Tropical Journal of Pharmaceutical Research June 2024; 23 (6): 941-949
ISSN: 1596-5996 (print); 1596-9827 (electronic)

Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v23i6.4

Original Research Article

Synergistic antifungal activity of combinations of *Artemisia annua* extract with fluconazole against resistant strains of *Candida albicans*

Maria I Ngwu[1], Godwin I Ngwu[2]*, Chibundo N Okorie[1], Stephen C Emencheta[1], Damian C Odimegwu[1], Anthony A Attama[3], Emmanuel C Ibezim[3]
[1]Department of Pharmaceutical Microbiology and Biotechnology, [2]Department of Zoology and Environmental Biology, [3]Department of Pharmaceutics, University of Nigeria, Nsukka, Enugu State, Nigeria

For correspondence:* ***Email:* *godwin.ngwu@unn.edu.ng*

Sent for review: 3 December 2023 *Revised accepted: 2 June 2024*

Abstract

Purpose: *To elucidate how fluconazole and the methanol extract of Artemisia annua interact to inhibit the growth of fungi.*
Methods: *Five hundred clinical samples of Candida albicans were isolated from patients attending three tertiary health facilities in Enugu State, Nigeria, namely, University of Nigeria Teaching Hospital (UNTH), Ituku Ozala, Bishop Shanahan Hospital (BSH), Nsukka, and Enugu State University Teaching Hospital (ESUTH), Enugu. Out of the five hundred clinical samples collected, eight genetically proven C. albicans isolates were employed for this study. The synergy between Artemisia annua and fluconazole was characterized by checkerboard analyses and fractional inhibitory concentration indices (FIC).*
Results: *Minimum inhibitory concentration (MIC) testing results show that most isolates were resistant to selected azoles employed in the study. Based on fractional inhibitory concentration (FIC) index, specific mixtures of the extract and FLU had synergistic effects on isolates of resistant Candida albicans.*
Conclusion: *When combined with fluconazole, the extract of A. annua demonstrated better inhibition than fluconazole alone against resistant C. albicans. This suggests a promising strategy for tackling the challenge of emerging fungal antibiotic resistance.*

Keywords: *Candidiasis, Azole, Fluconazole, Artemisia annua, Synergy, Checkerboard*

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, Web of Science, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

The main factors that pose severe threats to human and animal health worldwide are the growth of multidrug-resistant (MDR) bacterial and fungal infections [1]. Moreover, antibiotics are used in excess in animals and humans because they are easily gotten over the counter. Open defecation and manure application into the soil release antibiotics or residues into different environmental compartments [2].

A total of 93,309 tonnes of antimicrobials was used worldwide in livestock production in 2017 and the number is projected to increase to 104, 079 tonnes (representing 11.5 % increase) by

2030 [2,3]. The emergence of resistance and the adverse side effects associated with conventional drugs have led to the continuous search for new drugs and strategies [4]. A possible treatment approach would involve employing antifungals in combination with natural herbs to overcome resistance, highlighting the need for innovative antifungal agents, especially from natural sources such as plants [5,6].

Fluconazole is the most popular first-line treatment and preventive drug for *Candida albicans* infections. However, many fluconazole-resistant strains are being discovered due to its broad use in clinical settings [7].

This present work aims to study the synergistic effect of fluconazole combined with the methanol leaf extract of *Artemisia annua* for use against fluconazole-resistant *C. albicans* isolates.

METHODS

Microorganisms

Clinical samples of *C. albicans* were collected from patients visiting three hospitals in Enugu State namely the University of Nigeria Teaching Hospital (UNTH), Ituku Ozala, Bishop Shanahan Hospital (BSH), Nsukka, and Enugu State University Teaching Hospital (ESUTH), Enugu. Out of the five hundred (500) clinical samples collected, eight (8) azole-resistant isolates were identified and genetically confirmed. The isolates were characterized according to the clinical laboratory institutes' CLSI M27-A2 for yeasts. The standard strain of *C. albicans* (Sc5314) employed in the investigation as a positive control was sourced from a culture collection center in New York, USA.

Ethical approval

Ethical approval/permission, reference no. MH/MSD/EC/0176, was obtained from the Ministry of Health, Enugu State, Nigeria. Informed consent of patients involved in this study were also obtained.

Preparation and extraction of plant materials

Dried *Artemisia annua* leaves were purchased from Annamed (Germany). For 24 to 48 h, 30 g of *A. annua* leaf powder was soaked in 300 mL of 80 % methanol (Sigma, Germany) before being sieved with muslin cloth and subsequently filtered using Whatman filter paper. A watch glass was used to dispense the filtrate, which was left to air-dry at room temperature.

