South Asian Journal of Research in Microbiology

Volume 18, Issue 4, Page 35-47, 2024; Article no.SAJRM.114928
ISSN: 2582-1989

Synergistic Potential Sof Cinnamon Extracts, Antibiotics, and Alum on Selected Clinical Microbes

Benjamin, Gideon [a*], Amadi, Lawrence O. [a] and T. Sampson [a]

[a] Department of Microbiology, Faculty of Sciences, Rivers State University, Nkpolu-Oroworukwo, Port Harcourt. Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/SAJRM/2024/v18i4357

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/114928

Original Research Article

Received: 01/02/2024
Accepted: 03/04/2024
Published: 08/04/2024

ABSTRACT

Antibiotics are natural or synthetic substances used to inhibit or kill susceptible microorganisms. Due to the increase rate of bacterial resistance to antibiotics, there is the need to venture in other methods involving the coalescence effect of antibiotics and other substances such as cinnamon and alum to effectively eradicate these pathogenic organisms using less concentrated conventional antibiotics. The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of the selected antimicrobial agents were determined using the broth dilution method, following standards and procedures set by the national council of clinical laboratory standards (NCCLS). The synergistic potentials of all the antimicrobics used were analyzed by the checkerboard assay. The MIC values of 25, 0.25, 0.75,25mg/ml were obtained for cinnamon extracts, gentamicin, penicillin G, and aq. Alum respectively against S*. aureus.* For *E. coli O157H:H7,* the MIC values of cinnamon extracts, gentamicin, penicillin G, and aq. Alum were 12.5, 0.25, 0.75 and 25mg/ml respectively,s while the MIC values of cinnamon extracts alum and

**Corresponding author: Email: Benjasgideo@Gmail.Com;*

fluconazole for *C. albicans* were 12.5, 25 and 0.05mg/ml respectively. There is a reduction in the MIC values for the various combinations against the same clinical microbes. All the antimicrobics used in this study inhibited the growth of the clinical microbes. All the microbes used in this study inhibited the tested microbes in dose dependent manner. The combinations of these agents resulted in reduction in MIC and MBC values, with an additive, indifference, and antagonistic effects using checkerboard assay which resulted to a fractional inhibitory concentration index which ranged from 0.56 to 7.9. Therefore, application of cinnamon, alum, and their combinations with conventional antibiotics can be used as treatment measure against infections caused by *S. aureus, E. coli O157H:H7,* and *C. albicans*.

Keywords: Alum; cinnamon extracts; fractional inhibitory concentration index; minimum bactericidal concentration; synergistic effects.

1. INTRODUCTION

Antimicrobial agents are substance that are used to suppress the growth of microorganisms while antibiotics are natural or synthetic agents that are used to inhibit or kill microorganisms [1]. Antibiotics having the ability to kill microbes are known as Bactericidal agents, whereas those therapeutic agents having the ability to inhibit/stop the growth and proliferation of microorganisms are known as Bacteriostatic agents [2]. The misuse and overuse of antibiotics in recent years has increased the number of microbes that are resistant to antibiotics which is a major challenge to clinicians and public health practitioners [3]. The world health organization (WHO) has compiled a list of multidrug resistant microbes with a call for urgent discovery of new therapeutic agents Miethke et al. [4]. Initially the synthesis of antimicrobial agent was mainly based on single natural substance which was obtained from several sources such as trees/plants, animal, and microorganisms (fungi and bacteria) [5].

Cinnamon powder is a spice obtained from the bark of the tree *Cinnamomum zeylanicum*. It is used as a preservative due its antimicrobial properties. Cinnamon is mostly found in countries such as China, Sri Lanka Indonesia, and India [6]. cinnamaldehyde and eugonol are the two major antimicrobial compounds found in cinnamon resulting to its antimicrobial activity against a variety of Gram negative and Gram-positive bacteria as well as fungi. Cinnamaldehyde which is one of the major bioactive molecules in cinnamon is electronegative in nature and can interfere with cellular biological processes, especially the nitrogen containing compounds including the nucleic acids and proteins [7]. Also, cinnamon and its essential oil (EO) has been shown to inhibit bacterial growth through the following mechanisms: ATPase, Biofilm formation membrane porin, anti-quorum sensing effects, cell division inhibitions, and alterations of the cell membrane, these mechanisms could cause the reduction in susceptibility of bacteria to antibiotics, decreasing the dose of antibiotic necessary for the effective treatment, hence a decrease in the toxic side effect of the drug EL Atki et al. [8], Nabavi et al. [7]. However, there is an increased keen interest in using active compound from organic and inorganic material as source of active ingredient for the synthesis of antimicrobial agents. cinnamon tree has shown a promising source of natural active compound necessary for the synthesis of new antimicrobial drugs [9]. This is due to the fact that many studied have been devoted on it, and it has been shown that cinnamon and its derivatives have a strong antimicrobial effect on microbes [10]. Furthermore, it has also been shown that the combine effect of cinnamon and antibiotic produces a better and increased antimicrobial effect on tested clinical microbes.

Alum is an inorganic compound formed from natural substances, Alum is generally known as a hydrated double salt of aluminum with general formulae $XAl(SO_4)_2.12H_2O$. Alum can exist in nature as pure and impure form [5]. Alum is a compound formed from the combination of two or more separate elements, there are various types of alum for example potash alum is a combination of potassium, aluminum, Sulphur, and oxygen, also known as potassium aluminum sulphate [11]. It is used as antisepsis and as astringent in several food preparatory and preserving processes/procedures as well as water purification [12]. Alum can also be used in the following ways: acts as mordant (binder) use in dyeing, fixation of dye to cotton, as well as other fabrics, as fire extinguisher, as astringent in medicine, and adjuvant in vaccine production. The antimicrobial effect of

alum has been reported and documented [13,12].

