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ANTIBACTERIAL EFFECTS OF KITCHEN SOOT BLENDED WITH SHEA BUTTER AND ANTIBIOTICS

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ABSTRACT

The increasing resistance of microorganisms to conventional antimicrobials necessitate the research for the discovery of newer or alternative antimicrobial agents. Kitchen soot and shea butter are often used by most locals for treatment of various disease conditions, especially skin diseases. Therefore, this study assessed the synergistic antimicrobial effects of kitchen soot, shea butter and antibiotics. Eight different kitchen soot samples were collected from various locations within Samaru campus of the Ahmadu Bello University, Zaria, and transported to the diagnostic bacteriology laboratory, Department of Veterinary Microbiology, Ahmadu Bello University, Zaria. The sterility and purity of the kitchen soot and shea butter were determined in accordance with standard procedures. Soot and soot blended with shea butter concentrations were made by dissolving 1 g of the soot powder in 9 mL of distilled water, and 0.5 g of soot and 0.5 mL of molten shea butter in 9 mL of sterile distilled water to make a concentration of 100 mg/mL, respectively, and thereafter 50 mg/mL, 25 mg/mL, 12.5 mg/mL, and 6.25 mg/mL, respectively. The different concentrations were tested against Citrobaacter freundii, Staphylococcus aureus, Salmonella enterica, Klebsiella pneumoniae, Escherichia coli, Corynebacteriun pseudotuberculosis and Streptococcus pyogenes using agar well diffusion method. Findings revealed that the soot alone or blended with shea butter had no antimicrobial activity against the selected bacteria. However, soot blended with shea butter and antibiotics produced synergistic antibacterial effects against C. pseudotuberculosis, Citrobacter freundii & S. aureus. The active constituent of the soot and shea butter should be established, while further studies to determine the minimum inhibitory concentrations of the different combinations are recommended.

Keywords: Antimicrobials, Bacteria, Kitchen soot, Shea butter, Zaria.

INTRODUCTION

Infectious diseases caused by antimicrobial resistant bacteria are one of the most important problems limiting routine clinical practice worldwide (Tomoo & Yamaguchi, 2009). In Nigeria, information outlining the full burden of antimicrobial resistance and its economic impact on both humans and animals is limited. In situations where multidrug-resistant bacteria have spread widely, options for treatment with conventional antimicrobial agents are restricted, and the number of newer antimicrobials placed in the market is decreasing (Tomoo & Yamaguchi, 2009). This increasing resistance of microorganisms to conventional antibiotics highlights the pressing need for research to discover newer or alternative antimicrobial agents (Jia *et al*., 2005). Pharmaceutical companies and researchers have great interest for new, low cost, less irritant and effective agents for resistant pathogens. Over the past few years, various nanomaterials have been evaluated as antimicrobial agents (Jia *et al*., 2005). Similarly, carbon-based nanoparticles (CNPs) have demonstrated appreciable antimicrobial effects, and can effectively help plants to combat fungal or virus-induced biotic stress (Hao *et al*., 2017; Aloysuis *et al*., 2019). Kitchen soot, is a crude CNP that has been employed as an antimicrobial agent since ancient times, hence it is called "old woman's remedy" (Varghese *et al.,* 2013). Likewise, Shea butter, an oleaginous material obtained from the kernel of the shea nut from shea tree (*Vitellaria paradoxa*) had been used as an edible fat, antimicrobial and moisturizer in the food, pharmaceutical and cosmetic industries among other benefits (Abdul-Mumeen *et al.* 2019). Therefore, this study aimed to evaluate the synergistic antimicrobial effects of kitchen soot blended with shea butter and antibiotics on selected pathogenic bacteria.

MATERIALS AND METHODS

Soot collection, transportation and storage: Eight kitchen soots were collected from different locations within Samaru campus of Ahmadu Bello University (ABU), Zaria by scrapping the soot from kitchen roofs using a sterile scalpel blade and gloves into sterile aluminum foil paper. The collected samples were placed inside sterile envelope sealed with masking tape, then transported to the diagnostic bacteriology laboratory, Department of Veterinary Microbiology, ABU Zaria and stored at room temperature.

