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Antimicrobial Activity of *Citrus aurantifolia* Peel Essential Oil Against Clinically Relevant Bacteria and Fungi

[1]Olufemi L. Okunye, [2]Oluwaseun E. Adewole, [3]Comfort B. Kotun, [3]Ayedun S. JoJoshua [4]Osalewa H. Adewoyin, [5]Brendan I. Chijioke, [6]Titilayo T. Kolade and [7]Peter O. Ajayi

[1]Department of Pharmaceutical Microbiology, Faculty of Pharmacy, Olabisi Onabanjo University, Ogun State, Nigeria.
[2]Department of Microbiology, Faculty of Science, University of Ilesa, Ilesa Osun state, Nigeria.
[3]Department of Biological Sciences and Biotechnology, College of Pure and Applied Sciences, Caleb University, Imota, Lagos State, Nigeria.
[4]Department of Environmental and Occupational Health Faculty of Public Health. University of Medical Sciences, Ondo, Ondo state, Nigeria.
[5]Department of Pharmaceutical Microbiology, Faculty of Pharmacy, Olabisi Onabanjo University, Ogun State, Nigeria.
[6]Department of Biological Science, Yaba College of Technology, Yaba, Lagos, Nigeria
[7]Department of Pharmaceutical Microbiology, Faculty of Pharmacy, Olabisi Onabanjo University, Ogun State, Nigeria

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ABSTRACT

Background: The increasing tolerance of several microorganisms against commonly used antibiotics represents a challenge for microbiologists to find alternative ways for the treatment of such infections. A study was carried out to determine the effect of *Citrus aurantifolia* oil extract on clinical bacteria and fungi obtained from routine laboratory benches of the department of pharmaceutical microbiology, University of Ibadan.

Methods: *Citrus aurantifolia* peels oils were extracted by hydro-distillation using a Clevenger-type apparatus, distillates of essential oils were dried over anhydrous sodium sulfate, filtered and concentrated at 30^{o}C using a rotary evaporator. Conventional biochemical characterization of the isolates and antibacterial assay of the extract on the isolates was carried out.

Results: The antibacterial activities of the essential oil from *Citrus aurantifolia* peels on test isolates tested exhibited zones of inhibition that ranged between 15mm and 20mm. The minimum inhibitory concentration, minimum bactericidal concentration and minimum fungicidal concentration showed antimicrobial and anti-fungicidal activity on all tested isolates, with MIC values range between 0.39 mg/mL as obtained for *Salmonella typhi* and *Bacillus subtilis* and 3.125mg/mL as recorded in *Staphylococcus aureus* and *Klebsiella pneumonia* while the MFC values that range between 0.391 and 0.78 as were recorded in *Trichophyton rubrum* and *Candida albicans*.

Conclusion: Essential oils of *Citrus aurantifolia* peels demonstrated remarkable antibacterial and anti-fungicidal activity on all the isolates tested, which is suggestive of its antimicrobial potential and may be used as therapeutic agent for the treatment of microbial infections when further purified.