Test for terpenoids and steroids

In a boiling water bath, a mixture of 9 mL of ethanol (Sigma, Germany) and 1 mL of the plant extract was concentrated to 2.5 mL, and 5 mL of hot water was added. The waxy material was filtered off after the mixture had stood for an hour. The filtrate was extracted using a separating funnel and 2.5 mL of chloroform. To create a lower layer, 1 mL of concentrated sulphuric acid was carefully added to 0.5 mL chloroform extract in a test tube. The presence of steroids was observed as a reddish-brown interface. Another 0.5 mL of the chloroform extract was heated with 3 mL of concentrated sulphuric acid (Sigma, Germany) for 10 minutes in a water bath before evaporating to dryness. The presence of terpenoids was confirmed by the appearance of a gray colour.

Antifungal sensitivity tests

The disk diffusion (M44-A) and micro-broth dilution (M27-A3) tests were performed according to the Clinical Laboratory Standard Institute (CLSI) methods. Mueller-Hinton agar (Oxoid, Lagos) mixed with 2 % glucose and 0.5 µg/L methylene was used to execute the disc diffusion procedure. Five distinct colonies of approximately 1 mm were picked from a 24 h culture. Colonies were suspended in 18.5 mL of sterile saline. This was vortexed and turbidity adjusted to 0.5 McF standard (0.5×10^3 to 2.5×10^3 cells/mL). A sterile cotton swab was dipped into the standardized inoculum to collect the organism and the excess fluid was discharged into the tube by rubbing the swab against the inner wall of the test tube. Thereafter, the entire surface of the agar plate was streaked with the swab in three different turns of angle 60^o between each streaking unit. The inocula were allowed to dry within 5 to 15 min with the petri dish lid in place. The discs were placed approximately 24 mm apart. This was incubated at 35 ± 2 ^{o}C and examined after 24 – 48 h. The antifungal discs and their respective concentrations were as follows: ketoconazole (30 µg/disc), miconazole (30 µg/disc), clotrimazole (10 µg/disc), itraconazole (30 µg/disc), voriconazole (1 µg /disc) and fluconazole (25 µg/disc).

On the other hand, the micro-broth dilution method was performed using the Rosewell Park Memorial Institute (RPMI) Medium as follows: The overnight culture was first diluted with 0.9 % NaCl to 1.5×10^6 cfu/mL. Thereafter, a spectrophotometer (Bibby Scientific Ltd, UK) was used to compare the yeast inocula to the isolate density standard. The yeast suspensions were

then further diluted in RPMI-1640 (with L-glutamine, without sodium bicarbonate) and supplemented with 0.165 M morpholino-propane sulfonic acid (MOPS) (Sigma, Germany), resulting in a final inoculum that varied between 0.5 x 10^3 and 2.5 x 10^3 cfu/mL.

Preparation of antifungal agent

Fluconazole was dissolved in DMSO (Oxoid, Lagos) to a final concentration of 50 mg/mL. Concentrations ranging from 0.25 to 128 µg/mL were prepared in ten 2-fold serial dilutions in RPMI-1640 medium using the CLSI (M27-A3) standard. One hundred microliter (100 µL) of the yeast inoculum was mixed with 100 µL of the appropriate concentration of fluconazole in a microtiter plate. The wells used for the positive control were composed of 100 µL of the organism's inocula and 100 µL of RPMI, while the wells used for the negative control contained 200 µL of RPMI only. The setup was incubated for 24 h. The result of the MIC was read visually while the inhibitory optical density (OD) was read using an ELISA reader (Agilent Technologies, USA). Both readings were taken into consideration when determining the final result.

***In vitro* interaction studies of *Artemisia annua* with fluconazole**

The interaction study was performed using agar diffusion and the lowest inhibitory concentration (MIC). The checkerboard method investigated *A. annua*'s reactivity to fluconazole as a potential treatment option.

Checkerboard assay

The MIC values for *Artemisia annua* were determined using the agar well diffusion method. At the same time, the MIC for fluconazole was established by applying the broth microdilution method outlined in the CLSI M27-A2 document for yeasts. The MICs and interactions between *A. annua* and fluconazole were assessed by determining their fractional inhibitory concentration (FIC) indices ratios. An FIC index below 1 signifies synergism; equals 1, signifies indifference; and ≥ 2, antagonism.

