

# Comparative Analyses of the Efficacy of Dry and Fresh *Mangifera indica* Ethanolic Extracts on *E. coli* and *S. aureus*

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## Abstract

**Background:** The need to identify and characterize new antimicrobial agents is important due to the increasing development of resistance by microorganisms to the existing antimicrobial agents. **Aim:** This study examined the efficacies of *Mangifera indica* on *Escherichia coli* and *Staphylococcus aureus*. **Method:** Three parts (leaf [L], root [R], and bark [B]) of the plant were analyzed. The extraction of the samples was performed by aseptically grinding the samples, dissolving in absolute ethanol, and filtering through whatman filter paper. The efficacy of the extracts both single and combined was determined using agar well diffusion assay with gentamycin [10 µl] (*E. coli*) and vancomycin [30 µl] (*S. aureus*) as control antibiotics. **Results:** The higher concentration ( $C_2 = 3.0$  g/ml) showed more antibacterial effectiveness than the lower concentration ( $C_1 = 1.5$  g/ml) against both bacterial isolates with significant differences ( $P < 0.05$ ) in all extracts except for single extracts (*E. coli* dry leaf extract; fresh bark extract), double extracts (*S. aureus*: dry and fresh leaf extracts) and triple extract (*E. coli* and *S. aureus* dry extracts). For the single extracts the bacteria has the following significant results: *E. coli* L (dry  $6.3 \pm 2.5$  mm, fresh  $14.7 \pm 0.6$  mm,  $P = 0.0050$ ), R (dry  $11.3 \pm 1.5$  mm, fresh  $7.3 \pm 1.5$  mm,  $P = 0.0327$ ); for *S. aureus* L (dry  $7.0 \pm 1.7$  mm, fresh  $11.0 \pm 1.0$  mm,  $P = 0.0257$ ), R (dry  $7.0 \pm 2.0$  mm, fresh  $11.7 \pm 1.5$  mm,  $P = 0.0325$ ), and B (dry  $5.0 \pm 1.0$  mm, fresh  $16.0 \pm 1.0$  mm,  $P = 0.0002$ ). For the double extracts the bacteria has the following significant results: *E. coli* L + R (dry  $15.7 \pm 2.3$  mm, fresh  $1.7 \pm 1.5$  mm,  $P = 0.0070$ ), R + B (dry  $18.7 \pm 1.5$  mm, fresh  $9.7 \pm 1.5$  mm,  $P = 0.0020$ ), and L + B (dry  $9.7 \pm 1.5$  mm, fresh  $6.3 \pm 0.6$  mm,  $P = 0.0241$ ); *S. aureus* L + R (dry  $14.7 \pm 1.5$  mm, fresh  $7.0 \pm 1.0$  mm,  $P = 0.0019$ ), R + B (dry  $15.3 \pm 1.5$  mm, fresh  $11.7 \pm 1.5$  mm,  $P = 0.0424$ ). For the triple extracts, the fresh leaves showed significantly higher levels of efficacy than the dry for both *E. coli* L + R + B ( $P = 0.0101$ ) and *S. aureus* ( $P =$

0.0307). The fresh extracts showed higher levels of efficacy than dry extracts against both bacteria for all the single and three combined conditions. **Conclusions:** Fresh extracts show better efficacies against *E. coli* while dry extracts show greater efficacies against *S. aureus* for both single and triple combined extracts. The reverse is true for double combined extracts.

## Keywords

*Mangifera indica*, Susceptibility Testing, Herbal Drug, Single and Combination Therapy

## 1. Introduction

The use of phytochemicals, naturally occurring compounds found in plants, has been necessitated by the rapid increase in antimicrobial resistance across the globe [1]. For a very long period, medicines were obtained from plant sources [2]. These kinds of medicines have been advanced in developing nations as an alternative method of treating infectious diseases. A previous study has reported the antibacterial potencies of plant extracts such as the ethanolic extract of *Momordica charantia* inhibition of the growth of *Escherichia coli* and *Staphylococcus aureus* [3].

