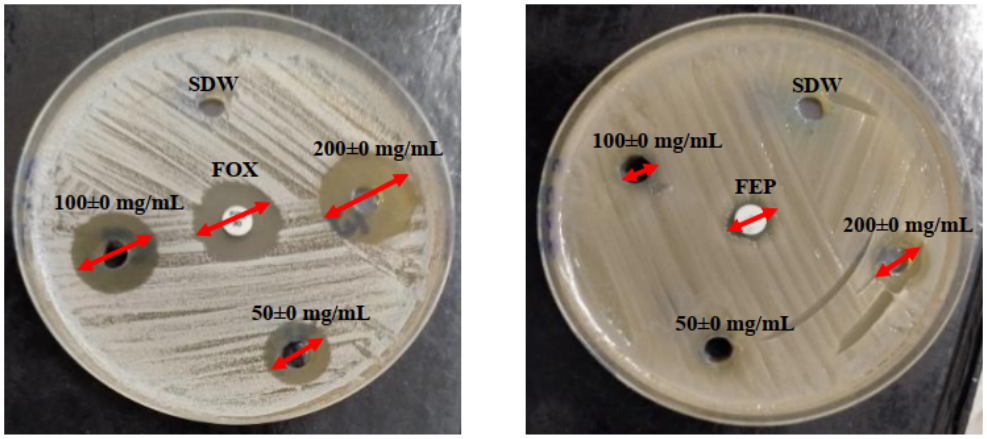
Bacterial strain	Vitamin C (mg/mL)			ATB (mm)	SDW (mm)
	200	100	50		
P. aeruginosa ATCC 27853	18.67 ± 0.5 (S)	13.67 ± 0.5 (S)	11.67±2.08 (S)	CZD: 23 (S)	6 (R)
E. coli ATCC 25922	17.33±1.53 (S)	15.00±0.10 (S)	12.00 (S)	FEP: 31 (S)	6 (R)
S. aureus ATCC 25923	17.33±0.58 (S)	12.00±0.60 (S)	10.00±1.00 (S)	FOX: 30 (S)	6 (R)
S. aureus 976/24	22.00 (S)	19.00 (S)	6.00 (R)	FOX: 18 (R)	6 (R)
K. pneumoniae 2055/24	10.67±0.57 (S)	9.00 (S)	6.00 (R)	FEP: 11 (R)	6 (R)
E. coli 988/24	10.50 (S)	8.67 ± 1.15 (R)	6.00 (R)	FEP: 6 (R)	6 (R)

Note: Tp, Positive control; ATB, Antibiotics; FOX, Cefoxitin; CZD, Ceftazidime–avibactam; FEP, Cefepime; Tn, Negative control; SDW, Sterile distilled water; S, Susceptible; R, Resistant.

Table 2
Antibacterial activity parameters and mode of action of Vitamin C on susceptible bacterial isolates.

Bacterial Strain	MIC (mg/mL)	MBC (mg/mL)	MBC/MIC	Mode of action
P. aeruginosa ATCC 27853	3.125	3.125	1	Bactericidal
E. coli ATCC 25922	3.125	3.125	1	Bactericidal
S. aureus ATCC 25923	3.125	6.25	2	Bactericidal
S. aureus 976/24	25.00	50.00	2	Bactericidal
K. pneumoniae 2055/24	25.00	50.00	2	Bactericidal
E. coli 988/24	25.00	50.00	2	Bactericidal

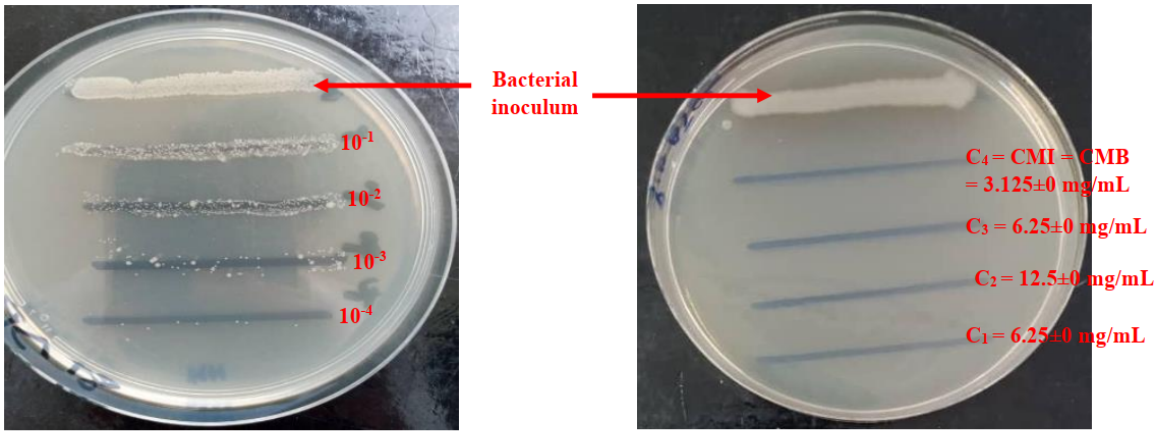
Note: MIC, Minimum Inhibitory Concentration; MBC, Minimum Bactericidal Concentration.