Assessment of the combined activity of *A. annua* extract and fluconazole

Stock solutions of *A. annua* extract (76 µg/mL) and fluconazole (64 µg/mL) were prepared. Fluconazole was dissolved in DMSO, while *A. annua* was dissolved in a mixture of RPMI and DMSO at a concentration of 20 %. Following the continuous variation checkerboard approach [9], a mixture was prepared with ratios of *A. annua* and fluconazole ranging from *A. annua* 0:10 fluconazole to *A. annua* 10:0 fluconazole. The RPMI was used to serially dilute each herbal–antifungal combination ratio, resulting in a twofold decrease in concentration. Thereafter, 1 mL of the respective combination of medication was mixed with 19 mL of sterile Sabouraud dextrose agar (SDA) and placed in a Petri dish. The mixture in the Petri dish was kept on the bench to harden.

On the surface of the SDA plates, MacFarland standards (0.5 mL), representing several isolated *C. albicans* strains, were streaked. Triplicates of the MacFarland standards preparation and control without the antifungals were set up and the test was carried out. After incubation at 35 ^oC for 24 h, the results were checked to determine if any growth had occurred. The MICs of the different combinations were determined and an analysis of the interactions between *A. annua* and fluconazole was done by calculating the fractional inhibitory concentration (FIC) index for each combination using Eq 1 - 4.

FIC index = FIC A + FIC B …… (1)

FIC A = (MIC of drug A + drug B)/(MIC of drug A alone) …… (2)

FIC B = (MIC of drug B + drug A)/(MIC of drug B alone) …….. (3)

Activity index (AI) = log FIC index …….. (4)

where A and B = Combined *A. annua* and fluconazole; MIC = Minimum inhibitory concentration; and Drug = Antimicrobial agent

RESULTS

Table 1: The MIC of fluconazole with micro-broth dilution

S/N	Isolate	MIC (µg/mL)
1	SHL7	≥128
2	C57	≥128
3	C6	≥128
4	C80	128
5	SH12[6]	≥128
6	PL12	32
7	SH39[5]	4
8	SH12[5]	16
9	SC5314 (positive control)	32
Breakpoint range	(BP) of MIC	0.125 - 64 µg/ml

Table 1 and Table 2 present the MICs of nine (9) resistant *C. albicans*. The MIC (mg/mL) for fluconazole ranges from 4 µg/mL to ≥ 128 µg/mL,

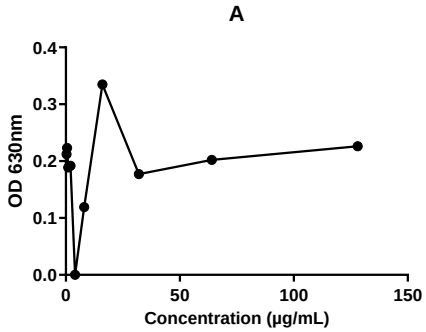
while that of *A. annua* ranges from 40.74 to 76.03 µg/mL. However, for the inhibition assay with fluconazole, the plotted graphs of absorption versus the concentration are presented in Figure 1 and Figure 2.

Checkerboard analysis

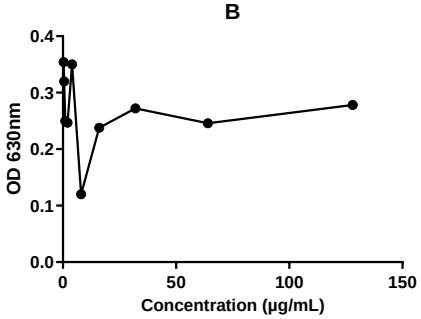
The Checkerboard analysis results are presented in Table 3. The index of the fluconazole inhibitory concentration showed that *A. annua*, in combination with fluconazole (FLU), produced synergistic effects against the resistant *Candida albicans* isolates at various ratios except at ratio 9:1 (*A. annua*: flu) for SHL7, PL13, C74, and SH44[6]. The other isolates are shown in their respective Figures. Furthermore, the combined effect of methanol extract of *A. annua* and fluconazole against C43 and Type C (SC5314) are presented in Figure 2 and Figure 3.