*Mangifera indica* L. is commonly referred to as mango. It is a member of the *Anacardiaceae*. This family consists of sixty genera and six hundred species [4]. Phytochemical screening of *M. indica* has indicated that the leaves consist of gallotannins polyphenols, phenolic acids [5], alcohols such as methyl, ethyl and isobutyl, terpenes, phenylpropenes, and sterols [6]. The roots constitute of triterpenes and triterpenoids and sterols [5]. The bark is composed of all the previously named phytochemicals of the roots in addition to gallotannins, halogenated amide and amino acids [5] [7].

The current study has selected two common opportunistic pathogens, *E. coli* and *S. aureus*. These microorganisms belong to the two major spectra of bacteria; gram-negative and gram-positive bacteria. Previous studies have examined the antimicrobial efficacy of some parts of *M. indica*. Mutua *et al.* [8] have demonstrated that the seed extract of *M. indica* possesses antimicrobial efficacy against *E. coli* and *Candida albican*. Similar observations were noted by Alok *et al.* [9] for the effect of the seed extract on *Pseudomonas aeruginosa* and *S. aureus*. Another study has also demonstrated that the leaf extract potentiates the inhibition of growth of *Streptococcus agalactiae*, *Bacillus megaterium*, *B. subtilis*, and *Lactobacillus bulgaricus* [10]. Some researches on this plant were from the antidiarrheal, antidiabetic [11], anticancer and anti-inflammatory [12] points of view. In this report, the comparative analyses of the antibacterial activities of dry and fresh leaf, root, and bark extracts of *M. indica* were studied on *E. coli* and *S. aureus*. This study further analyzed whether the combination of the various extracts worked synergistically or antagonistically.

## 2. Methods

### 2.1. Collection and Preparation of the *M. indica* Samples

Samples of *M. indica* were collected from Barako in Gokana of Rivers State, Nigeria. Barako is located at latitude 4°40'5"N, and longitude 4°43'5" East. The plant samples (leaf, root, and bark) were collected by plucking the leaves, scraping off the bark, and digging the soil off the root before cutting the exposed roots. The samples were immediately transported in plastic bags to the Department of Medical Laboratory Science Laboratory of Rivers State University, Port Harcourt for processing.

The samples were washed thoroughly to remove dirt and further sterilized to destroy any contaminating microorganisms. The fresh extracts were prepared by grinding 600 g of leaves, roots, and bark in a sterile mortar. Each sample was soaked in 200 ml of absolute ethanol for 72 h at room temperature in an enclosed container to extract the phytochemical components. The samples were first filtered through a sterilized wire net to sift the debris from the solution. The solution was finally filtered through a sterile no.1 Whatman filter paper. The filtrate was evaporated to dryness in a water bath at 80°C. The dried samples were put in specimen bottles and reconstituted with 200 ml of sterile distilled water. The samples were stored in a refrigerator at 4°C until further use. To prepare the dry extracts, 600 g of leaves, roots, and bark dried in an incubated at 25°C for four (4) weeks. The samples were then grounded in a sterile mortar and the procedure for the extraction of fresh samples was followed. The combination of the extract before efficacy testing was in the ratio of 1:1.

### 2.2. Bacterial Isolates and Growth Conditions

Microorganisms used for the experiment were *E. coli* ATCC 252922 and *S. aureus* ATCC 29213 which were identified using PCR amplification of the 16S rRNA at Lahor Research Laboratories, Benin, Edo State, Nigeria. The organisms were stored in 10% glycerol and kept at -20°C.

### 2.3. Media Preparation

Tryptic Soy Agar (TSA), Mueller-Hinton agar (MHA) and Tryptic Soy Broth (TSB) were prepared according to the manufacturer's instruction and sterilized by autoclaving at 15 psi or 121°C for 15 minutes. The solid media were allowed to cool to about 50°C, aseptically poured into a sterile Petri dish and allowed to solidify at room temperature before storage at 4°C for subsequent use. The TSB was stored at room temperature.