***Keywords*:** Antimicrobial activity, *Citrus aurantifolia,* Essential oil, Fungi, Pathogenic bacteria.

***Corresponding author: Email:** okunyelionel@oouagoiwoye.edu.ng: **Phone: +2349066611198**

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1.0 INTRODUCTION

The use of plants and herbs as an alternative therapy in the treatment of diseases in various composition and forms has been in practice from time immemorial and it is gaining more popularity worldwide due to low cost, ease of access and experimental knowledge of use and reliability. It has been estimated by the World Health Organization that approximately 80% of the world population depends mainly on traditional medicines or plant-derived products for their primary health care, while the remaining 20% are from advanced countries. However, less than 20% of African medicinal plants have been investigated phytochemically and tested for bioactivity [1]. *Citrus aurantiifolia* is a shrubby tree, growing to 5 meters (16 feet), with many thorns. Dwarf varieties exist that can be grown indoors during winter months and in colder climates. Its trunk, which rarely grows straight, has many branches, and they often originate quite far down on the trunk. The leaves are ovate, 2.5–9 centimeters (1–3.5 inches) long, resembling orange leaves (the scientific name *aurantiifolia* referring to this resemblance to the leaves of *Citrus aurantium*). The flowers are 2.5 cm (1 in) in diameter, are yellowish white with a light purple tinge on the margins. Flowers and fruit appear throughout the year but are most abundant from May to September in the Northern Hemisphere. *Citrus. aurantiifolia* is native to Southeast Asia and are largely by fertile land climate and every agro-supportive factor in many geographical zones in Nigeria [2]. The antimicrobials obtained from plants have been given serious attention due to the development of resistance to conventional antibiotics by some microorganisms. It has been reported that a significant number of the world's population depend on traditional medicine for primary healthcare. Over the years, essential oils and other plant extracts have stirred up curiosity as sources of natural products and have thus been screened for their potential uses as alternatives for the treatment of many infectious diseases [3]. Essential oils are complex mixtures of organic compounds that give characteristics odor and flavor. They possess a wide spectrum of different impressive qualities including antiphlogistic, spasmolytic and antioxidant activity. Moreover, they exert immunomodulant, psychotropics, acaricide and expectorant effects. Due to their multi-functionality, essential oils find a huge application in medicine and arotherapy [4]. Essential oils have been shown to possess significant antimicrobial properties against a wide range of Gram-positive and Gram-negative bacteria [5]. Therefore, this study aimed to evaluate the antibacterial and antifungal activity of essential oil from peel of *Citrus aurantifolia* against clinically relevant bacteria and fungi obtained from routine laboratory benches.

2.0. MATERIALS AND METHODS

2.1 Materials

2.1.1 Biological materials

Bacteria and fungi isolates comprise of *Staphylococcus aureus, Salmonella typhi, Klebsiella spp, Bacillus subtilis, Trychophyton rubrum* and *Candida albicans*

2.1.2 Chemicals and reagents

Methanol (Analar grade), distilled water, normal saline, peptone water, Sensitivity Test Agar, Nutrient agar, Mannitol Salt Agar, Eosin Methylene Blue Agar, Saboraud Dextrose Agar, *Salmonella-Shigella* Agar, MacConkey Agar, Crystal violet, Safranin, hydrogen peroxide.

2.1.3 Equipment and other materials

Clevenger apparatus, blending machine, Petri-dishes, Bunsen burner, sterile universal containers, conical flasks retort stand, graduated test tubes and beakers, cotton wool, inoculating loop, incubator, oven, laminar flow hood, Winchester bottles, refrigerator and autoclave. Conical flasks, spirit lamp, rotary evaporator and laboratory benches.

2.2 Methods

2.2.1. Study area

The *Citrus aurantifolia* fruits were collected from farm around Second Avian, New garage axis in Oluyole local government Ibadan, within the Latitude 7.3775 ^{o}N and Longitude 3.9470 ^{o}E in Oyo state, Nigeria.

2.2.2. *Sample Collection*

The *Citrus aurantifolia* fruits were collected from farm around Second Avian, New garage axis in Oluyole local government Ibadan. The entire specimen was authenticated at herbarium laboratory of the Department of Botany, University of Ibadan where the voucher was deposited.

2.2.3 Extraction of Essential oil

Exactly 900 grams of the *Citrus aurantifolia* fruits peels were chopped in a blender, and thereafter hydro-distillated using a Clevenger apparatus for 5hrs for isolation of oils. Mixture of *(Citrus aurantifolia)* oils and water incorporated were separated, and the isolated oil. were dried over anhydrous sodium sulfate, filtered and then concentrated at 30 ℃ using a rotary evaporator (Buchi R-124, Flavil, Sweden). The resulting yellowish essential oil was subsequently stored in dark brown sealed vials at -20 ℃ until use.

2.2.4 Sterility test of the extract

Exactly, 2mls of the extracts were added to 8mls of sterile peptone water and incubated at 37 ºC for 24 hours. It was subculture to various selective and differential media; MSA, MCA, NA, SDA and incubated at 37ºC for 24 hours and plates were observed for growth; but no growth was found.

2.2.5 Collection of clinical isolates

Clinical isolates of bacteria which included *Staphylococcus aureus, Salmonella typhi, Klebsiella spp, Bacillus subtilis, Trychophyton rubrum* and *Candida albicans* were collected from the routine laboratory bench work from the department of pharmaceutical microbiology and biotechnology of the University of Ibadan, Ibadan, Nigeria.