Table 2: MIC of *Artemisia annua* obtained by agar well diffusion

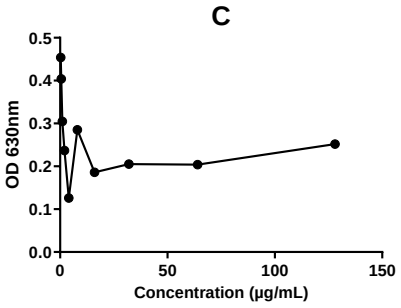
S/N	Isolate	MIC (µg/mL)
1	SHL7	62.66
2	C57	76.03
3	C6	73.28
4	C80	62.66
5	SH12[6]	69.82
6	PL12	40.74
7	SH39[5]	68.87
8	SH12[5]	73.28
9	SC5314 (positive control)	71.94
10	SH33[6] (control)	69.50



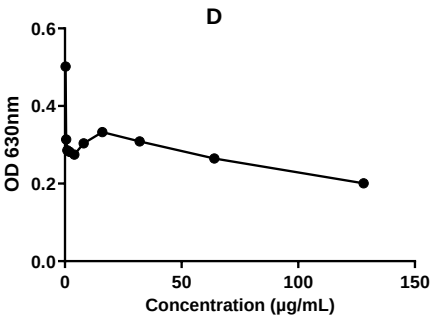
Fluconazole Inhibition of Candida isolate SHL7



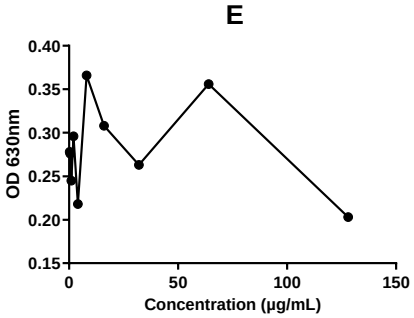
Fluconazole Inhibition of Candida Isolate C57



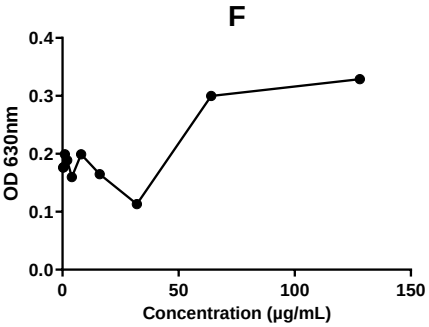
Fluconazole Inhibition of Candida Isolate C6



Fluconazole Inhibition of Candida Isolate C80

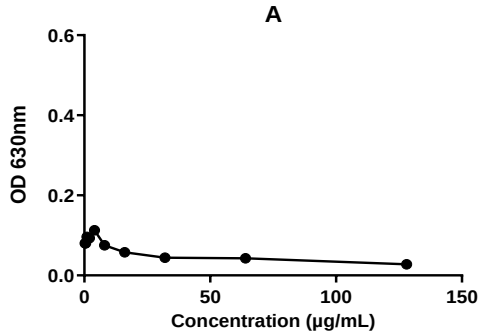


Fluconazole Inhibition of Candida Isolate SH12[6]

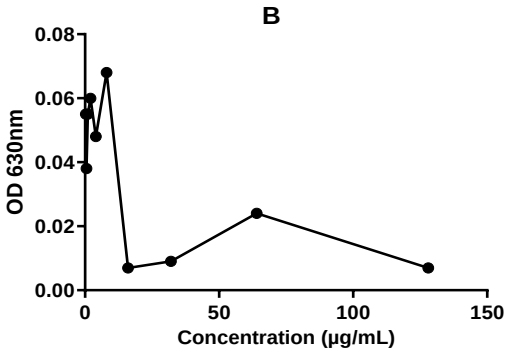


Fluconazole Inhibition of Candida Isolate PL12

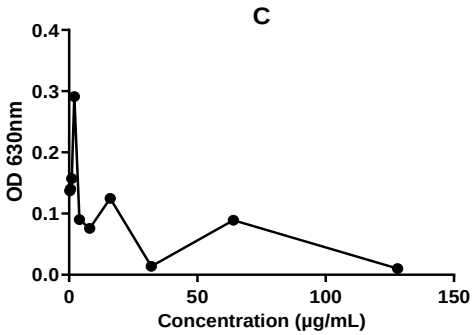
Figure 1: Inhibition of Candida isolates by fluconazole



Fluconazole Inhibition of Candida Isolate SH39[5]



Fluconazole Inhibition of Candida Isolate SH12[5]