Staphylococcus aureus ATCC 25923 | *Klebsiella pneumoniae* 2055/24

↔: Diameter of the bacterial growth inhibition zone at a given concentration; FOX, Cefoxitin; FEP, Cefepime; SDW, Sterile distilled water.

Fig. 3. Comparative effect of Vitamin C and controls on *Staphylococcus aureus* ATCC 25923 and *Klebsiella pneumoniae* 2055/24.



Box A | Box B

Fig. 4. MBC determination of Vitamin C against *Pseudomonas aeruginosa* ATCC 27853 based on colony density comparison.

3.3. Antifungal profile of Vitamin C

Table 3 and Figure 5 illustrate the antifungal profile of Vitamin C, which exhibits generally low activity against the tested fungal isolates.

Based on the results, no complete minimum inhibitory concentration (MIC_{100}) is below 125 mg/mL, a value far exceeding the threshold (1.6 mg/mL) generally considered indicative of strong antifungal activity [11].

Among the tested strains, *Candida glabrata* (*C. glabrata*) MY24216 exhibited the most notable sensitivity, with an MIC_{50} of 46.87 mg/mL, which may be considered moderately low. However, its MIC_{100} remained high (125 mg/mL), indicating a partial fungistatic effect rather than a clear fungicidal action. This relative sensitivity suggests potential interest for combination therapy with other antifungal agents, in which Vitamin C could enhance their activity [18]. In contrast, strains of *C. albicans* (NR-50363 and MY24264), as well as *C. parapsilosis* and *C. tropicalis*, displayed marked resistance, with very high MIC_{100} values ranging from 125 to 1000 mg/mL, confirming their low susceptibility to ascorbic acid. This resistance may be attributed to the low permeability of the fungal cell wall, the rapid degradation of Vitamin C in

aqueous media, or the ability of certain *Candida* strains to neutralize reactive oxygen species (ROS) via antioxidant enzymes such as catalase and superoxide dismutase [19].

Table 4 illustrates the antifungal activity of the reference control, fluconazole, whose efficacy is markedly higher than that observed with Vitamin C. Fluconazole demonstrates exceptional antifungal potency against all tested strains, with inhibitory concentrations (MIC_{50} and MIC_{100}) extremely low, ranging from 0.001 to 0.002 mg/mL and 0.004 to 0.008 mg/mL, respectively.

Table 3
Evaluation of the antifungal activity of Vitamin C.

Species	MIC_{50} (mg/mL)	MIC_{100} (mg/mL)	Activity evaluation
C. parapsilosis ATCC22019	62.5	500	Weak
C. albicans NR-50363	125	125	Weak
C. albicans MY24264	125	1000	Very weak
C. tropicalis MY24203	93.75	500	Weak
C. glabrata MY24216	46.87	125	Moderate low

Note: MIC_{100}, Minimum concentration of Vitamin C completely inhibiting fungal growth; MIC_{50}, Minimum concentration of Vitamin C inhibiting 50% of fungal growth.