2.2.6 *Isolation and Identification of Microorganism*

The isolates were confirmed by a different selective media unique for the growth of each bacterium and fungi and thereafter incubated at optimum temperature for 24-48 ℃ for each isolate. The plates were thereafter observed for morphological growth. Gram staining and other conventional biochemical test which include; catalase test, coagulase test, indole test, methyl red test, Voges proskauer test, citrate test, oxidase test, salts and sugars utilization tests and other relevant tests that are specifics for each species was carried out. The isolates obtained were preserved at 4 ℃ on nutrient slant s in the refrigerator for further use.

2.2.7 *Antimicrobial Assay: (Agar Diffusion Techniques)*

The screening of antimicrobial activities of essential oils extract on the tested bacteria and fungi in this study was determined on sensitivity test agar media, using agar well diffusion methods. Wells of 6mm diameter and 4mm depth were made on the solid agar using a sterile cork borer. A volume of 100uL of essential oils of *Citrus aurantifolia* extract was inoculated onto wells already impregnated in spread plate cultures of each microbial isolates (each microbial concentration was made 10^6 CFU/ml). The plates were allowed to stand on the laboratory bench between 1 - 2 h to allow proper inflow of the solution into the medium before incubating the plates at 37˚C for 24 h. The plates were performed in triplicates. The diameters of the zone of inhibitions were measured by measuring scale in millimeter (mm).

2.2.8 *Minimum inhibitory concentration (MIC).*

The stock solutions of *Citrus aurantifolia* peels oil were prepared by dissolving it in growth medium containing (2% DMSO) to obtain a final concentration of 200 mg/mL. The stock solutions (200 µL) of the extract were dispensed into the wells of a 96-well micro-titre plate, followed by two-fold serial dilutions with the growth medium. Microbial suspensions were prepared by adjusting turbidity to 0.5 McFarland standard and subsequently diluted 1:100 in growth medium to achieve a concentration of 10^6 CFU/mL for bacterial isolates and 10^4 CFU/ mL for fungal isolates. Then, 100 µL of the microbial suspension was inoculated into each well, resulting in a final inoculum concentration of 0.5×10^6 CFU/mL for bacteria and 0.5×10^4 CFU/mL for fungi. The microtitreplates were incubated under the specific optimum conditions aerobically. The MIC was defined as the lowest concentration of each tested sample that visibly inhibited the growth of the isolates was determined by identifying the wells that remained clear (non-turbid) in comparison to the growth observed in the control wells (i.e., growth control).

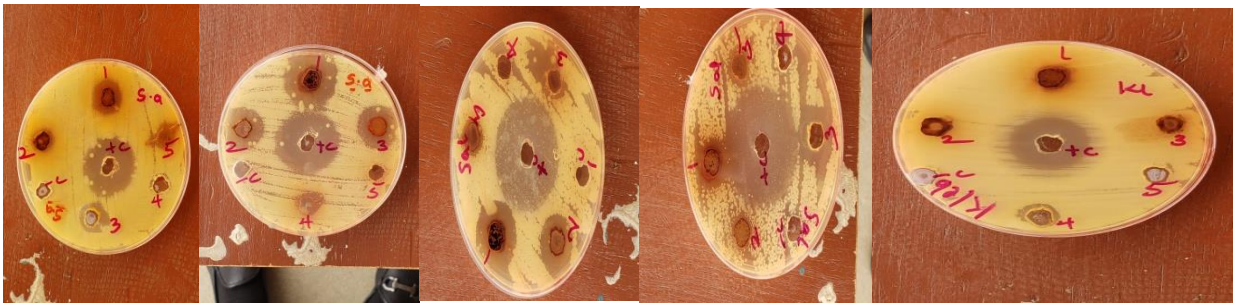
2.2.9. Minimum Bactericidal Concentration (MBC) or Minimum Fungicidal Concentration (MFC) Determination.

The MBC and MFC values were determined by taking 10 µL aliquots from wells showing no visible growth (non-turbid) and inoculated onto the appropriate agar medium. The plates were incubated under aerobic condition and colony formation was evaluated after incubation. The MBC and MFC as the lowest concentration of the tested samples that resulted in complete microbial killing was determined.