### 2.4. Minimum Inhibitory Concentration (MIC) of Extracts

The MIC assay of the extracts was performed using the broth dilution method in the TSB as described by the Clinical and Laboratory Standard Institute (CLSI) [13]. The 3-(4,5-dimethylthiazol-2,5-diphenyltetrazolium bromide (MTT) was used as an indicator as it is utilized by physiologically viable bacteria. The MIC

was read as the lowest concentration of the extract that resulted in the production of color change. The study performed a negative control in 100  $\mu\text{l}$  of 0.4% dimethylsulfoxide (DMSO).

### 2.5. Agar Well Diffusion Assay

The antibacterial susceptibility of the bacterial isolates was determined using agar well diffusion assay. The overnight cultures of *E. coli* and *S. aureus* were diluted to produce an optical density (OD) of 0.5 at 590 nm and 100  $\mu\text{l}$  of the diluted overnight cultures are spread on MHA. Sterilized Pasteur pipettes were used to make wells 6 mm in diameter. 100  $\mu\text{l}$  of extracts ( $C_1 = 1.5$  g/ml,  $C_2 = 3$  g/ml) were added into the wells and incubated at 37°C for 24 h in an upright position. The zones of clearance around the wells were measured in millimeters. Gentamycin [10  $\mu\text{l}$ ] and vancomycin [30  $\mu\text{l}$ ] disks were used as controls for both *E. coli* and *S. aureus* respectively.

### 2.6. Statistical Analysis

The data obtained in this study were represented as mean  $\pm$  SD (standard deviation) for  $n = 3$ . The statistical analysis used was a t-test for comparisons between two variables and ANOVA for more than two variables. The post analysis was performed by ANOVA. *P*-value was considered to be statistically significant at  $P < 0.05$ .