Fluconazole Inhibition of Candida Isolate Type C

Figure 2: Inhibition of Candida isolates by fluconazole

Table 3: Combined effect of *Artemisia annua* extract and fluconazole

Comb. Ratio Arte:Flu	MIC of Arte (µg/mL)	MIC of Flu (µg/mL)	FIC Artemisia	FIC Fluconazole	FIC Index	Activity Index	Effect
against SHL7							
0:10	0.0000	0.4000	0.0000	0.0063	0.0063	-2.204	
1:9	0.1900	1.4400	0.0025	0.0225	0.0250	-1.602	Synergistic
2:8	0.1900	0.6400	0.0025	0.0100	0.0125	-1.903	Synergistic
3:7	0.2850	0.5600	0.0038	0.0088	0.0126	-1.901	Synergistic
4:6	0.0950	0.1200	0.0013	0.0019	0.0159	-1.805	Synergistic
5:5	0.2375	0.2000	0.0031	0.0031	0.0062	-2.206	Synergistic
6:4	0.5700	0.3200	0.0075	0.0050	0.0125	-1.903	Synergistic
7:3	0.3325	0.1200	0.0044	0.0019	0.0063	-2.202	Synergistic
8:2	3.0400	0.6400	0.0400	0.0100	0.0500	-1.301	Synergistic
9:1	0.0000	0.0000	0.0000	0.0000	0.0000	0.000	
10:0	0.4750	0.0000	0.0063	0.0000	0.0063	-2.204	
against C29							
.0:10	ND	ND	ND	ND	ND	ND	
1:9	ND	ND	ND	ND	ND	ND	ND
2:08	0.76	2.56	0.01	0.04	0.05	-1.301	Synergistic
3:7	1.14	2.24	0.015	0.035	0.05	-1.301	Synergistic
4:6	ND	ND	ND	ND	ND	ND	ND
5:5	1.9	1.6	15.0.025	0.025	0.05	-1.301	Synergistic
6:4	2.28	1.28	0.03	0.02	0.05	-1.301	Synergistic
7:3	ND	ND	20.ND	ND	ND	ND	ND
8:2	3.04	0.64	0.04	0.01	0.05	-1.301	Synergistic
9:1	ND	ND	ND	ND	ND	ND	ND
10:0	ND	ND	ND	ND	ND	ND	

Arte = *Artemisia annua*; Flu = Fluconazole; ND = not determined

Table 3: Combined effect of *Artemisia annua* extract and fluconazole **continued**

Comb. Ratio Arte:Flu	MIC of Arte (µg/mL)	MIC of Flu (µg/mL)	FIC Artemisia	FIC Fluconazole	FIC Index	Activity Index	Effect
against SH12[6]							
0:10	0.000	3.200	0.000	0.050	0.050	-1.301	
1:9	0.380	2.880	0.005	0.045	0.050	-1.301	Synergistic
2:8	ND	ND	ND	ND	ND	ND	ND
3:7	ND	ND	ND	ND	ND	ND	ND
4:6	1.520	1.920	0.020	0.030	0.050	-1.301	Synergistic
5:5	ND	ND	ND	ND	ND	ND	ND
6:4	ND	ND	ND	ND	ND	ND	ND
7:3	ND	ND	ND	ND	ND	ND	ND
8:2	ND	ND	ND	ND	ND	ND	ND
9:1	3.800	0.000	0.050	0.000	35.005	-1.301	Synergistic
10:0	ND	ND	ND	ND	ND	ND	
against PL13							
0:10	0.0000	0.4000	0.0000	0.0063	0.0063	-2.204	
1:9	0.1900	1.4400	0.0025	0.0225	0.0250	-1.602	Synergistic
2:8	0.0475	0.1600	0.0006	0.0025	0.0031	-2.505	Synergistic
3:7	0.2850	0.5600	0.0038	0.0088	0.0125	-1.903	Synergistic
4:6	0.3800	0.4800	0.0050	0.0075	0.0125	-1.903	Synergistic
5:5	0.2375	0.2000	0.0031	0.0031	0.0063	-2.204	Synergistic
6:4	0.2850	0.1600	0.0038	0.0025	0.0063	-2.204	Synergistic
7:3	0.6650	0.2400	0.0088	0.0038	0.0125	-1.903	Synergistic
8:2	0.7600	0.1600	0.0100	0.0025	0.0125	-1.903	Synergistic
9:1	0.4275	0.0400	0.0056	0.0006	0.0063	-2.204	Synergistic
10:0	1.9000	0.0000	0.0250	0.0000	0.0250	-1.602	
against C74							
0:10	0.0000	0.4000	0.0000	0.0063	0.0063	-2.204	
1:9	0.1900	1.4400	0.0025	0.0225	0.0250	-1.602	Synergistic
2:8	0.0475	0.1600	0.0006	0.0025	0.0031	-2.505	Synergistic
3:7	0.2850	0.5600	0.0038	0.0088	0.0125	-1.903	Synergistic
4:6	0.3800	0.4800	0.0050	0.0075	0.0125	-1.903	Synergistic
5:5	0.2375	0.2000	0.0031	0.0031	0.0063	-2.204	Synergistic
6:4	0.2850	0.1600	0.0038	0.0025	0.0063	-2.204	Synergistic
7:3	0.6650	0.2400	0.0088	0.0038	0.0125	-1.903	Synergistic
8:2	0.7600	0.1600	0.0100	0.0025	0.0125	-1.903	Synergistic
9:1	0.4275	0.0400	0.0056	0.0006	0.0063	-2.204	Synergistic
10:0	1.9000	0.0000	0.0250	0.0000	0.0250	-1.602	
against C6							
0:10	ND	ND	ND	ND	ND	ND	ND
1:9	ND	ND	ND	ND	ND	ND	ND
2:8	0.0475	0.1600	0.0006	0.0025	0.0031	-2.505	Synergism
3:7	ND	ND	ND	ND	ND	ND	ND
4:6	ND	ND	ND	ND	ND	ND	ND
5:5	ND	ND	ND	ND	ND	ND	ND
6:4	ND	ND	ND	ND	ND	ND	ND
7:3	ND	ND	ND	ND	ND	ND	ND
8:2	ND	ND	ND	ND	ND	ND	ND
9:1	3.4200	0.3200	0.0450	0.0050	0.0500	-1.301	Synergism
10:0	3.8000	0.0000	0.0500	0.0000	0.0500	-1.301	Synergism
against SH44[6]							
0:10	0.0000	0.2000	0.0000	0.0031	0.0031	-2.505	
1:9	0.0475	0.3600	0.0006	0.0056	0.0063	-2.204	Synergistic
2:8	0.1900	0.6400	0.0025	0.0100	0.0125	-1.903	Synergistic
3:7	0.2850	0.5600	0.0038	0.0088	0.0125	-1.903	Synergistic
4:6	0.1900	0.2400	0.0025	0.0038	0.0063	-2.204	Synergistic
5:5	0.4750	0.4000	0.0063	0.0063	0.0125	-1.903	Synergistic
6:4	0.5700	0.3200	0.0075	0.0050	0.0125	-1.903	Synergistic
7:3	1.3300	0.4800	0.0175	0.0075	0.0125	-1.602	Synergistic
8:2	1.5200	0.3200	0.0200	0.0050	0.0250	-1.602	Synergistic
9:1	ND	ND	ND	ND	ND	ND	ND
10:0	ND	ND	ND	ND	ND	ND	