Also, its medical benefits as immune enhancer, used as adjuvant in vaccine production such as hepatitis A & B (Bestoon, 2012),[12]. Often times a single antimicrobial agent is not sufficient to effectively eradicate an infectious agent, therefore in order to achieve the desired antimicrobial affect, a combination of drugs or agents is used to produce the desired antimicrobial effect not obtained with a single antimicrobial agent [12]. Finally, there is a limited research report on the coalescence of cinnamon, and antibiotics which shows a strong synergistic effect against several clinical microbes [8], Umar et al. 2017) However, to the best of my knowledge there is no available research data about the combine antimicrobial effect of cinnamon, antibiotics, and Alum against *Staphylococcus aureus, E. coli O157H:H7* and *Candida albicans*. Hence the aim of this research is to investigate the possible synergistic antimicrobial effects of cinnamon, antibiotic (gentamicin and penicillin G) and Alum against *Staphylococcus aureus, E. coli 0157H:H7* and *Candida albicans.*

2. MATERIALS AND METHODS

2.1 Experimental Design

Crude cinnamon bark extracts (aqueous and ethanolic) of the plant *Cinnamomum zeylanicum* were prepared in a Soxhlet assembly using hot extraction method. Analytical grade alum was purchased from the chemical store with the required standard concentration and was diluted serially using distilled water as solvent. The antimicrobials (antifungal and antibiotics) - Gentamicin, Penicillin G and Fluconazole were purchased from the pharmaceutical store. The antimicrobials (crude aqueous and ethanolic cinnamon bark extracts, aqueous alum, Gentamicin, Penicillin G, and Fluconazole) were then screened, and their antimicrobial activity were evaluated using Broth dilution assay to determine the Minimum inhibitory concentration (MIC[mg/ml]), minimum microcidal (bactericidal/fungicidal) concentration [mg/ml] against selected clinical microbes *(S. aureus* = Gram positive, *E. coli O157H:H7* = Gram negative*, C albicans* = fungi). The MIC values obtained were used to calculate the fractional inhibitory concentration index (FICI) of the antimicrobial agents, which were subsequently used to classify the antimicrobial effects as synergistic, additive, indifference or antagonistic.

2.2 Preparation and Extraction of Cinnamon (*Cinnamomun Zeylanicum*) Bark

About 300 grams of *Cinnamomun zeylanicum* was purchased from the Mile 3 market, Port Harcourt, Nigeria. After sterilization of the blender by autoclaving at 121^{O}C for 15 minutes, allowed to cool, and thereafter used to grind the *Cinnamomun zeylanicum* bark into powder form, 100 grams of the powdered *Cinnamomun zeylanicum* was weighed out and was used for ethanol extraction while another 100 grams was used for the aqueous extraction. The extraction was performed using the Soxhlet extractor at the ratio of 50 mg: to 1mL, that is 2000 mL of ethanol was used for 100grams (100,000mg) of the *Cinnamomun zeylanicum* powder. Furthermore, ethanol available in the extract was removed/ eliminated by the use of rotary evaporator, leaving a sediment of crude extract with a concentration of 50mg/ml. the crude extract was then stored at an appropriate temperature until it was used on the selected clinical isolates. The same procedures and method were used for the aqueous extraction. same volume of solvent and mass of *Cinnamomun zeylanicum* but in place of ethanol, distilled water was used.

2.3 Preparation of Aqueous Alum

Analytical grade alum was purchased from the chemical store, manufactured by Vicker's Laboratories LTD. Bunley in Wharfedale west Yorkshire England. 5000mg was dissolved in 50ml of distilled water to produce an initial concentration of 100 mg /ml. This concentration was further diluted serially. The serially diluted alum with varying concentrations was tested on the selected clinical isolates to ascertain the antimicrobial properties of alum on the selected pathogens.

2.4 Sources of Selected Clinical Microbes

The Microorganism used this study were *S. aureus* = Gram positive, *E. coli O157H:H7* = Gram negative*, C albicans*= fungi) obtained from different site of the body of an infected patient. *S. aureus* isolated from topical wound infection, *E. coli O157H:H7* was isolated from stool sample, and *C albicans* was isolated from oral thrush patent. These clinical isolates were procured from the University of Port Harcourt Teaching

Hospital (UPTH). The Selected Clinical Microbes were cultured on different media where colonial morphology were used to confirm the identity of the microorganisms.

2.5 Culturing of *E. coli O157H:H7*

Escherichia coli serotype O157H:7H was inoculated on sorbitol MacConkey agar prepared accurately according to the manufacturer's s instruction. Sorbitol MacConkey agar used is a selective, differential media which was used for the detection and differentiation of the *Escherichia coli* serotype O157H:7H from other strains of *Escherichia coli*. The media was autoclaved at 121^{O}C for 15 minutes, and was poured on a sterile petri dishes, allowed to solidify. The plates were dried and the organisms was inoculated by streak plate method. The inoculated plates were incubated at 37^{O}C for 24 hours. The growth of colorless colonies was an indication of *Escherichia coli* serotype O157H:H7. However, other strains of *Escherichia coli* other than O157H:H7 were pink colonies growth due to the ability to ferment sorbitol. The media inhibits the growth of other Gram-positive bacteria such as *Staphylococcus aureus*.

2.6 Culturing of *Candida albicans*

Sabouraud Dextrose agar was used for the growth and maintenance of the pathogenic fungi C*andida albicans* using the cultural morphology and color. The Sabouraud dextrose agar was prepared according to the manufacturer's instructions, incorporated in the agar was 50mg of chloramphenicol and 5mg of gentamicin to inhibit bacteria growth. The media was autoclaved at 121^{O}C for 15 minutes and was poured on a sterile petri dishes then allowed to cool at room temperature. The organisms were inoculated by streak plate method and was incubated for 24 hours at 37^{O}C. the result showed creamy pasty colonies with good growth for *Candida albicans*, and there was no growth for *Escherichia coli* and *Staphylococcus aureus*. Other morphological and biochemical investigations was performed to confirmed the identity of the fungi.