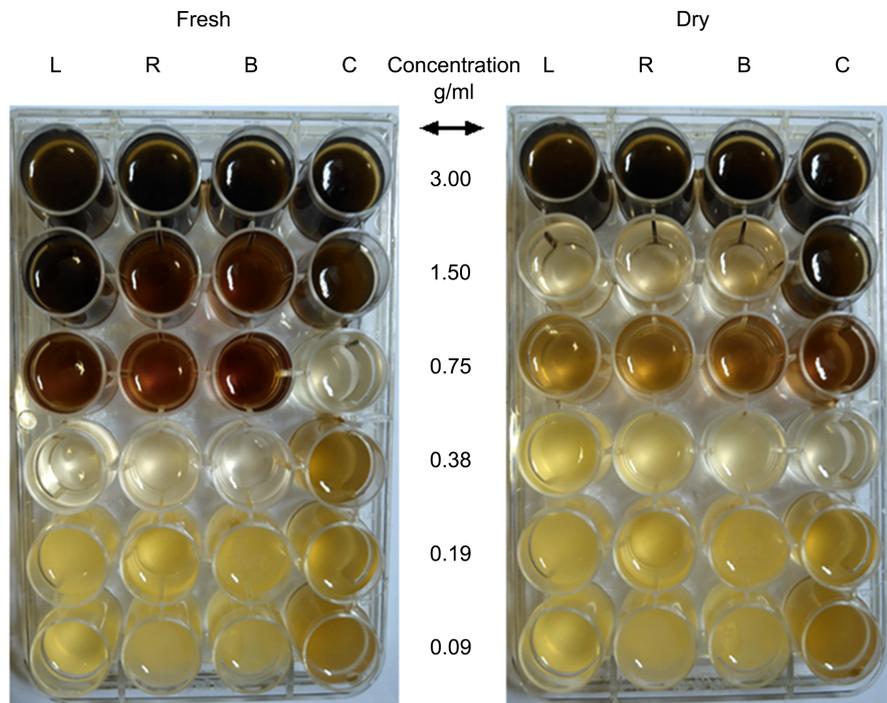
## 3. Results

In all the experiments in this study, the control drugs, gentamycin [10  $\mu\text{l}$ ] for *E. coli* and vancomycin for *S. aureus*, showed significantly ( $P < 0.05$ ) higher levels of zones of inhibition compared to either of the concentrations of the extracts used (represented as superscripts a and b in **Tables 2-5** and **Figure 2, Figure 3**). The results were analyzed by intra-comparison of the variations within the group (dry or fresh leaf extracts) using ANOVA analysis or between the two groups using a t-test. The comparison of the efficacy of the different concentrations of extracts on both *E. coli* and *S. aureus* showed significant variation ( $P = < 0.0001$ ) in their mode of actions. The MIC for the extracts on *E. coli* (leaf, root, and bark) and *S. aureus* (leaf, root, and bark) are shown in **Table 1**. **Figure 1** shows a sample of the 24-wells plate for the broth dilution method. The wells showing clear solution were adopted as the MIC.

**Table 1.** Minimum inhibitory concentration of extracts.

Bacteria	MIC (g/ml)					
	Leaf		Root		Bark	
	D	F	D	F	D	F
<i>E. coli</i>	1.50	0.38	1.50	0.38	1.5	0.38
<i>S. aureus</i>	1.50	0.38	1.50	0.38	1.5	0.75

Key: D—Dry, F—Fresh, MIC—Minimum inhibitory concentration.



**Figure 1.** Minimum inhibitory concentration of leaf, root, and bark on bacteria. Key: L—Leaf, R—Root, B—Bark, C—Control.

### 3.1. Efficacy of Single Component of *M. indica* Extracts on *E. coli* and *S. aureus*

**Table 2** represents the comparison of the antibacterial efficacies of fresh and dry single-component of *M. indica* on *E. coli*. The higher concentrations ( $C_2 = 3.0$  g/ml) of the dry extract of root and bark were significantly (represented by the superscript c) higher than the lower concentrations ( $C_1 = 1.5$  g/ml) but the difference was not significant ( $P > 0.05$ ) in the dry leaf extract. Also, the higher concentrations of the fresh leaf, root, and bark extracts were significantly ( $P < 0.05$ ) higher than the lower concentrations. A comparative analysis of the dry and fresh extracts between corresponding concentrations indicates that the fresh leaf, root, and bark extracts were significantly ( $P$  values: leaf;  $C_1 = 0.0533$ ,  $C_2 = 0.0050$ ; root  $C_1 = 0.0080$ ,  $C_2 = 0.0327$ ; bark  $C_1 = 0.0249$ ) effective than the dry extracts except for the higher concentration of the bark extract which is not significant ( $P = 0.1481$ ).

**Table 3** represents the comparison of the antibacterial efficacies of fresh and dry single-component of *M. indica* on *S. aureus*. The higher concentrations ( $C_2 = 3.0$  g/ml) of the dry and extracts of leaf, root, bark were significantly (represented by the superscript c) higher than the lower concentrations ( $C_1 = 1.5$  g/ml). Relatively comparison of the dry and fresh extracts between corresponding concentrations indicates that the fresh leaf, root and bark extracts were significantly ( $P$  values: leaf;  $C_1 = 0.0111$ ,  $C_2 = 0.0257$ ; root  $C_2 = 0.0325$ ; bark  $C_1 = 0.0088$ ,  $C_2 = 0.0002$ ) effective than the dry extracts except for the lower concentration of the root extract which is not significant ( $P = 0.1890$ ).