Arte = *Artemisia annua*; Flu = Fluconazole; ND = not determined

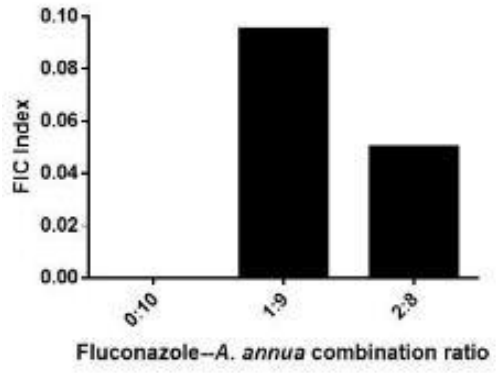


Figure 3: The combined effect of methanol extract of *Artemisia annua* and fluconazole against C43

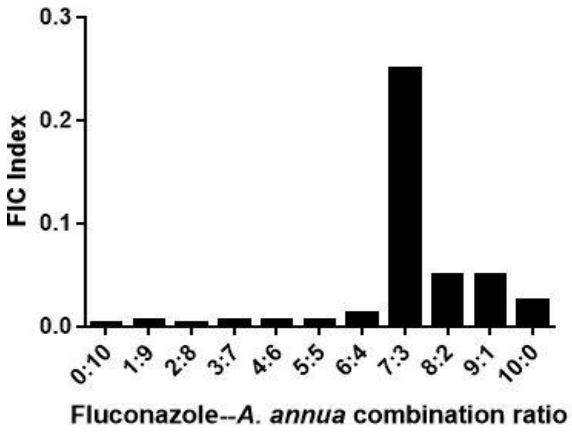


Figure 4: The combined effect of methanol extract of *Artemisia annua* and fluconazole against Type C (SC5314)

DISCUSSION

Infections caused by fungi have emerged as a significant problem in the past few decades, yet, there are only few antifungal medications available. In addition, drug-resistant strains of fungi are rapidly evolving which has further execrated the menace of antifungal infection. This could result in fungal infections that cannot be cured. It has been reported that combination therapy is effective against disease pathogens, including those linked with fungal infection [10,11]. Therapists therefore recommend combination therapy in the treatment of some fungal infections. This is particularly the case when optimal dose cannot be achieved due to toxicity and when organisms that possess unique fungal susceptibility patterns are removed by empirical treatment [12].