2.7 Determination of Minimal Inhibitory Concentration

Mueller Hinton broth was prepared according to the manufacturer's instruction, it was autoclaved at 121^{O}C for 15 minutes, and 0.5ml each of the sterilized broth was pipette into different test tubes. Exactly 0.5ml of the antimicrobial agent was added to the broth in the first test tube, the pipette was discarded, with a fresh pipette, the content of the first test tube was mixed thoroughly and 0.5ml was transferred into the second test tube containing 0.5ml of the sterile broth, again the pipette was discarded and a fresh pipette was used to mixed the content of the second test tube, and 0.5 ml was transferred to the third test tube; the dilution process was continued throughout the test tube number. However, the antimicrobial agent was not added to the test tubes labelled positive growth control and the sterility control. After the content of the last test tube was mixed, 0.5 ml was removed from the tube and was discarded. So that the content of all the test tube be uniformed, that is, 0.5ml in all test tubes.

From a plate culture of the clinical isolates a suspension of the organism was prepared in 5ml of normal saline which is equivalent to 0.5 MarFarland standard. With the aid of a sterile pipette 0.1ml of the microbe in broth was transferred to 9.9ml of normal saline. With a fresh pipette the content of the test tube was mixed, and 0.1ml of the inoculum were transferred to the test tubes containing the antibiotics, and to the test tube labelled growth control; however, none was added to the test tube labelled sterility control. The test tubes were mixed by gently shaking the test tubes. The tubes were incubated at 37^{O}C for a duration of 1 8-24 hours. After the said duration elapsed, the tubes with the lowest concentration of the microbial agent with no visible growth was considered. And the concentration of the tube was reported as the minimal inhibitory concentration of the antimicrobial agent, as there was visible growth on the positive growth control tube and no growth on the sterility control tube. The various concentrations of antimicrobial agents used were Cinnamon extracts (50, 25, 12.5, 6.25 and 3.13mg/ml), Alum (100, 50, 25, 12.5, 6.25 and 3.13mg/ml), Gentamicin (0.25, 0.13, 0.06 and 0.03mg/ml), Penicillin G (1.50, 0.75, 0.37 and 0.19) and fluconazole (0.2, 0.1, 0.05, 0.025 and 0.013mg/ml).

2.8 Determination of Minimal Bactericidal Concentration

The minimal lethal concentration was determined by the transfer of sample from the tubes with no visible growth from the test tube of the minimal inhibitory concentration test onto a sterile agar

medium on Petri dishes by streak plate method, and thereafter incubated for 24- 48 hours at $37^{O}C$. the lowest concentration or the highest dilution of antimicrobial agent that do not show any visible growth on the agar plate was considered the minimal bactericidal concentration.

2.9 Evaluation of Interaction Between the Antimicrobials

This checkerboard assay was used to evaluate the interactions between cinnamon extracts, antibiotics, and alum. The procedure was performed according to the methods conducted by Moody et al., 2003 with modifications AL ALK et al., 2019. Briefly, six concentrations of alum, and five concentrations of each cinnamon extracts were prepared in a sterilized tube by serial dilutions. Subsequently, Alum at decreasing concentrations going from 100mg/ml through 3.13mg/ml were added introduced horizontally into the tubes. In the same manner the cinnamon extracts at decreasing concentrations starting from 50mg/ml through 3.13mg/ml were introduced vertically. The final volume in each tube was 8ml, comprising of 1ml of each alum and cinnamon extracts, as well as 6ml of Mueller Hinton (MH) media consisting of 10^{6}CFU/ml of the selected clinical microbe suspension. The tubes were incubated at $37^{O}C$ for 24 hours. The analysis of the consortiums were obtained by calculating the FICI (AL ALK et al. 2019); [5]. The same methods and procedures were repeated by combining Alum or Cinnamon extracts with different concentrations of Gentamicin(0.25, 0.13, 0.06, and 0.03mg/ml), Penicillin G (1.50, 0.75, 0.37, 0.19) and fluconazole (0.2, 0.1, 0.05, 0.025, 0.013mg/ml), against *S. aureus*, *E. coli O157H:H7, C albicans.* The fractional inhibitory concentration was calculated using the following formula.

$$FICI = FIC_A + FIC_B$$

$$= A/MIC_A + B/MIC_B$$

Where A and B are the minimal inhibitory concentration of antimicrobial A and B combined in the same tube, whereas MIC_A and MIC_B were the minimal inhibitory concentration of each of the two antimicrobial agents respectively. The value of the fractional inhibitory concentration index was used to classify the interaction between the two antimicrobial agents combined together using the following statements as guideline [14].

1. The two antimicrobial agents have a synergistic effect when the fractional inhibitory concentration index is less than 0.5 (FICI≤0.5)
2. When the value of the fractional inhibitory concentration index is between 0.5 and 1, the two antimicrobial agents have an additive effect.
3. The two antimicrobial agents have an indifferent effect when the fractional inhibitory concentration index is greater than 1 and less than 4 (1<FICI>4)
4. The combination of two antimicrobial agents was considered antagonistic when the fractional inhibitory concentration index is greater than 4 (FICI ≥ 4

3. RESULTS

3.1 Minimum Inhibitory Concentration MIC

The MIC values of the crude cinnamon extracts for *S. aureus* were 25mg/ml, 0.25mg/ml for gentamicin, 25mg/ml for Aqueous Alum and 0.19mg/ml for Penicillin G. The lowest MIC value was with Penicillin G against *S. aureus* (0.19mg/ml), and the highest MIC value for *S. aureus* were 25mg/ml with cinnamon extracts and Aqueous Alum. For *E. coli O157H:H7,* the MIC values ranged from 0.25 to 25mg/ml for all the antimicrobial agents used, with the lowest MIC value of 0.25mg/ml for gentamicin and the highest MIC value of 25mg/ml with Aqueous Alum and cinnamon extracts. The recorded MIC value of the antimicrobial agents against *Candida albicans* was between 0.05 and 25mg/ml, Fluconazole showed the lowest MIC value of 0.05mg/ml and the highest was 12.5mg/ml with Aqueous Alum for the uncombined antimicrobial agents. The combinations of these antimicrobial agents resulted in a lower MIC values when compared to the individual antimicrobial agents.

