



Full Length Research Paper

ANTIBACTERIAL ACTIVITY OF CINNAMON AND CLOVE OIL ON DIFFERENT FOOD BORNE BACTERIAL ISOLATES

Y.O. Adesiji^{1*}, O.A.T. Alli², A.A. Shittu² and A.S. Oluremi²

¹Department of Medical Microbiology and Parasitology, College of Health Sciences, Ladoke Akintola University of Technology, Osogbo, Osun state, Nigeria.

²Department of Biomedical Sciences College of Health Sciences, Ladoke Akintola University of Technology, Osogbo, Osun state, Nigeria.

*Correspondence: drmrsadesiji@yahoo.com, yoadesiji@lautech.edu.ng
Tel: +2348032948270

ABSTRACT

The prevalence of antimicrobial resistance among food-borne pathogens has increased during recent decades. This has led to the growing concern about food safety that recently resulting into the use of natural antimicrobials to control food borne pathogens and spoilage bacteria. This study was aimed at assessing the *in vitro* antibacterial potentials of the cinnamon and clove oil against the leading food borne pathogenic bacteria (*E. coli* 0157:H7, *Salmonella spp* and *Arcobacter spp*). The antimicrobial susceptibility testing of 14 bacteria isolates, containing 10 *Escherichia coli* isolates, 2 *Salmonella* and 2 *Arcobacter* from meat in Osogbo was investigated using disc diffusion method, minimum inhibitory concentration (MIC) and maximum bactericidal concentration (MBC) using cinnamon and clove oil as antibacterial agents. Screening of cinnamon and clove extracts showed varying antimicrobial activity against the test organisms. Cinnamon oil exhibits bacterial activity with MIC at dilution $\geq 1/256$ equivalent to ≥ 0.004 % and MBC at dilution $\geq 1/8$ equivalent to ≥ 0.125 % while the result of antimicrobial activity of Clove oil exhibits MIC at dilution $\geq 1/32$ equivalent to ≥ 0.1 $\mu\text{g/ml}$ and MBC at dilution $\geq 1/8$ equivalent to ≥ 0.3 $\mu\text{g/ml}$. This study provided the evidence that both the cinnamon and Clove oil could be useful as an important natural alternative to control some food borne pathogens.

Keywords: Food borne pathogens, Antibacterial activity, MIC, MBC, Essential oil

INTRODUCTION

The growing concern about food safety has recently led to the development of chemical additives to control food borne pathogens and spoilage bacteria (Ali *et al.*, 2005). Essential oils which have been known to show numerous medicinal properties are key in the preservation of food. They have been recognized for their antibacterial, antifungal and antiviral properties (Al Yousef, 2014). Verocytotoxigenic *E. coli*, *Salmonella* species and *Arcobacter* species belong to a group of prioritized, extended list of food and water borne zoonoses. *E. coli* (O157:H7) is responsible for majority of outbreaks of gastrointestinal illness relating to *E. coli* infections. *Salmonella spp* are members of the *Enterobacteriaceae* which are implicated in the worldwide incidence of human non-typhoidal salmonellosis (Kariuki *et al.*, 2006). *Arcobacter* are regarded as one of the key emerging food borne pathogens particularly in developing countries and they have been frequently isolated from various food sources in Nigeria (Adesiji *et al.*, 2011a).

Clove and cinnamon oil are amongst the major essential oils that are known to show a broad spectrum antimicrobial activity (Ismail *et al.*, 2011). They are known to be more efficient in killing bacteria than most other available essential oils (Vani and Lakshmi, 2014). Apart from the ability of essential oils to help in food control, they also help in preventing food borne diseases which drastically increases the cost of health care in developing countries if considering the fact that a food borne illnesses could cost a developed country like United States about 152 billion dollars per year in healthcare, workplace and other economic losses (CDC, 2012).

Cinnamon oil could be extracted or distilled from cinnamon trees which belong to the family *Lauraceae*, with about 250 known species among the genus *Cinnamomum* (Sangal, 2011). The main components of cinnamon oil are cinnamaldehyde and trans-cinnamaldehyde (Cin), which contributes greatly to their biological activities and fragrance (Yeh *et al.*, 2013). Other components cinnamon which have been extracted include; linalool, limonene, α -terpineol, terpinen-4-ol, γ -terpinene, α -terpinene and 1,8-cineole and methyl eugenol (Abdelwahab *et al.*, 2014).