The result of this study demonstrated that the combination of fluconazole and the methanol extract of *Artemisia annua* produced synergistic effects against the resistant isolates at various ratios. The most pronounced synergy between artesunate and miconazole was previously reported [13]. However, since crude methanol extract of *Artemisia annua* was used, the tendency of other extract derivatives acting in synergy cannot be ruled out. *Artemisia annua* is reported to possess potent antifungal effect in addition to its known antimalarial properties [14]. Previous studies have shown that artemisinin derivatives possess *in vitro* antifungal action against *Candida albicans* [15,16]. In this study, the bioactive effect of fluconazole was enhanced by combination with methanol extract of *A. annua* as shown by the checkerboard assay. Complemental combinations involving artemisinins with conventional antifungal drugs have been advocated as a viable strategy for identifying new antifungal medicines in the future [17]. A combination of three chitosans with fluconazole-resistant strains which achieved great antifungal synergistic activity against *C. albicans* was previously reported [18] and agrees with this study. This finding also agrees with investigations by Hui Li *et al* [19] on the *in vitro* interactions between fluconazole and minocycline against mixed *Candida albicans* and *Staphylococcus aureus* cultures. They found that a combination of Fluconazole and minocycline had synergistic effects on a strain of *C. albicans* resistant to fluconazole.

Developing novel therapies that use combinations of current antifungal drugs is an important choice that should be considered for treating resistant microbiological conditions, such as candidiasis. Good antimicrobial tracking exercises and related antimicrobial screening activities would be effective to provide efficient remedies employing combination medications. This is because antimicrobial resistance can develop quickly. This would then supplant the void produced by the ever-widening pandemic of recurrent and newer emerging Candida infections.

CONCLUSION

This investigation demonstrates that fluconazole combined with *Artemisia annua* has a synergistic antifungal effect and, thus, suggests a promising strategy for tackling the challenge of emerging fungal antibiotic resistance.

DECLARATIONS

Acknowledgements

The authors wish to express their gratitude to the Department of Pharmaceutical Microbiology and Biotechnology, Department of Pharmaceutics, Department of Zoology and Environmental

Biology and Department of Microbiology, University of Nigeria, Nsukka, Nigeria. The authors also wish to acknowledge the Department of Veterinary Pathology and Microbiology University of Nigeria, Nsukka for providing their diagnostic laboratory where part of this work was done. Professors Adikwu and Esimone.

Funding

None provided.

Ethical approval

None provided.

Availability of data and materials

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

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

We declare that this work was done by the author(s) named in this article and all liabilities about claims relating to the content of this article will be borne by the authors. Conceptualization: Maria I Ngwu, Anthony A Attama, Emmanuel C Ibezim, Godwin I. Ngwu, Damian C Odimegwu; Data curation: Chibundo N Okorie, Stephen C Emencheta; Formal analysis: Maria I Ngwu, Anthony A Attama, Emmanuel C Ibezim, Godwin I Ngwu, Chibundo N Okorie, Stephen C Emencheta, Damian C Odimegwu, Funding: Maria I Ngwu, Chibundo N Okorie, Stephen C Emencheta, Damian C Odimegwu, Godwin I Ngwu, Investigation: Maria I Ngwu, Anthony A Attama, Emmanuel C Ibezim, Godwin I. Ngwu, Damian C Odimegwu, Chibundo N Okorie, Stephen C Emencheta; Methodology: Maria I Ngwu, Anthony A Attama, Emmanuel C Ibezim, Godwin I. Ngwu, Damian C Odimegwu; Project administration: Maria I Ngwu, Stephen C Emencheta; Validation: Chibundo N Okorie, Godwin I. Ngwu; Writing – original draft: Maria I Ngwu, Godwin I Ngwu; Writing – review & editing: Maria I Ngwu, Godwin I Ngwu, Anthony A Attama, Emmanuel C Ibezim.