3.2 Combinations of the Various Antimicrobials

The result of the various combinations with exact concentrations, showed a reduction in MICs and MBCs values compared to individual antimicrobial agents. The MIC of Gentamicin against *S. aureus* and *E. coli O157H:H7* was 0.25mg/ml, but in combination with Penicillin G decreased to 0.08mg/ml against *E. coli O157H:H7*, and 0.13mg/ml against *S. aureus* Table 4. The MIC of Gentamicin against *S.*

aureus was 0.25mg/ml, but in combination with aqueous cinnamon extracts decreased to 0.08mg/ml. The MIC of Aqueous cinnamon extracts alone were 25mg/ml against *S. aureus* and *E. coli O157H:H7*, but in combination with Gentamicin reduced to 12.5mg/ml Table 6. The MIC of ethanolic cinnamon extracts alone were 12mg/ml against *C. albicans* and *E. coli O157H:H7*, but in combination with Aqueous Alum reduced to 6.25mg/ml against *C. albicans* table 8. The various alterations in MICs values as a result of different consortiums of cinnamon extracts, antibiotics and alum against *S. aureus, E. coli O157H:H7* and *C. albicans* are shown in Table 4-8.

	100	**50**	**25**	**12.5**	**6.25**	**3.13**	**Alum mg/ml**
50	—	—	—	—	—	—	
25	—	—	—	—	—	—	
12.5	—	—	—	—	—	—	MIC of cinnamon
6.25	—	—	—	—	+	+	+
3.13	—	—	—	+	+	+	+
Cinnamon extracts mg/ml			MIC of alum				

Fig. 1. Evaluation of the interactions between different concentrations of ethanolic cinnamon extracts and aqueous alum against *Candida albicans* using the checker board assay. * + = microbial growth, — = no microbial growth, = positive control, = sterility control.

Table 1. MIC and MBC values of different concentrations of aqueous and ethanolic *Cinnamomun zeylanicum* extracts on *S. aureus, E. coli O157H:H7*, and *C. albicans*

Antimicrobial Agent	**Organisms**	**MIC and MBC values mg/ml**	
		MIC	MBC
Aqueous Extract	*S. aureus*	25	50
	E. coli O157H:H7	12.5	25
	C. albicans	12.5	12.5
Ethanolic Extract	*S. aureus*	25	50
	E. coli O157H:H7	12.5	25
	C. albicans	12.5	12.5

Table 2. MIC and MBC values of different concentrations of gentamicin, penicillin G, and fluconazole Against *S. aureus, E. coli O157H:H7*, and *C. albicans*

Antimicrobial Agent	**Organisms**	**MIC and MBC values mg/ml**	
		MIC	**MBC**
Gentamicin	*S. aureus*	0.25	0.5
	E. coli O157H:H7	0.25	0.5
Penicillin G	*S. aureus*	0.19	0.37
	E. coli O157H:H7	0.75	1.5
Fluconazole	*C. albicans*	0.05	0.1

Table 3. MIC and MBC values of different concentrations of Alum on *S. aureus*, *E. coli O157H:H7*, and *C. albicans*

antimicrobial agent	**Organisms**	**MIC and MBC values mg/ml**	
		MIC	MBC
Alum	*S. aureus*	25	50
	E. coli O157H:H7	25	50
	C. albicans	25	12.5

Table 4. MIC and MBC values of different combinations and concentrations of gentamicin, penicillin G, and fluconazole on *S. aureus, E. coli O157H:H7,* and *C. albicans*

Antimicrobial Agent	Organisms	MIC and MBC values mg/ml	
		MIC	MBC
Gentamicin	*S. aureus*	0.13+0.75	0.25+1.15
+	*E. coli O157H:H7*	0.08+0.37	0.13+0.75
Penicillin G	*C. albicans*	-	-
Gentamicin	S. aureus	0.13+0.10	0.25+0.20
+	E. coli O157H:H7	0.13+0.10	0.25+0.20
Fluconazole	*C. albicans*	0.08+0.05	0.13+0.10
Penicillin G	*S. aureus*	0.75+0.10	1.50+0.20
+	*E. coli O157H:H7*	0.75+0.10	1.50+0.20
Fluconazole	*C. albicans*	0.37+0.05	0.75+0.10

Table 5. MIC and MBC values of different combinations and concentrations of gentamicin, penicillin G, fluconazole and aqueous Alum on *S. aureus, E. coli O157H:H7*, and *C. albicans*

Antimicrobial Agent	Organisms	MIC and MBC values mg/ml	
		MIC	**MBC**
Gentamicin	*S. aureus*	0.08+25	0.13+50
+	*E. coli O157H:H7*	0.08+25	0.13+50
Alum	*C. albicans*	0.13+50	0.25+100
Penicillin G	*S. aureus*	0.37+25	0.75+50
+	*E. coli O157H:H7*	0.37+25	0.75+50
Alum	*C. albicans*	0.75+50	1.50+100
Fluconazole	*S. aureus*	0.10+50	0.20+100
+	*E. coli O157H:H7*	0.10+50	0.20+100
Alum	*C. albicans*	0.05+25	0.10+50

Table 6. MIC and MBC values of different combinations and concentrations of gentamicin, penicillin G, fluconazole and aqueous extract of *Cinnamomun zeylanicum* on *S. aureus, E. coli O157H:H7*, and *C. albicans*