Clove oil is obtained from distillation of the flowers, stems, and leaves of the clove tree (*Eugenia aromatica*) (Safrudin *et al.*, 2015). Amongst other components of the clove oil is the phenylpropene eugenol which is responsible for the distinctive aroma of the clove oil, with eugenol making up about 70-90% of the clove oil (Safrudin *et al.*, 2015). Clove oil has been reported to have various degrees of antimicrobial activity against pathogens (Mishra and Kalyani, 2014).

MATERIALS AND METHODS

Study site: This research was carried out in the month of December- 2014 -February, 2015 at the Department of Biomedical sciences, College of Health sciences, Ladoke Akintola University of Technology, Mercyland campus, Osogbo, Osun state, Nigeria.

Bacterial identification: A total of 14 bacterial isolates of frequently reported food borne pathogens and food spoilage bacteria, including *Escherichia coli* O157: H7, *Salmonella Enteritidis* and *Arcobacter butzleri* were freshly isolated and used in this study. The stock cultures of the test organisms in 30% glycerol-containing medium in cryogenic vials were kept

at 25°C. Working cultures were kept at 4°C on Mueller Hinton agar slant and were periodically transferred to fresh slants.

Antimicrobial study: Preliminary investigation of antibacterial activities of the oils was determined by using paper disk diffusion method to screen for the efficacy of the oils among all samples. Those that exhibited antimicrobial property were later selected for the determination of minimum inhibitory concentration using micro-dilution method. The organism that showed resistance to the oils was also compared with the standard antibiotics. The essential oils were diluted with analytical grade ethanol at the following concentration 1:1, 1:10, 1:20, 1:40 (v/v). A volume of 20 µl of each concentration was respectively, impregnated into the paper disk with 5 mm diameter (Whatmann®), and then placed onto Mueller-Hinton agar (MHA) plates (Oxoid, UK), which were previously inoculated on the surface agar with 20 µl of 10^6 cfu/mL suspension for each tested bacterium. Ethanol was used as a control. Three standard antibiotics, cefoxitin (30 µg), ceftaxidime (30 µg), and Amoxicillin/clavulanate (30 µg), were used as reference controls for the tested bacteria. The plates were then incubated at 37°C for 24h for *Arcobacter butzleri*, *S. Typhimurium* and *E. coli* O157. While Mueller Hinton agar was used to perform sensitivity testing of the two oils, by using disk diffusion method, against bacterial isolates. The antibacterial activity was evaluated by measuring the diameter of inhibitory zones in millimetres using transparent meter rule and the means were expressed as the results of five determinations microbiological Standards and NCCLS guidelines for interpretation for conventional antimicrobials (NCCLS, 2012). The essential oils, which exhibited the best antimicrobial activity in the paper disk diffusion assay, and some individual constituents, were selected for determining the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) using broth dilution method. One colony of each bacterial strain was sampled with a loop, then inoculated in 25 mL BHI broth and incubated for 18–24h at 37°C in order to get a bacterial suspension of 10^9 cfu/mL. Each stock solution was diluted with buffered peptone water (Oxoid) to obtain 10^5 cfu/mL bacterial suspensions. Serial dilutions of essential oils (0.125–5mL/L) were prepared with BHI broth medium in test tube and mixed with bacterial suspensions to give a volume of 4mL and a final concentration of bacteria 10^4 cfu/mL. Final solutions were incubated at the temperature mentioned earlier. The MIC was considered as the lowest concentration that prevented the visible growth. The MBC was determined by subculturing 100mL from each negative test tube onto plate count agar (PCA) plates. MBC was defined as the lowest concentration resulting in a negative subculture or giving presence of only one colony after incubation. The experiments were carried out in four replicates to find the mean.

RESULTS AND DISCUSSION

A total number of 14 bacterial isolates and two different essential oils were used for this work. Antibacterial potential of the two oils were tested against the freshly prepare isolates using standard antibiotic discs of known concentration. Table 1 shows the antibacterial activity of cinnamon oil against the tested food borne pathogens.

Table 1. Antibacterial activity of Cinnamon oil against Food borne bacteria using disc Diffusion method.

	Disc Conc.	1	1/1	1/10	1/20	1/40	Positive control	Negative control
S/N	Org/Diameter	mm	Mm		Mm	mm	Mm	mm
1	EC.1	28	0	25	20	16	FOX-8, AMC-10	0
2	EC.2	26	24	16	14	8	FOX-16, AMC-10	0
3	EC.3	25	15	13	9	5	-	0
4	EC.4	28	24	18	14	11	CAZ- -, AMC-14	0
5	EC.5	30	26	21	18	14	FOX-16	0
6	SL.1	10	8	6	4	2	CAZ--, AMC-6	0
7	ARC.1	14	8	6	4	2	FOX-14, AMC-12	0
8	Control 1	16	10	8	6	4	0	0

KEY: EC- *E. coli* 0157, SL - *Salmonella typhimurium* and ARC- *Arcobacter butzleri*.
 1, 1/1, 1/10, 1/20, 1/40 - Concentration of cinnamon oil in Absolute alcohol (v/v).
 0 – No- antimicrobial activity.
 AMC -Amoxicillin-clavulanate (30µg), FOX – Cefoxitin (30µg) and CAZ - Cefazidime (30µg).