Open Access

REFERENCES

1. *Francis JB, Alia SR, Bayan S, Galyah A, Noor BA, Sayed A. Emerging status of multidrug-resistant bacteria and fungi in the Arabian Peninsula. Biology (Base) 2021; 10(11): 1144. doi: 10.3390/biology10111144*
2. *Van Boeckel TP, Glennon EE, Chen D, Gilbert M, Robinson TP, Grenfell BT, Levin SA, Bonhoeffer S, Laxminarayan R. Reducing antimicrobial use in food animals. Science 2017; 357(6358):1350–1352. doi: 10.1126/science. aao1495*
3. *Tiseo K, Huber L, Gilbert M, Robinson TP, Van Boeckel, TP. Global trends in antimicrobial use in food animals from 2017 to 2030. Antibiotics (Basel) 2020; 9-918. doi: 10.3390/antibiotics9120918.*
4. *Sanglard D, Sanglard D. Emerging threats in antifungal-resistant fungal pathogens. Front Med 2016; 3-1–10.*
5. *Mareshah A, Sydney S, Linna G, Adrienne S, Tiffany SR, Keely P, Jeffrey L. Antimicrobial activity of the volatile substances from essential oils. BMC Complement Med Ther 2021; 21: 124.*
6. *Nasrin A, Batool SN, Sedigheh YN, Sadegh T, Yusef Y, Ehsan A. In vitro anti-candidial activity of some Iranian medicinal plants against candida species. Int J Enteric Pathog 2021; 9(2): 52-58.*
7. *Ruhnke, MA, Westhausen S, Morschh¨auser J. Development of simultaneous resistance to fluconazole in Candida albicans and Candida dubliniensis in a patient with AIDS. J Antimicrobial Chemo 2000; 46(2): 291–295.*
8. *Trease, GE, Evans, WC 2002. Pharmacognosy. 15th Edition, Saunders Publishers, London, 42-44, 221-229, 246-249, 304-306, 331-332, 391-393.*
9. *Evaluation of chemical antimicrobial agents. In: Okore VC. Pharmaceutical Microbiology: Principles of Pharmaceutical Applications of Antimicrobial Agents. 1st edition. Enugu: Demak Publisher 2005; p 6-64.*
10. *Shaista K, Mohd I, Mohammed I, Nuzhat P. Antimicrobial activity of various ethanolic plant extracts against pathogenic multi drug resistant Candida spp. Bioinformation 2017; 13(3): 67–72.*
11. *Síle FM, Cecilia K, Robert SH, Angela L, Charles K, Duncan C, Sayoki M, Elvis T, Shabir L, Sokoine L, et al. Antifungal combinations for treatment of cryptococcal meningitis in Africa. N Engl J Med 2018; 378(11): 1004-1017 doi: 10.1056/NEJMoa1710922.*
12. *Allen UD. Canadian Paediatric Society ID and, Committee. I. Antifungal agents for the treatment of*

systemic fungal infections in children. Paediatr Child Heal. 2010; 15(9): 603–9.

13. *De Cremer K, Lanckacker E, Cools TL, Bax M, De Brucker K, Cos P, Cammue BP, Thevissen K. Artemisinins, new miconazole potentiators resulting in increased activity against Candida albicans biofilms. Antimicrob Agents Chemother. 2015; 59(1):421–6.*
14. *Liu CH, Zou WX, Lu H, Tan RX. Antifungal activity of Artemisia annua endophyte cultures against phytopathogenic fungi. J Biotechnol. 2001; 88(3):277–82.*
15. *Dogra S, Singh J, Koul B, Yadav D. Artemisia vestita: A folk medicine with hidden herbal fortune. Molecules 2023;28(6):2788; https://doi.org/10.3390/molecules28062788*
16. *World Health Organization. Malaria Unit. (1998). The use of artemisinin and its derivatives as anti-malarial drugs: report of a Joint CTD/DMP/TDR informal consultation, Geneva, 10-12 June 1998. World Health Organization. https://apps.who.int/iris/handle/10665/65382.*
17. *Bhattacharya A, Kaur H, Lal NK, Bharti N. Repurposing antimalarial artemisinin against candidiasis. Int J Pharmaceut Sci Rev Res 2015; 32(1):143-147.*
18. *Lo W-H, Deng F-S, Deng F-S, Chang C-J, Lin C-H. Synergistic Antifungal activity of chitosan with fluconazole against Candida albicans, Candida tropicalis, and Fluconazole-resistant strains. Molecules 2020; 25(21): 5114; https://doi.org/10.3390/molecules25215114.*
19. *Li H, Zhang C, Liu P, Liu W, Gao Y, Sun S. In vitro interactions between fluconazole and minocycline against mixed cultures of Candida albicans and Staphylococcus aureus Journal of Microbiology, Immunol Infect 2015; 48: 655e661.*