2.3. Statistical Analysis

Data were expressed as mean ± SD and differences between sets obtained were determined using ONE WAY ANOVA followed by Duncan post Hoc Test with the use of SPSS v 20 software. Differences were considered significant at $p < 0.05$

3.0 RESULTS



1.S. aureus *2. S. aureus* *3. Sal. typhi* *4. Sal. typhi* *5. Klebsiella spp*

6. C. albicans *7. T. rubrum* *8 T. rubrum* *9. Bacillus subtilis*

Figure 1-9: Culture plates evidence of bacteria and fungi exposed to *C. aurantifolia* peel essential oil.

Table 1: Sensitivity Testing of the Extract on the isolates. Size of the sterile cork borer 6mm

The average of the zones of growth inhibition for tested isolates of bacteria and fungi were recorded to be relatively varied from each organisms as shown in Table 1 below.

S/N	Test isolates	Zones of Inhibition
1	*S.aureus (A1)*	20
2	*S.aureus(A2)*	19
3	*S.typhi (B3)*	15
4	*S.typhi(B4)*	16
5	*K. pneumonia(K5)*	22
6	*B.subtilis (S6)*	20
7	*T. rubrum (T7)*	15
8	*T. rubrum (T8)*	17
9	*C.albicans (C9)*	20

Table 2: Test Statistics

Mean	Variance	SD
23.2222	13.444	3.66667

F ratio = 108.931, p value = <0.001

Table 3: F and P ratios

Bacterial Isolate	Mean ± SD	95% CI
S.aureus (A1)	4.69 ± 2.21	-15.17 – 24.54
S.aureus (A2)	7.82 ± 6.63	-51.71 – 67.34
S.typhi (B3)	7.03 ± 7.73	-62.47 – 76.53
S.typhi (B4)	0.98 ± 0.83	-6.45 – 8.40
K.pneumonia (K5)	4.69 ± 2.21	-15.17 – 24 54
B.subtilis (S6)	0.98 ± 0.83	-6.45 – 8.40

F. ratio = 0.881, p value = 0.545

- **F-ratio (0.881):** The F-statistic is less than 1, suggesting that the variation observed between the different bacterial species is actually less than the variation occurring within the groups themselves.
- **P-value (0.545):** The p-value is much higher than the 0.05 significance threshold. Consequently, there is no statistical evidence to suggest that the treatment or conditions affected these isolates differently.
  Though the clinically relevant isolates are potential pathogens, All tested bacterial isolates namely —*S. aureus, S. typhi, K. pneumoniae,* and *B. subtilis*—responded in a statistically similar manner under the experimental conditions. And despite the numerical differences in mean values, the results are not statistically significant.

Table 3: The MIC, MBC and MFC of the extracts on the isolates. Note: All tests were performed in triplicates(n=3)

	Citrus aurantifolia essential oil (mg/mL)		
Bacterial isolates	MIC	MBC	MBC:MIC ratio
S.aureus (A1)	3.125	6.250	2
S.aureus(A2)	3.13	12.50	8
S.typhi (B3)	1.56	12.50	8
S.typhi(B4)	0.391	1.560	4
K. pneumonia(K5)	3.125	6.250	2
B.subtilis (S6)	0.391	3.125	8
Fungal isolates	MIC	MFC	MFC/MIC ratio
T. rubrum (T7)	0.391	1.56	4
T. rubrum (T8)	0.78	1.56	2
C.albicans (C9)	0.78	1.56	2

Table 4: Comparative analysis between MIC and MBC for Bacterial isolate

	Mean	95% CI
MIC	1.95 ± 1.35	0.53 – 3.37
MBC	6.77 ± 4.91	1.62 – 11.92

F ratio = 5.367, p value = 0.043*

The comparative analysis between the **Minimum Inhibitory Concentration (MIC)** and the **Minimum Bactericidal Concentration (MBC)** for the bacterial isolates reveals a statistically significant difference between the two parameters.