Antimicrobial Agent	Organisms	MIC and MBC values mg/ml	
		MIC	**MBC**
Gentamicin	*S. aureus*	0.08+12.5	0.13+25
+	*E. coli O157H:H7*	0.08+12.5	0.13+25
Aqueous Cinnamon Extract	*C. albicans*	0.13+25	0.25+50
Penicillin G	*S. aureus*	0.19+6.25	0.37+12.5
+	*E. coli O157H:H7*	0.37+12.5	0.75+25
Aqueous Cinnamon Extract	*C. albicans*	0.75+25	1.50+50
Fluconazole	*S. aureus*	0.75+25	1.50+50
+	*E. coli O157H:H7*	0.75+25	1.50+50
Aqueous Cinnamon Extract	*C. albicans*	0.37+12.5	0.75+25

3.3 Minimum Bactericidal Concentration (mg/ml)

The highest dilution of antimicrobial agent that yielded no antimicrobial growth as evidence of absent turbidity on the broth. The range of MBC in this investigation was 0.1 to 50mg/ml for all the antimicrobial agents conducted alone. The lowest MBC value of 0.1mg/ml was for Fluconazole against *C. albicans*, and the highest (50mg/ml) for *S. aureus* with cinnamon extracts.

3.4 Fractional Inhibitory Concentration Index (FICI)

Fractional inhibitory concentration index is the sum of the fractional inhibitory concentration FICs of each drug tested when used in combination. FIC is calculated by dividing each

drug's MIC in combination by each drug's MIC when used alone. The fractional inhibitory concentration index showed a ranged of 0.56 to 7.9. gentamicin + penicillin G showed a FICI of 0.56 as the lowest with *E. coli O157H:H7*, and the highest FICI was 7.9 with a combination of Fluconazole + aqueous cinnamon extract against *C. albicans*. The result for this present study showed additive effect (0.5<FICI>1) Indifferent effect(1<FICI>4), and antagonistic effect (FICI≥4). This present study has also shown that combination of cinnamon extracts antibiotics (gentamicin, and penicillin G) and Aqueous Alum resulted in an additive, indifferent and antagonist effects against *S. aureus, E. coli O157H:H7,* and *C. albicans.*

4. DISCUSSIONS

The MIC and the MBC values of the aqueous and ethanolic cinnamon extracts was greater against *S. aureus* 25mg/ml and 50mg/ml

Table 7. MIC and MBC values of different combinations and concentrations of gentamicin, penicillin G, fluconazole and ethanolic extract of Cinnamomun zeylanicum on *S. aureus, E. coli O157H:H7*, and *C. albicans*

Antimicrobial Agent	Organisms	MIC and MBC values mg/ml	
		MIC	MBC
Gentamicin	*S. aureus*	0.06+12.5	0.13+25
+	*E. coli O157H:H7*	0.06+12.5	0.13+25
Ethanolic Cinnamon Extract	*C. albicans*	0.13+25	0.25+50
Penicillin G	*S. aureus*	0.19+6.25	0.37+12.5
+	*E. coli O157H:H7*	0.37+12.5	0.75+25
Ethanolic Cinnamon Extract	*C. albicans*	0.75+25	0.20+50
Fluconazole	*S. aureus*	0.10+25	0.20+50
+	*E. coli O157H:H7*	0.05+12	0.10+25
Ethanolic Cinnamon Extract	*C. albicans*	0.03+6.25	0.05+12.5

Table 8. MIC and MBC values of different combinations and concentrations of aqueous alum, aqueous and ethanolic extract of *Cinnamomun zeylanicum* on *S. aureus, E. coli O157H:H7,* and *C. albicans*

Antimicrobial Agent	Organisms	MIC and MBC values mg/ml	
		MIC	MBC
Ethanolic Cinnamon Extract	*S. aureus*	25+50	50+100
+	*E. coli O157H:H7*	12.5+25	25+50
Alum	*C. albicans*	6.25+12.5	12.5+25
Aqueous Cinnamon Extract	*S. aureus*	25+50	50+100
+	*E. coli O157H:H7*	12.5+25	25+50
Alum	*C. albicans*	6.25+12.5	12.5+25

Table 9. Fractional inhibitory concentration index (FICI) of various combinations of gentamicin, penicillin and fluconazole against S. aureus, *E. coli O157H:H7,* and *C. albicans*

Antimicrobial Agent	Organisms	FIC_A	FIC_B	FICI	S, A, I, AN
Gentamicin	*S. aureus*	0.52	0.5	1.02	I
+	*E. coli O157H:H7*	0.3	0.2	0.56	A
Penicillin G	*C. albicans*	-	-	-	NT
Gentamicin	*S. aureus*	0.52	-	0.52	NT
+	*E. coli O157H:H7*	0.52	-	0.52	NT
Fluconazole	*C. albicans*	-	1	1.00	NT
Penicillin G	*S. aureus*	0.5	-	0.5	NT
+	*E. coli O157H:H7*	1	-	1.0	NT
Fluconazole	*C. albicans*	-	1	1.0	NT

Key: FIC_A = Fractional inhibitory concentration of drug A, FIC_B= Fractional inhibitory concentration of drug B, A=Additive, S=Synergistic, I = indifference, AN = antagonistic, FICI= Fractional inhibitory concentration index

respectively, and the lowest was with C. albicans at 12.5mg/ml, when the same concentrations of antimicrobial agents were applied. Aqueous and ethanolic cinnamon extracts were more active against *C. albicans* with lower MIC value than *S. aureus.* different organisms have different MIC value when cinnamon was used as an antimicrobial agent. This is in accordance with EL Atki et al. [8] which showed that the cinnamon essential oil (EO) resulted to a lower MIC values ranging from 19.53ug/ml to 4.4ug/ml. Also, Raeisi et al. 2015 showed that the MIC value of cinnamon essential oil against *E. coli and S aureus was* 2500ug/ml.