**Table 2.** Antimicrobial effect of single component of *Mangifera indica* on *E. coli*.

	Dry (n = 3)	Fresh (n = 3)	P-value (t-test)
Zone of Inhibition (mm)			
<b>Leaf</b>			
C <sub>0</sub>	29.0 ± 1.0	29.0 ± 1.0	
C <sub>1</sub>	2.3 ± 1.5 <sup>a</sup>	5.3 ± 1.2 <sup>ac</sup>	0.0533
C <sub>2</sub>	6.3 ± 2.5 <sup>b</sup>	14.7 ± 0.6 <sup>bc</sup>	0.0050
P-value (ANOVA)	<0.0001	<0.0001	
<b>Root</b>			
C <sub>0</sub>	29.0 ± 1.0	29.0 ± 1.0	
C <sub>1</sub>	5.0 ± 1.0 <sup>ac</sup>	1.0 ± 1.0 <sup>ac</sup>	0.0080
C <sub>2</sub>	11.3 ± 1.5 <sup>bc</sup>	7.3 ± 1.5 <sup>bc</sup>	0.0327
P-value (ANOVA)	<0.0001	<0.0001	
<b>Bark</b>			
C <sub>0</sub>	29.0 ± 1.0	29.0 ± 1.0	
C <sub>1</sub>	5.0 ± 1.0 <sup>ac</sup>	7.3 ± 0.6 <sup>a</sup>	0.0249
C <sub>2</sub>	8.7 ± 0.6 <sup>bc</sup>	11.3 ± 2.5 <sup>b</sup>	0.1481
P-value (ANOVA)	<0.0001	<0.0001	

Key: C<sub>0</sub>—gentamycin [10 µl], C<sub>1</sub>—1.5 g of extract, C<sub>2</sub>—3.0 g of extract. Superscripts a, b, c show significant comparison ( $P < 0.05$ ) between C<sub>0</sub> vs C<sub>1</sub>, C<sub>0</sub> vs C<sub>2</sub>, and C<sub>1</sub> vs C<sub>2</sub> respectively.

**Table 3.** Antimicrobial effect of single components of *Manifera indica* on *S. aureus*.

	Dry (n = 3)	Fresh (n = 3)	P-value (t-test)
Zone of Inhibition (mm)			
<b>Leaf</b>			
C <sub>0</sub>	30.0 ± 1.0	30.0 ± 1.0	
C <sub>1</sub>	1.3 ± 0.6 <sup>ac</sup>	4.7 ± 1.2 <sup>ac</sup>	0.0111
C <sub>2</sub>	7.0 ± 1.7 <sup>bc</sup>	11.0 ± 1.0 <sup>bc</sup>	0.0257
P-value (ANOVA)	<0.0001	<0.0001	
<b>Root</b>			
C <sub>0</sub>	30.0 ± 1.0	30.0 ± 1.0	
C <sub>1</sub>	1.3 ± 0.6 <sup>ac</sup>	3.0 ± 1.7 <sup>ac</sup>	0.1890
C <sub>2</sub>	7.0 ± 2.0 <sup>bc</sup>	11.7 ± 1.5 <sup>bc</sup>	0.0325
P-value (ANOVA)	<0.0001	<0.0001	
<b>Bark</b>			
C <sub>0</sub>	30.0 ± 1.0	30.0 ± 1.0	
C <sub>1</sub>	1.3 ± 0.6 <sup>ac</sup>	10.3 ± 3.2 <sup>ac</sup>	0.0088
C <sub>2</sub>	5.0 ± 1.0 <sup>bc</sup>	16.0 ± 1.0 <sup>bc</sup>	0.0002
P-value (ANOVA)	<0.0001	<0.0001	

Key: C<sub>0</sub>—Vancomycin, C<sub>1</sub>—1.5 g of extract, C<sub>2</sub>—3.0 g of extract. Superscripts a, b, c show significant comparison ( $P < 0.05$ ) between C<sub>0</sub> vs C<sub>1</sub>, C<sub>0</sub> vs C<sub>2</sub>, and C<sub>1</sub> vs C<sub>2</sub> respectively.

### 3.2. Efficacy of Two Combined Components of *M. indica* Extracts on *E. coli* and *S. aureus*

**Table 4** represents the comparison of the antibacterial efficacies of fresh and dry two mixed components of *M. indica* on *E. coli*. The higher concentrations ( $C_2 = 3.0$  g/ml) of the fresh and dry combined extracts of leaf, root, and bark were significantly ( $[P < 0.05]$  represented by the superscript c) higher than the lower concentrations ( $C_1 = 1.5$  g/ml). The comparative evaluation shows that all the dry extracts exhibited higher efficacies than the fresh extracts for both matching concentrations with significant differences seen in  $C_1$  and  $C_2$  ( $P = 0.0021$  and  $0.0020$  respectively) for only root + bark and  $C_2$  for leaf + root and leaf + bark ( $P = 0.0070$  and  $0.0241$ ).