Results showed that at higher concentrations of alcoholic extracts of cinnamon oil, there was a higher level of antibacterial activities. In addition, at highest concentrations of cinnamon oil, moderate antibacterial activity was exhibited by *E. coli* followed by *Arcobacter* and *Salmonella*, respectively. Table 2 shows the antibacterial activity of essential oil of clove against the tested food borne pathogens.

Table 2. Antibacterial activity of Essential oil of clove against Food borne bacteria using disc diffusion method.

S/N	Disc Conc.	1	1/1	1/10	1/20	1/40	Positive control	Negative control
	Org/Diam eter	mm	mm	mm	mm	mm	Mm	mm
9	EC.6	16	12	6	4	2	AMC-26	0
10	EC.7	20	16	10	8	2	CAZ--,AMC-10	0
11	EC.8	16	12	8	6	4	AMC-8	0
12	EC.9	20	16	12	8	6	FOX-16,AMC-10	0
13	EC.10	26	12	10	8	6	FOX-16,CAZ--	0
14	SL.2	30	25	20	16	12	AMC-16,FOX-16	0
15	ARC.2	30	28	20	16	9	FOX-16, AMC- 10	0
16	Control 2	14	10	4	0	0	0	0

KEY : EC- *E. coli* 0157:H7, SL - *Salmonella* species and ARC- *Arcobacter* species.

1, 1/1, 1/10, 1/20, 1/40 - Concentration of clove oil in Absolute alcohol (v/v).

0 – No- antimicrobial activity.

AMC - Amoxicillin-clavulanate (30µg), FOX – Cefoxitin (30µg) and CAZ - Ceftazidime (30µg).

Comparison of cinnamon oil with clove oil revealed that the higher the concentration of the alcoholic clove oil, the higher the antibacterial activity. The essential oil of clove showed the highest level of antibacterial activity against *Arcobacter* and *Salmonella*.

The increase in cases of antibiotic resistance abilities of pathogens as well as the emergence of new food borne pathogens such as *Arcobacter*, has led to the increase in the study of natural edible oils with antimicrobial properties that could help battle these pathogens both *in vivo* and *in vitro*. Results of this study have clearly revealed that clove and cinnamon oils are effective against food borne pathogens. They have the potentials to inhibit the growth of *Arcobacter*, *E. coli* and *Salmonella*. The antibacterial activity of these oil against *Arcobacter* is noteworthy, as to the best of our knowledge few literature has been able to show the effect of essential oils on this organism which has become an emerging food borne pathogen in Nigeria (Adesiji *et al.*, 2011b). The antibacterial ability of these oils have been largely attributed to the presence of cinnamaldehyde (Abdelwahab *et al.*, 2014) and eugenol (Safrudin *et al.*, 2015) in cinnamon oil and clove oil respectively. This is well complemented by the ability of these essential oils to penetrate the membranes of bacteria, leading to their lysis (Vani and Lakshmi, 2014).

A particularly interesting fact in this study is the increase in the effectiveness of the antibacterial abilities of the essential oils as the concentration of alcoholic extract of clove and cinnamon increases. This is similar to reports by Ismail *et al.* (2012) where it was revealed that an increase in the concentrations of alcoholic extracts of clove, cinnamon and thyme showed a remarkable increase in antifungal effect on the tested yeast and mold strains.

Although clove and cinnamon oils showed similar level of antibacterial activity, oil of clove was more effective in curtailing the growth of *Arcobacter*, compared to cinnamon oil which at the same concentration of ethanolic extracts exhibited a lower level of antibacterial activity. This might be due to the fact that the initial concentration of cinnamon oil was not known or reports by Dhamvir *et al.* (2014) which indicated that oil of clove had a generally higher antibacterial ability than cinnamon oil, with this oil generally known to have a broad spectrum antimicrobial activity (Shoaib *et al.*, 2014).

CONCLUSION

The need for further studies on the antimicrobial activity of essential oils against *Arcobacter* cannot be over emphasized, as it is fast becoming a food borne pathogen with great importance in Nigeria. This study showed that Essential oils of clove and cinnamon exhibit antibacterial activity on food borne bacteria and thus could be used as food additive to reduce spoilage.

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