Table 10. Fractional inhibitory concentration index (FICI) of various combinations of gentamicin, penicillin, fluconazole and ethanolic cinnamon extract against *S. aureus*, *E. coli O157H:H7,* and *C. albicans*

Antimicrobial Agent	Organisms	FIC_A	FIC_B	FICI	A, S, I, AN
Gentamicin +	*S. aureus*	0.24	0.48	0.72	A
Ethanolic Cinnamon	*E. coli O157H:H7*	0.24	0.96	1.2	I
Extract	*C. albicans*	-	1	1.0	NT
Penicillin G +	*S. aureus*	1	0.25	1.25	I
Ethanolic Cinnamon	*E. coli O157H:H7*	0.493333	0.96	1.453333	I
Extract	*C. albicans*	-	2	2.0	NT
Fluconazole +	*S. aureus*	-	1	1.0	NT
Ethanolic Cinnamon	*E. coli O157H:H7*	-	0.96	0.96	NT
Extract	*C. albicans*	0.6	0.5	1.1	I

Key: FIC_A = Fractional inhibitory concentration of drug A, FIC_B= Fractional inhibitory concentration of drug B, A=Additive, S=Synergistic, I = indifference, AN = antagonistic, FICI= Fractional inhibitory concentration index

Table 11. Fractional inhibitory concentration index (FICI) of various combinations of gentamicin, penicillin, fluconazole and Aqueous Alum against S. aureus, *E. coli O157H:H7,* and *C. albicans*

Antimicrobial Agent	Organisms	FIC_A	FIC_B	FICI	S, A, I
Gentamicin +	*S. aureus*	0.32	0.5	0.82	A
Alum	*E. coli O157H:H7*	0.32	0.5	0.82	A
	C. albicans	-	2	2.13	NT
Penicillin g +	*S. aureus*	1.947368	0.5	2.44	I
Alum	*E. coli O157H:H7*	0.493333	0.5	0.99	A
	C. albicans	-	2	2.0	NT
Fluconazole+	*S. aureus*	-	1	1.1	NT
Alum	*E. coli O157H:H7*	-	1	1.1	NT
	C. albicans	0.05	1	1.05	I

Key: FIC_A = Fractional inhibitory concentration of drug A, FIC_B= Fractional inhibitory concentration of drug B, A=Additive, S=Synergistic, I = indifference, AN = antagonistic, FICI= Fractional inhibitory concentration index

Table 12. Fractional inhibitory concentration index (FICI) of various combinations of gentamicin, penicillin, fluconazole and Aqueous cinnamon extract against S. aureus, *E. coli O157H:H7,* and *C. albicans*

Antimicrobial Agent	Organisms	FIC_A	FIC_B	FICI	S, A, IAN
Gentamicin +	*S. aureus*	0.32	0.5	0.82	A
Aq. Cinnamon Extract	*E. coli O157H:H7*	0.32	1	1.32	I
	C. albicans	-	2	2.0	NT
Penicillin G +	*S. aureus*	1	0.25	1.25	I
Aq Cinnamon Extract	*E. coli O157H:H7*	0.49	1	1.49	I
	C. albicans	-	2	2.0	I
Fluconazole +	*S. aureus*	-	0.5	0.5	NT
Aq. Cinnamon Extract	*E. coli O157H:H7*	-	0.5	0.5	NT
	C. albicans	7.4	0.5	7.9	AN

Key: FIC_A = Fractional inhibitory concentration of drug A, FIC_B= Fractional inhibitory concentration of drug B, A=Additive, S=Synergistic, I = indifference, AN = antagonistic, FICI= Fractional inhibitory concentration index

Table 13. Fractional inhibitory concentration index (FICI) of various combinations of ethanolic cinnamon extract, Alum, and Aqueous cinnamon extract against S. aureus, *E. coli O157H:H7,* and *C. albicans*

Antimicrobial Agent	Organisms	FIC_A	FIC_B	FICI	S, A, I
AQ. Cinnamon Extract	*S. aureus*	1	1	2	I
+	*E.coli O157H:H7*	1	0.5	1.5	I
Alum	*C. albicans*	0.5	0.5	1	A
Eth. Cinnamon Extract	*S. aureus*	1	1	2	I
+	*E.coli O157H:H7*	1	0.5	1.5	I
Alum	*C. albicans*	0.5	0.5	1	A

Key: FIC_A = Fractional inhibitory concentration of drug A, FIC_B= Fractional inhibitory concentration of drug B, A=Additive, S=Synergistic, I = indifference, AN = antagonistic, FICI= Fractional inhibitory concentration index

when gentamicin was tested *on S. aureus* and *E. coli O157H:H7* it resulted to an MIC and MBC values of 0.25mg/ml and 0.50mg/ml respectively. While Penicillin G against *S. aureus* and *E. coli O157H:H7* resulted to an MICs of 0.19mg/ml and 0.75mg/ml and MBCs of 0.37mg/ml and 1.50mg/ml respectively. Fluconazole against *C. albicans* resulted to an MIC and MBC of 0.05mg/ml and 0.1ng/ml respectively. Hence the study conducted by Jaccobs et al., 1998; Dilnessa, 2019; Glajzner et al., 2022 showed that the MIC and methicillin susceptible *Staphylococcus aureus* are between 0.06-128ug/ml, and the MIC of methicillin resistant Staphylococcus aureus for penicillin G is between 32-128ug/ml. The MIC of gentamicin methicillin susceptible *Staphylococcus aureus* are between 0.06-16ug/ml, and between 0.06-64ug/ml for methicillin resistant *Staphylococcus aureus*. susceptible *S. aureus* to penicillin G, also means that it is susceptible to other beta lactam antibiotics. Furthermore, a study conducted by Gomes et al.,2019 showed an MIC value between 0.4 -24ug/ml for penicillin G against all strains of *S. aureus*. A study conducted by sabee et al. 2020 showed that the MIC of gentamicin against *S. aureus* is 0.02mg/ml, same MIC with *E. coli*. Landman et al. [15] also showed that MIC(90) of gentamicin was 32mg/L against most isolates of *E coli.*