**Table 5** displays the assessment of the antibacterial efficacies of fresh and dry two mixed components of *M. indica* on *S. aureus*. All the higher concentrations ( $C_2 = 3.0$  g/ml) of the double-combined fresh and dry combined extracts of leaf, root, and bark were significantly ( $[P < 0.05]$  represented by the superscript c) higher than the lower concentrations ( $C_1 = 1.5$  g/ml). A comparative analysis shows that just about all the dry extracts exhibited higher efficacies than the fresh extracts for both matching concentrations with significant changes observed in  $C_1$  and  $C_2$  for only root + bark ( $P = 0.0101$  and  $0.0424$  respectively),  $C_2$  for leaf + root and leaf + bark ( $P = 0.0019$ ) and  $C_1$  for leaf + bark ( $P = 0.0295$ ). The exception to this rule was the  $C_1$  of the leaf + root which had non-significantly ( $P = 0.2051$ ) lower efficacy of the dry compared to the fresh extract.

**Table 4.** Antimicrobial effect of double components of *Mangifera indica* on *E. coli*.

	Dry (n = 3)	Fresh (n = 3)	P-value (t-test)
Zone of Inhibition (mm)			
<b>Leaf + Root</b>			
$C_0$	29.0 ± 1.0	29.0 ± 1.0	
$C_1$	2.3 ± 1.2 <sup>ac</sup>	1.7 ± 1.5 <sup>ac</sup>	0.5185
$C_2$	15.7 ± 2.3 <sup>bc</sup>	8.7 ± 0.6 <sup>bc</sup>	0.0070
P-value (ANOVA)	<0.0001	<0.0001	
<b>Root + Bark</b>			
$C_0$	29.0 ± 1.0	29.0 ± 1.0	
$C_1$	12.3 ± 1.5 <sup>ac</sup>	5.7 ± 0.6 <sup>ac</sup>	0.0021
$C_2$	18.7 ± 1.5 <sup>bc</sup>	9.7 ± 1.5 <sup>bc</sup>	0.0020
P-value (ANOVA)	<0.0001	<0.0001	
<b>Leaf + Bark</b>			
$C_0$	29.0 ± 1.0	29.0 ± 1.0	
$C_1$	2.7 ± 0.6 <sup>ac</sup>	2.0 ± 0.5 <sup>ac</sup>	0.2051
$C_2$	9.7 ± 1.5 <sup>bc</sup>	6.3 ± 0.6 <sup>bc</sup>	0.0241
P-value (ANOVA)	<0.0001	<0.0001	

Key:  $C_0$ —gentamycin [10 µl],  $C_1$ —1.5 g of extract,  $C_2$ —3.0 g of extract. Superscripts a, b, c show significant comparison ( $P < 0.05$ ) between  $C_0$  vs  $C_1$ ,  $C_0$  vs  $C_2$  and  $C_1$  vs  $C_2$  respectively.