Media preparation **:** A calculated amount of dehydrated blood, MacConkey, nutrient and biochemical media were dissolved in required distilled water based on the manufacturer's instructions. The solutions were autoclaved at 121°C for 15 min, then allowed to cool to about 45-50°C before pouring into petri dishes and test tubes.

Soot and shea butter purification The soot was purified as per method reported by Aloysuis *et al*. (2019) with little modification. Briefly; 25 g of each collected soot sample was mixed and washed repeatedly with sterile distilled water and acetone solution at a ratio of 1:3. This method of cleansing removed floating and dissolvable impurities. The purified soot was heated in a hot air oven at 60°C for 1 hour to remove moisture. The product obtained was then grinded with mortar and pestle into fine particles and thereafter calcined in an oven at 180°C for 3 hours. The shea butter was sterilized by autoclaving at 121°C for 15 minutes, then stored at 4°C until usage.

Preparation of soot and soot blended with shea butter Suspension : Soot and soot blended with shea butter concentrations were made by dissolving 1g of the soot powder in 9 mL of distilled water, and 0.5 g of soot and 0.5 mL of molten shea butter in 9 mL of sterile distilled water to make a concentration of 100 mg/mL, respectively. A two-fold dilution was made into four subsequent test tubes, each containing 5ml of distilled water to obtain 50 mg/mL, 25 mg/mL, 12.5 mg/mL, and 6.25 mg/mL, respectively.

Antimicrobial susceptibility test: Conventional antimicrobial susceptibility testing was performed using the Kirby-Bauer disc diffusion method. A 0.5 McFerland standard suspension of selected bacteria (*Citrobaacter freundii, Staphylococcus aureus, Salmonella enterica, Klebsiella pneumoniae, Escherichia coli, Corynebacteriun pseudotuberculosis,* and *Streptococcus pyogenes*) sourced from Antimicrobial Resistance Sentinel Laboratory of Veterinary Teaching Hospital, Ahmadu Bello University, Zaria was made. A swab stick was used to lawn the organisms on a Mueller-Hinton agar plate. Ten different antimicrobials, namely, Cefoxitin (30 µg), Bacitracin (10 iu), Cefixime (5 µg), Amoxicillin/Clavulanic acid (30 µg), Carbenicillin (100 µg), Azithromycin (15 µg), Doxycycline (30 µg) Ciprofloxacin (5 µg), Chloramphenicol (30 µg) and Gentamicin (30 µg) were placed on the lawned plates and the plates were incubated aerobically at 37°C for 24-48 hours. The zone of diameters of inhibition were measured in millimetres using metre rule. Results were interpreted using Clinical and Laboratory Standard Institute (CLSI) 2018 guidelines.

Antimicrobial susceptibility test of soot, soot blended with shea butter, and with antibiotics: Standard suspensions of the seven selected bacteria were made. Each bacterium was lawn on three Mueller Hinton agar plates, and five wells were created on each plate. Five different concentrations (100 mg/mL, 50 mg/mL, 25 mg/mL, 12.5 mg/mL and 6.25 mg/mL) of soot and soot blended with shea butter respectively, and standard concentrations of soot with shea butter blended with standard concentrations of antibiotics discs respectively were inoculated. The plates were then incubated aerobically at 37°C for 24-48 hours. The well with the lowest concentration and with zones of clearing was recorded as the minimum inhibitory concentration to kill or inhibit the growth of bacteria.