Aqueous Alum was active against the three selected clinical isolates used the MICs of aqueous alum against *S. aureus* and *E. coli O157H:H7* and *C. albicans were* 25mg/ml, 25mg/ml and 12.5mg/ml respectively, and the MBCs aqueous alum against *S. aureus* and *E. coli O157H:H7* and *C. albicans* were 50mg/ml, 50mg/ml and 25mg/ml respectively this showed that the MIC and MBC of alum was low against *C. albicans* compared to *S. aureus*, this is in accordance with a study conducted by *Ali , 2018* revealed that the MIC and MBC of Aqueous Alum against *S. aureus* was 20%, the MIC of alum against *E. coli* was 10% while the MBC was 20%. The same study by Ali, [5]; Lee et al. [16], also revealed that the MIC and MBC of aqueous Alum against *C. albicans* was 10%. The MIC of alum against *E. coli* was reported to be 10mg/ml [17]. Ahmed, 2011 Showed that Alum has different MIC value for different pathogens, and that the MIC value of alum against Proteus mirabilis was 0.8g//ml.

4.1 Interactions Between the Antimicrobials

The combination of penicillin G, gentamicin, and fluconazole resulted in the additive and indifferent effects with a FICI ranging from 0.56 to 1.02, lowest FICI of 0.56 was with gentamicin and penicillin G against *E. coli O157H:H7* and the highest FICI was 1.37 with the combination of penicillin G and gentamicin against *E. coli O157H:H7.*

For the combination of ethanolic cinnamon extracts with gentamicin, penicillin G and Fluconazole, there was an additive and indifferent effects against the tested clinical isolates with a FICI ranging from 0.72 to 1.45, the lowest FICI of 0.72 obtained from the combination of gentamicin + ethanolic cinnamon extracts against *S aureus,* and the highest FICI of 1.45 obtained from the combination of Penicillin G + Ethanolic cinnamon extracts against *E. coli O157H:H7*. Reports from Utchariyakiat et al. [18], Yap et al. [19] & Mahadlet et al. [20], showed that combinations of *Cinnamomum zeylanicum* and antibiotics expresses additive and synergistic effects against a variety of microorganisms. This is in confirmation with the study conducted by Guerra et al. 2012, that a combination of *Cinnamomun zeylanicum* with antibiotics shows an additive effect.

The combinations aqueous Alum Gentamicin Penicillin G and fluconazole tested on the selected clinical isolates resulted to both additive and indifferent effects with a FICI between 0.82 to 2.44. fluconazole + aqueous Alum resulted in an indifferent effect against *C. albicans w*ith a FICI of 1.05 while the combination of gentamicin + aqueous Alum and combinations of Penicillin G + aqueous Alum produced both additive and indifferent effects against *S. aureus, E. coli O157H:H7..* For the combination aqueous cinnamon extracts with Gentamicin, Penicillin G, and fluconazole against *S. aureus, E. coli O157H:H7,* and *C. albicans* produced an additive, indifferent, and antagonistic effects with a FICI ranging from 0.82 to 7.9. The combination of fluconazole + aqueous cinnamon extracts resulted to an antagonistic effect against *C. albicans* with FICI of 7.9. Furthermore, the combinations aqueous Alum, with Ethanolic Aqueous cinnamon extracts produced an additive and indifferent effects against the three tested clinical isolates used in this study, with a FICI ranging from 1 to 2. The combinations produced an additive effect against *C. albicans* and indifferent effects against *S. aureus* and *E. coli O157H:H7*. EL Atki et al. [8] showed that the combination of cinnamon essential oil (EO) with streptomycin resulted in an additive effect against strains of *E coli* and *S. aureus* with FICI of 1.0 to 0.75 respectively. The same study also revealed that the coalescence effects of cinnamon EO+Ampicillin resulted in an indifferent effect against *E. coli* with a FICI of 1.2. this showed that the combination of cinnamon extracts and different conventional antibiotics produced different effects ranging from synergistic to indifferent effects depending on the organism and antibiotics used. Mahadlek et al. [20], also showed that the combination of cinnamon EO + Ciprofloxacin resulted to an additive effect against S. aureus using the checkerboard assay. Furthermore, Lu et al*.* [21]; Utchariyakiat et al. [18] revealed that the combination of cinnamon EO + Clove displayed both additive and indifferent effects against a variety of bacteria; both clinical and foodborne bacteria.

5. CONCLUSION

This research study showed that all the antimicrobial agents used were effective against the selected clinical isolates. The combination of these antimicrobial agents showed a reduction in the minimal inhibitory concentration against the tested microorganisms. The various combinations showed additive, indifferent and antagonistic effects when analyzed using the checkerboard assay.

The various antimicrobial combinations can be used as alternative therapeutic medicine for topical and internal applications for the treatment of infections caused by *S. aureus, E coli O157H:H7, and Candida albicans.* These combinations could reduce the minimal effective dose of the antimicrobial agents, thereby decreasing their potential side effect and treatment cost.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. AL-Khikani F, Zaraa D, Abbas H, Musa H, Dahir H, Alhusayni A. Evaluation of the antibacterial activity of potassium aluminium sulphate (Alum) combined with other antibiotics. Microbes and infectious diseases; 2023.
Available:https:doi.org/10.21608/mid.2023.206322.1514
2. Tosun M, Taylan G, Demirel Zorba N. Antibacterial and antibiofilm activities of some plant essential oils and synergistic effects of cinnamon essential oil with vancomycin against Clostridioides difficile: *in vitro* study. Letters In Applied Microbiology. 2022;75(3):598-606.
Available:https://doi.org/10.1111/lam.13747
3. Becerril R, Nerín C, Gómez-Lus R. Evaluation of bacterial resistance to essential oils and antibiotics After Exposure to Oregano and Cinnamon Essential Oils. Foodborne Pathogens and Disease. 2012;9(8):699-705.
Available:https://doi.org/10.1089/fpd.2011.1097.
4. Miethke M, Pieroni M, Weber T, Brönstrup M, Hammann P, Halby L, Arimondo PB, Glaser P, Aigle B, Bode HB, Moreira R, Li Y, Luzhetskyy A, Medema MH, Pernodet J-L, Stadler M, Tormo JR, Genilloud O, Truman AW, Müller R. Towards the sustainable discovery and development of new antibiotics. Nature Reviews Chemistry. 2021;5(10):726–749.