**Table 5.** Antimicrobial effect of double components of *Mangifera indica* on *S. aureus*.

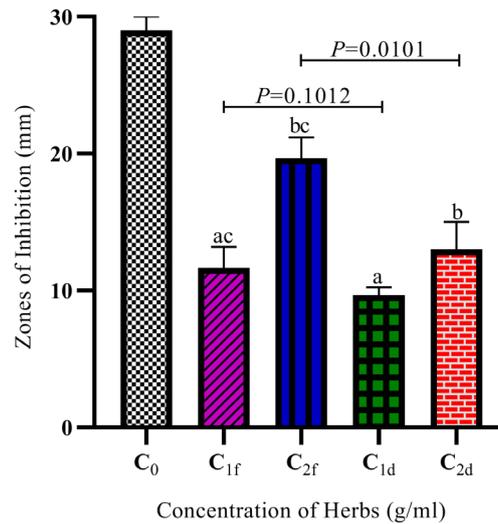
	Dry (n = 3)	Fresh (n = 3)	P-value (t-test)
Zone of Inhibition (mm)			
<b>Leaf + Root</b>			
C <sub>0</sub>	30.0 ± 1.0	30.0 ± 1.0	
C <sub>1</sub>	1.0 ± 1.0 <sup>ac</sup>	2.3 ± 1.2 <sup>ac</sup>	0.2051
C <sub>2</sub>	14.7 ± 1.5 <sup>bc</sup>	7.0 ± 1.0 <sup>bc</sup>	0.0019
P-value (ANOVA)	<0.0001	<0.0001	
<b>Root + Bark</b>			
C <sub>0</sub>	30.0 ± 1.0	30.0 ± 1.0	
C <sub>1</sub>	11.7 ± 1.5 <sup>ac</sup>	7.3 ± 0.6 <sup>ac</sup>	0.0101
C <sub>2</sub>	15.3 ± 1.5 <sup>bc</sup>	11.7 ± 1.5 <sup>bc</sup>	0.0424
P-value (ANOVA)	<0.0001	<0.0001	
<b>Leaf + Bark</b>			
C <sub>0</sub>	30.0 ± 1.0	30.0 ± 1.0	
C <sub>1</sub>	7.2 ± 0.8 <sup>a</sup>	5.3 ± 0.6 <sup>a</sup>	0.0295
C <sub>2</sub>	7.3 ± 1.5 <sup>b</sup>	6.7 ± 1.5 <sup>b</sup>	0.6213
P-value (ANOVA)	<0.0001	<0.0001	

Key: C<sub>0</sub>—Vancomycin, C<sub>1</sub>—1.5 g of extract, C<sub>2</sub>—3.0 g of extract. Superscripts a, b, c show significant comparison ( $P < 0.05$ ) between C<sub>0</sub> vs C<sub>1</sub>, C<sub>0</sub> vs C<sub>2</sub>, and C<sub>1</sub> vs C<sub>2</sub> respectively.

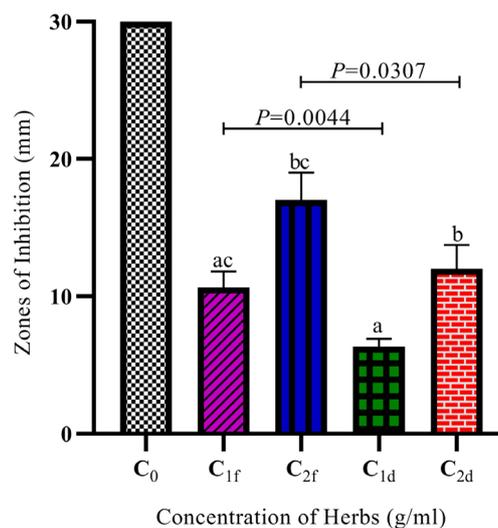
### 3.3. Antimicrobial Efficacy of Combined *M. indica* Leaf, Root, and Bark on *E. coli* and *S. aureus*

**Figure 2**, **Figure 3** display the antibacterial efficacies of fresh and dry three-mixed components of *M. indica* on *E. coli* and *S. aureus* respectively. **Figure 2** shows that the higher concentration (C<sub>2</sub> = 3.0 g/ml) of the three-combined fresh extracts of leaf, root, and bark was significantly ( $[P < 0.05]$  represented by the superscript c) higher than the lower concentration (C<sub>1</sub> = 1.5 g/ml) while the variations in that of the dry extract were not significant ( $P > 0.05$ ). A comparative analysis for both matching concentrations shows that the fresh extract exhibited higher efficacies than the dry extract with significant changes observed in C<sub>2</sub> of leaf + root + bark ( $P = 0.0101$ ), while the C<sub>1</sub> of the fresh extract had non-significantly ( $P = 0.1012$ ) higher antimicrobial efficacy compared to the dry extract.