- **F-ratio (5.367):** The F-statistic indicates that the variance between the MIC and MBC groups is greater than the variance within the groups themselves.
- **P-value (0.043):** Because the p-value is less than the 0.05 significance level, the result is considered statistically significant. Instead

There is a significant difference between the inhibitory and bactericidal concentrations. On average, the concentration required to kill the bacterial isolates is more than three times higher than the concentration required inhibiting them

Table 5: Turkey's multiple comparisons test for bacterial

Isolates	Isolates	Mean Difference	95% CI	Adj. p. value	Remark
S.aureus (A1)	*S.aureus (A2)*	-3.12750	-20.50 – 14.29	0.97	No
	S.typhi (B3)	-2.34250	-19.76 – 15.07	0.99	No
	S.typhi (B4)	3.71200	-13.70 – 21.13	0.95	No
	K.pneumonia (K5)	0.00000	-17.41 – 17.41	1.00	No
	B.subtilis (S6)	3.71200	-13.70 – 21.13	0.95	No
S.aureus (A2)	*S.aureus (A1)*	3.12750	-14.29 – 20.54	0.97	No
	S.typhi (B3)	0.78500	-16.63 – 18.20	1.00	No
	S.typhi (B4)	6.83950	-10.57 – 24.25	0.65	No
	K.pneumonia (K5)	3.12750	-14.29 – 20.54	0.97	No
	B.subtilis (S6)	6.83950	-10.57 – 24.25	0.65	No
S.typhi (B3)	*S.aureus (A1)*	2.34250	-15.07 – 19.76	0.99	No
	S.aureus (A2)	-.78500	-18.20 – 16.63	1.00	No
	S.typhi (B4)	6.05450	-11.36 – 23.47	0.74	No
	K.pneumonia (K5)	2.34250	-15.07 – 19.76	0.99	No
	B.subtilis (S6)	6.05450	-11.36 – 23.47	0.74	No
S.typhi (B4)	*S.aureus (A1)*	-3.71200	-21.13 – 13.70	0.95	No
	S.aureus (A2)	-6.83950	-24.25 – 10.57	0.65	No
	S.typhi (B3)	-6.05450	-23.47 – 11.36	0.74	No
	K.pneumonia (K5)	-3.71200	-21.13 – 13.70	0.95	No
	B.subtilis (S6)	0.00000	-17.41 – 17.41	1.00	No
K.pneumonia (K5)	*S.aureus (A1)*	0.00000	-17.41 – 17.41	1.00	No
	S.aureus (A2)	-3.12750	-20.54 – 14.29	0.97	No
	S.typhi (B3)	-2.34250	-19.76 – 15.07	0.99	No
	S.typhi (B4)	3.71200	-13.70 – 21.12	0.95	No
	B.subtilis (S6)	3.71200	-13.70 – 21.12	0.95	No
B.subtilis (S6)	*S.aureus (A1)*	-3.71200	-21.13 – 13.70	0.95	No
	S.aureus (A2)	-6.83950	-24.25 – 10.57	0.65	No
	S.typhi (B3)	-6.05450	-23.47 – 11.36	0.74	No
	S.typhi (B4)	0.00000	-17.41 – 17.41	1.00	No
	K.pneumonia (K5)	-3.71200	-21.13 – 13.70	0.94	No

The post-hoc analysis reveals a high degree of homogeneity in how the isolates responded:

- *Identical Mean Responses*: Several pairs showed a mean difference of 0.00 and an Adjusted P-value of 1.00. This includes the comparisons between *K. pneumoniae (K5)* vs. *S. aureus (A1)* and *B. subtilis (S6)* vs. *S. typhi (B4)*. These isolates performed identically under the experimental conditions.
- *Most Distinct (but non-significant) Pair:* The largest mean difference observed was 6.83950 (between *S. aureus (A2)* and *S. typhi (B4)* or *B. subtilis (S6)*). However, even this difference was not statistically significant ($p = .645$), as the variance within the groups was large enough to mask the difference between the means.
- *Staphylococcus and Salmonella comparisons:* Within-species variations (e.g., A1 vs. A2 or B3 vs. B4) were also non-significant, suggesting that the different strains of the same bacteria did not behave differently from one another.