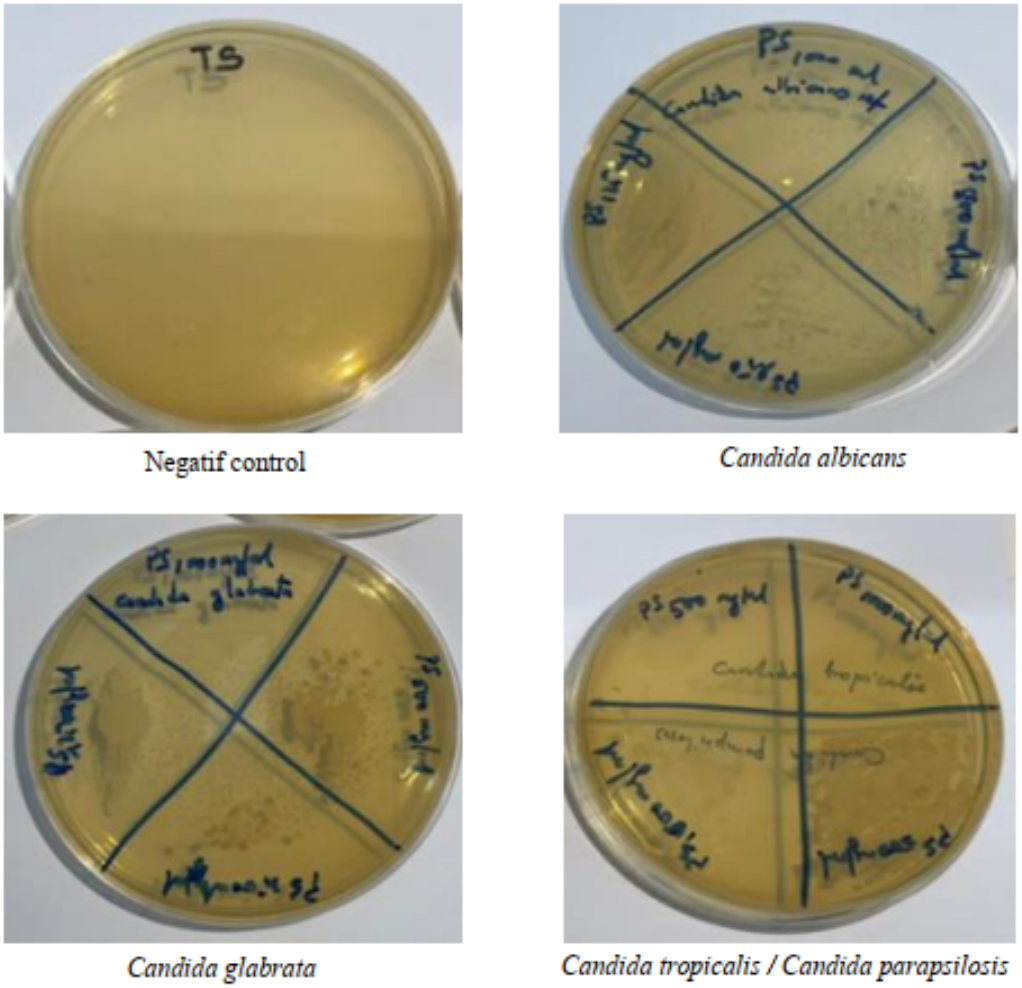


Fig. 5. Antifungal effect of Vitamin C on the tested strains.

Table 4
Assessment of the antifungal activity of fluconazole.

Species	MIC_{50} (mg/mL)	MIC_{100} (mg/mL)	Breakpoints EUCAST/CLSI	Interpretation
C. parapsilosis ATCC22019	0.002	0.004	S $\leq$ 2 mg/L	Susceptible
C. albicans NR-50363	0.002	0.004	S $\leq$ 2 mg/L	Susceptible
C. albicans MY24264	0.002	0.004	S $\leq$ 2 mg/L	Susceptible
C. tropicalis MY24203	0.001	0.004	S $\leq$ 2 mg/L	Susceptible
C. glabrata MY24216	0.001	0.008	SDD $\leq$ 32 mg/L (CLSI)	Susceptible dose-dependent

Note: MIC_{100}, Minimum concentration that completely inhibits fungal growth; MIC_{50}, Minimum concentration that inhibits 50% of fungal growth; EUCAST: European Committee on Antimicrobial Susceptibility Testing; CLSI, Clinical and Laboratory Standards Institute; S: Susceptible; SDD: Susceptible dose-dependent.

In accordance with EUCAST and CLSI reference criteria [12], all strains are classified as sensitive to fluconazole.

Strains of *Candida albicans* (NR-50363 and MY24264), *Candida parapsilosis* (ATCC22019), and *Candida tropicalis* (MY24203) exhibit complete susceptibility.

For the fungal strain *Candida glabrata* (MY24216), the measured MIC_{100} (0.008 mg/mL) indicates dose-dependent susceptibility (SDD) [20]. This classification, specific to this species, is due to the value reaching the upper limit of the accepted susceptibility range.

This comparison highlights the substantial difference in efficacy between Vitamin C, with MIC_{100} values ranging from 125 to 1000 mg/mL, and the reference antifungal, fluconazole. Fluconazole exhibits a potency several thousand times greater than that of Vitamin C. This remarkable efficacy confirms its status as a standard treatment for fungal infections and provides a solid benchmark for evaluating the potential of new molecules or therapeutic approaches, including strategies in which Vitamin C could act as an adjuvant to enhance antifungal activity.

4. Conclusion

Ascorbic acid exhibits noteworthy antibacterial activity, particularly at high concentrations, with a confirmed bactericidal effect against both reference and Ivorian bacterial strains, notably *Pseudomonas aeruginosa*, *Escherichia coli*, and *Staphylococcus aureus*. In contrast, its antifungal activity remains very limited and appears insufficient for direct therapeutic application against the tested Candida species. Nevertheless, its potential as a combined agent with other antimicrobial compounds warrants further investigation. Vitamin C could therefore be considered as an adjuvant or co-therapeutic agent, particularly in optimized formulations designed to enhance its stability and bioavailability.

In vivo studies and clinical trials will be required to confirm these findings and to evaluate the tolerance, bioavailability, and pharmacokinetic profile of Vitamin C. It would also be relevant to investigate, in Côte d'Ivoire, its combination with conventional antibiotics or antifungals in order to highlight possible synergistic effects, as suggested by previous studies.

Abbreviations

ATCC: American Type Culture Collection
ATB: Antibiotic
RC_{50}: Concentration that reduces 50% of DPPH
CZD: ceftazidime-avibactam
DPPH: 2,2-diphenyl-1-picrylhydrazyl
FEP: cefepime

FOX: cefoxitin
MH: Mueller-Hinton
MIC: Minimum Inhibitory Concentration
MBC: Minimum Bactericidal Concentration
PR: Percentage of DPPH reduction

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