Available:https://doi.org/10.1038/s41570-021-00313-1

5. Ali ZM. Synergistic antibacterial interaction between an alum and antibiotics on some microorganism. Scientific Journal of Medical Research. 2018;02(05):47–51.
Available:https://doi.org/10.37623/sjmr.2018.2510
6. Ravindran P, Shalaja M, Nirmal Babu K, Krisnamoorthy B. Botany and crop improvement of cinnamon cassia in Ravindran. CRC Press Boca Raton Fl, USA; 2004.
7. Nabavi S, Di Lorenzo A, Izadi M, Sobarzo-Sánchez E, Daglia M, Nabavi S. Antibacterial Effects of Cinnamon: From Farm to Food, Cosmetic and Pharmaceutical Industries; 2022.
Accessed on: 10 October 2022.
8. El Atki Y, Aouam I, El Kamari F, Taroq A, Nayme K, Timinouni M. Antibacterial activity of cinnamon essential oils and their synergistic potential with antibiotics. Journal Of Advanced Pharmaceutical Technology &Amp; Research. 2019;10(2), 63.
Available:https://doi.org/10.4103/japtr.japtr_366_18
9. Cava-Roda R, Taboada-Rodríguez A, López-Gómez A, Martínez-Hernández G, Marín-Iniesta F. Synergistic Antimicrobial Activities of Combinations of Vanillin and Essential Oils of Cinnamon Bark, Cinnamon Leaves, and Cloves. Foods. 2021;10(6):1406.
Available:https://doi.org/10.3390/foods10061406
10. Fadli M, Saad A, Sayadi S, Chevaier J, Mezrioui NE, Pages JM, Hassani L. antibacterial activity of thymus maroccanus and thymus broussonetii essential oil against nosocomial infection- bacteria and their synergistic potential with antibiotics. Phytomedicine: International Journal of Phytotherapy and Phytopharmacology. 2012;19(5):464-471.
Available:https://doi.org/10.1016/j.phymed.2011.12.003
11. Amadi LO. A Review of Antimicrobial Properties of Alum and Sundry Applications. Acta Scientific Microbiology. 2020;3(4):109-117.
Available:https://doi.org/10.31080/asmi.2020.03.0553
12. Amadi LO, Wanabia D, Amadi V. Synergistic effects of alum and guava (*Psidium guajava*) leaf extracts on some pathogens from clinical samples. International Journal of Current Research. 2016;8(05):31354-31358.
13. Bnyan IA, Alte'ee AH, Kadhum NH. Antibacterial Activity of Aluminium potassium sulphate and Syzgium aromaticum extracts against pathogenic microorganisms. Journal Natural Sciences Research, 2014;4(15):11-14.
14. Wendakoon CN, Sakaguchi M. Inhibition of Amino Acid Decaboxylase activity of Enterobacter aerogenes by active components in spices. Journal of food protection. 1995;58(3):280-283.
Available:https://doi.org/10.4315/0362-028x-58.3.280
15. Landman D, Babu E, Shah N, Kelly P, Backer m, Bratu S, Quale J. Activity of a novel aminoglycoside, ACHN-490, against clinical isolates of *Escherichia coli* and Klebsiella pneumoniae from New York City', Journal of Antimicrobial Chemotherapy. 2010;65(10):2123–2127.
DOI: 10.1093/jac/dkq278
16. Lee JH, Lee S-I, Chung CJ, Lee JH, Lee SC, Choi SR, Oh JN, Bae JY. The synergistic effect of gentamicin and clindamycin on rocuronium-induced neuromuscular blockade. Korean Journal of Anesthesiology. 2013;64(2):143.
Available:https://doi.org/10.4097/kjae.2013.64.2.143
17. Shahriari R, Salari S, Shahriari S. In Vitro Study Of Concentration-Effect And Time-Course Pattern Of White Alum On Escherichia Coli O157:H7 Growth. African Journal of Traditional, Complementary and Alternative Medicines. 2017;14(2):311-318.
Available:https://doi.org/10.21010/ajtcam.v14i2.32
18. Utchariyakiat I, Surassmo S, Jaturanpinyo M, Khuntayaporn P, Chomnawang MT. Efficacy of cinnamon bark oil and cinnamaldehyde on anti-multidrug resistant pseudomonas aeruginosa and the synergistic effects in combination with other antimicrobial agents. BMC Complementary and Alternative Medicine. 2016;16(1).
Available:https://doi.org/10.1186/s12906-016-1134-9
19. Yap PS, Lim SH, Hu CP, Yiap BC. Combination of essential oils and

antibiotics reduce antibiotic resistance in plasmid-conferred multidrug resistant bacteria. Phytomedicine. 2013;20(8–9):710–713.
Available:https://doi.org/10.1016/j.phymed.2013.02.013

20. Mahadlek J, Charoenteeraboon J, Phaechamud T. Combination effects of the antimicrobial agents and Cinnamon Oil. Advanced Materials Research. 2012;506:246–249.
Available:https://doi.org/10.4028/www.scientific.net/amr.506.246

21. Lu F, Ding YC, Ye XQ, Ding YT. Antibacterial effects of cinnamon oil combined with thyme or clove oil. Agricultural Science China. 2011;10:1482-7.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/114928