"No" remark across the entire table signifies that the null hypothesis—stating that all group means are equal—cannot be rejected. The extract or intervention tested appears to have a uniform effect across all Gram-positive and Gram-negative bacteria included in this study

Table 6: Statistics of Fungal isolates

Isolates	Mean ± SD	95% Confidence Interval
T. rubrum (T7)	0.98 ± 0.82	-6.45 – 8.40
T. rubrum (T8)	1.17 ± 0.55	-3.79 – 6.13
C. albicans (C9)	1.17 ± 0.55	-3.79 – 6.13

F ratio = 0.059, p value = 0.944

The 95% Confidence Intervals for all three groups are notably wide and overlap significantly, all encompassing the value of zero. This suggests a high degree of variability within each group relative to the sample size, indicating that the true population means could vary across a broad range. There is no statistically significant difference in the mean values among *T. rubrum (T7)*, *T. rubrum (T8)*, and *C. albicans (C9)*. The experimental conditions affected all three fungal isolates in a statistically similar manner.

Table 7: Comparative MIC and MBC for fungi

	Mean ± SD	95% CI
MIC	0.65 ± 0.22	0.09 – 1.21
MFC	1.56 ± 0.00	1.56 – 1.56

F ratio = 42.216, p value = 0.002*

F-ratio (42.216): This high F-statistic demonstrates that the difference between the MIC and MFC is much greater than the variation observed within the MIC group.

P-value (0.002): The p-value is significantly lower than the standard 0.05 threshold. This indicates a statistically significant difference

There is a statistically significant difference between the MIC and MFC values. The concentration required to kill the fungal isolates (MFC) is significantly higher—more than double—than the concentration required to merely inhibit their visible growth (MIC)

Table 8: Multiple Comparisons

Fungal isolates	Isolates paired	Mean Difference	Remark	P value	95% CI
T. rubrum (T7)	*T. rubrum (T8)*	-.19450	N.S	.953	-2.94 – 2.55
	C.albicans (C9)	-.19450	N.S	.953	-2.97 – 2.55
T. rubrum (T8)	*T. rubrum (T7)*	.19450	N.S	.953	-2.55 – 2.94
	C.albicans (C9)	.00000	N.S	1.000	-2.74 – 2.74
C.albicans (C9)	*T. rubrum (T7)*	.19450	N.S	.953	-2.55 – 2.94
	T. rubrum (T8)	.00000	N.S	1.000	-2.74 – 2.74

The statistical analysis of the fungal isolates T. rubrum (T7), T. rubrum (T8**)**, and C. albicans **(**C9**)** indicates that there are no significant differences between any of the groups tested.

In all pairwise comparisons, the p-values significantly exceed the standard alpha threshold of 0.05. Specifically, the comparisons between the two *T. rubrum* strains and between *T. rubrum* and *C. albicans* yielded p-values of 0.9530, while the comparison between *T. rubrum (T8)* and *C. albicans (C9)* showed a p-value of 1.000, representing an identical mean result. Furthermore, the 95% Confidence Intervals (CI) for every comparison cross the value of zero. This confirms the "Not Significant" (**N.S**) remark, as we cannot conclude that any one isolate performs differently from the others under the tested conditions

4.0 DISCUSSION

The increasing tolerance of several microorganisms against commonly used antibiotics represents a challenge for scientists to find alternative ways for the treatment of such infections. One of the main causes that provokes resistance of microorganisms is unguided and unrecommended use of antibiotics [6]. This study evaluated *C. aurantifolia peels* oil against isolates of bacteria and fungi obtained from varied clinical spectrum of infection via exposure to varied concentrations, integrating the minimum inhibitory concentration, minimum bactericidal concentration and minimum fungicidal concentration. The zones of growth inhibition of the clinical isolates bacteria and fungi exposed to *C. aurantifolia* peel essential oil as shown in the culture plates in Figure 1-9 informed the need for further findings on the antimicrobial activity of the crude extract of essential oil exposed. The variation in the zones of inhibition obtained that ranged from 15 mm as recorded in *Salmonella typhi* B3, and 20 mm as showed in *Bacillus subtilis* S6 and *Candida albicans* C9 (Table 1) could be attributed to strains variation, inherent genetic factors of each clinical isolates