RESULTS AND DISCUSSION

All the selected bacteria showed different multidrug resistance patterns to conventional antimicrobials, with the exception of *C. pseudotuberculosis* (table 1). Broadly, higher concentrations of 100 mg/mL & 50 mg/mL produced lower zones of inhibitions than lower concentrations (12.5 mg/mL & 6.25 mg/mL) of the soot blended with shea butter and antimicrobials. This could be due to the thickness of solution with higher concentration which reduces it rate of absorption and permeability into the media as against solutions with lower concentrations. The present study showed that kitchen soot alone or when blended with shea butter had no antimicrobial activity against the selected bacteria. This contradicts the findings of Varghese *et al.* (2013) and Aloysuis *et al.* (2019) who independently reported significant antimicrobial activity of kitchen soot (CNPs)

against *S. aureus* and some selected bacteria respectively. The exact reason for this disparity is not clear but might be associated with the variation of concentrations used in the different studies. When the soot was blended with shea butter and antibiotics, marked synergistic antibacterial effects were observed against *C. pseudotuberculosis*, *Citrobacter freundii & S. aureus*. Augmented zone of inhibitions from 10 to 23 mm, 21 to 23 mm, and 19 to 40 mm were observed on *C. pseudotuberculosis* when amoxicillin/clavulanic acid, azithromycin, and doxycycline were respectively blended with soot and shea butter at a concentration of 6.25mg/ml (table 2). These results are in line with the findings of Habiba *et al.* (2020) who observed synergistic antibacterial effect of soot blended with amoxicillin on *Salmonella enterica.*

Table 1: Antimicrobial resistance pattern of archived selected bacteria

Bacteria	Resistant pattern						No. of class
Corynebacterium pseudotuberculosis	FOX	B					2
Salmonella enterica	F OX	B	CFM	AMC	CAR		3
Citrobacter freundii	AZM	AMC	B	FOX	CAR	CFM	4
Staphylococcus aureus	CAR	CFM	B	FOX			3
klebsiella pneumoniae	CAR	AMC	CFM	B	FOX		3
Escherichia coli	CAR	AMC	CFM	B	FOX		3
Streptococcus pyogenes	CAR	CFM	FOX				2

Key: FOX=Cefoxitin, B=Bacitracin, CFM=Cefixime, AMC= Amoxicillin/Clavulanic acid, CAR=Carbenicillin, AZM=Azithromycin.

Table 2: Sensitivity test results of soot blended with shea butter and antimicrobials on selected bacteria

Bacteria	Antimicrobial	Z.I (mm)	Interpretation	SSBA (mg/ml)	Z.I (mm)	Interpretation
Corynebacterium pseudotuberculosis	Amoxycillin + clavulanic acid	10	R	100	25	S
				50	23	S
				25	23	S
				12.5	23	S
				6.25	23	S
	Azithromycin	21	S	100	31	S
				50	29	S
				25	23	S
				12.5	23	S
				6.25	23	S
	Doxycycline	19	S	100	25	S
				50	38	S
				25	38	S
				12.5	40	S
				6.25	40	S
Salmonella enterica	Doxycycline	19	S	100	20	S
				50	22	S
				25	22	S
				12.5	22	S
				6.25	20	S
Citrobacter freundii	Doxycycline	12	I	100	23	S
				50	25	S
				25	25	S
				12.5	25	S

				6.25	25	S
	Azithromycin	0	R	100	18	S
				50	17	S
				25	20	S
				12.5	20	S
				6.25	22	S
Staphylococcus aureus	Azithromycin	14	I	100	28	S
				50	29	S
				25	29	S
				12.5	29	S
				6.25	28	S
	Bacitracin	0	R	100	15	S
				50	14	S
				25	13	I
				12.5	11	R
				6.25	13	I

Key: SSBA = Soot blended with shea butter & antimicrobial, Z.I = Zone of inhibitions, I = Intermediate, R = Resistant, S = Susceptible.

CONCLUSION AND RECOMMENDATION

This study established that *C. pseudotuberculosis*, *Citrobacter freundii & S. aureus* are susceptible to kitchen soot blended with shea butter and Azithromycin. Likewise, soot blended with shea butter and doxycycline produced synergistic antimicrobial activity on *C. pseudotuberculosis* & *citrobacter freundii.* However, there is a need to establish the active ingredients in both soot and shea butter, and a need to further attempt lower concentrations so as to establish MIC for the different combinations.

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