or composition of the sensitivity agar test, which agrees with the study of Aibinu *et al.* (2007) on evaluation of the antimicrobial properties of different parts of *Citrus aurantifolia* used locally. The results of present study show that essential oils of *C. aurantifolia* peel had antibacterial activities on the tested isolates as indicated by the diameter of their zone of inhibition [7]. The p-value (0.545) obtained was much higher than the 0.05 significance threshold. Consequently, there is no statistical evidence to suggest that the treatment or conditions affected these isolates differently. Despite the numerical differences in mean values, the results are not statistically significant. All tested bacterial isolates—*S. aureus, S. typhi, K. pneumoniae,* and *B. subtilis*—responded in a statistically similar manner under the experimental conditions. The minimum inhibitory concentration, minimum bactericidal concentration and minimum fungicidal concentration showed remarkable antimicrobial activity on all tested isolates, with MIC values 0.39 mg/mL for *Salmonella typhi B3* and *Bacillus subtilis S6* and 3.125mg/mL for *Staphylococcus aureus* A1 and *Klebsiella pneumonia* K5 while the MFC values range between 0.391 mg/mL and 0.78 mg/mL as recorded for *Trichophyton rubrum T7* and *Candida albicans* C9 as shown in Table 3 [8]. The MBC/MFC values that range from 1.56 mg/mL to 12.5 mg/mL and the MBC/MIC ratios that range from 2 mg/mL and 8 mg/mL were obtained. This agrees with the study of Pintan *et.al.,* (2025) on chemical characterization and antimicrobial activities of *Citrus aurantifolia* peel oils and *Ocimum sanctum* ethanolic extract. Statistical comparative analysis between the Minimum Inhibitory Concentration (MIC) and the Minimum Bactericidal Concentration (MBC) for the bacterial isolates as shown in Table 4 reveal a statistically significant difference between the two parameters [9]. P-value (0.043): Because the p-value is less than the 0.05 significance level, the result is considered statistically significant. There is a significant difference between the inhibitory and bactericidal concentrations. On average, the concentration required to kill the bacterial isolates is more than three times higher than the concentration required inhibiting them [10]. The extract or intervention tested as shown in Table 5 Turkey's multiple comparisons test for bacterial appears to have a uniform effect across all Gram-positive and Gram-negative bacteria included in this study which means "no" remark across the entire table signifies null hypothesis—stating that all group means are equal—cannot be rejected. There is no statistically significant difference in the mean values among *T. rubrum (T7)*, *T. rubrum (T8)*, and *C. albicans (C9)*. The experimental conditions affected all three fungal isolates in a statistically similar manner as elicited in Table 6. The statistical analysis of the fungal isolates T. rubrum (T7), T. rubrum (T8**)**, and C. albicans **(**C9**)** indicates that there are no significant differences between any of the groups tested (Table 8) In all pairwise comparisons, the p-values significantly exceed the standard alpha threshold of 0.05. Specifically, the comparisons between the two *T. rubrum* strains and between *T. rubrum* and *C. albicans* yielded p-values of 0.9530, while the comparison between *T. rubrum (T8)* and *C. albicans (C9)* showed a p-value of 1.000, representing an identical mean result [11]. The mechanism of antimicrobial activity of the essential oil of *Citrus aurantifolia* peels studied could be attributed to its inhibitory potential against cellular integrity of bacteria and inherent ability to initiates oxidative stress and apostosis in clinical isolates exposed and coupled with membranous damage property against *Candida albicans and Trichphyton rubrum*, which agrees with the study of Enejoh, *et.al.,* (2015) on ethno-medical importance of *Citrus aurantifolia* [12].
The in-vitro activity of many experimental set up cannot be directly translated instantly to in-vivo use. In in-vivo study, parameters like allergies and some other further immunological reactions and every other factor that some users may develop should be considered.

5. CONCLUSION:

Citrus aurantifolia in this study showed broad spectrum of antimicrobial activity against the clinical isolates of bacteria and fungi tested, when purified, it can be incorporated into pharmaceutical preparations for therapeutic use.

DECLARATIONS

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Conflict of Interest: The authors declare no conflict of interest.

Authors Contributions
Okunye, Olufemi Lionel conceived and designed the study, conducted the data analysis, interpreted the findings, and prepared the manuscript. All co-authors contributed to the experimental work, data collection, and reviewed and approved the final version of the manuscript.

Ethical Approval:
Ethical approval with reference number NHREC/28/9/2025 was obtained from the Health Research Ethics Committee Office of the Hospital Management Board of the University Teaching Hospital before embarking on the study.

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