Again, **Figure 3** shows that the higher concentration (C<sub>2</sub> = 3.0 g/ml) of the three-combined fresh extracts of leaf, root, and bark was higher than the lower concentration (C<sub>1</sub> = 1.5 g/ml) but not significantly ( $P > 0.05$ ) while the variations in that of the dry extract were significant ( $P < 0.05$ ). A comparative analysis for both identical concentrations shows that the fresh extract exhibited higher efficacies than the dry extract with significant changes observed in both C<sub>1</sub> and C<sub>2</sub> of leaf + root + bark ( $P = 0.0044$  and  $0.0307$ ).



**Figure 2.** Antimicrobial efficacy of triple combined leaf, root, and bark on *E. coli*. C<sub>0</sub>—gentamycin [10 µl], C<sub>1</sub>—1.5 g of extract, C<sub>2</sub>—3.0 g of extract. Superscripts a, b, c shows significant comparison ( $P < 0.05$ ) ( $n = 3$ ) between C<sub>0</sub> vs C<sub>1</sub>, C<sub>0</sub> vs C<sub>2</sub>, and C<sub>1</sub> vs C<sub>2</sub> respectively.



**Figure 3.** Antimicrobial efficacy of triple combined leaf, root, and bark on *S. aureus*. C<sub>0</sub>—gentamycin [10 µl], C<sub>1</sub>—1.5 g of extract, C<sub>2</sub>—3.0 g of extract. Superscripts a, b, c shows significant comparison ( $P < 0.05$ ) ( $n = 3$ ) between C<sub>0</sub> vs C<sub>1</sub>, C<sub>0</sub> vs C<sub>2</sub>, and C<sub>1</sub> vs C<sub>2</sub> respectively.

#### 4. Discussion

All extracts of *M. indica* were found to have demonstrated some levels of efficacy against both *E. coli* and *S. aureus*, however, at different concentrations. This phenomenon could be explained by the zones of clearance seen in conditions treated with the extracts. These zones of inhibition vary directly with the concentration of extracts, that is, the effectiveness of the extracts is affected by the dilution of the extract. This similar concentration-dependent activity of herbal antimicrobial agent was observed by Matasyoh *et al.* [14].

A predominant observation on the antimicrobial efficacy pattern across both bacterial isolates was that the fresh extracts possess more antibacterial efficacy than the dry extracts for either the single, double and triple components of the extracts (Tables 2-4 and Figure 2, Figure 3). This could have resulted from the loss of some vital phytochemical compounds during the drying process [15] [16]. The exception to this observation is the effect of the dry extracts (double mixture) on *S. aureus* which were higher than the fresh extracts (Table 5). Since the single component of the extract showed higher efficacy for fresh over dry, the latter observations could be explained in terms of removal of the interfering component during drying which made the resultant dry extract have higher efficacy in the double-combined mixture over the single. The explanation from the action of the triple-combined extract in possessing better antibacterial activities than either the single or double combined mixtures could be synergistic effects of several components of the extracts. This phenomenon has been observed by Kuok *et al.* [17] in which different herbs were combined (*Verbena officinalis*, *Magnolia officinalis*, *Momordica charantia*, and *Daphne genkwa*) with oxacillin to produce a synergistic action against methicillin-resistant *S. aureus*. Diso *et al.* [18] also worked on the leaf, root, and stem of *M. indica* using only the individual extract components on *S. aureus* and noted much higher zones of inhibition than the current study.

The combination of the three extracts showed the highest level of efficacy (Figure 2, Figure 3). For both bacteria studied, the fresh extract of this tripartite extract showed significantly higher levels of zones of clearance compared to the dry extract especially for the higher concentration, that is, *E. coli* (fresh  $19.7 \pm 1.5$ , dry  $13.0 \pm 2.0$   $P = 0.0101$ ); *S. aureus* (fresh  $10.7 \pm 1.2$  dry  $12.0 \pm 1.7$   $P = 0.0307$ ). A logical explanation of this phenomenon could be a result of the synergistic kinetics of the bioactive components of the leaf, root, and bark. These higher zones of clearance are similar to the observation noted in the efficacies of the single extract which has been previously studied [8] [9]. However, the zones of clearance by the tripartite mixtures were nearly double that of the single extracts. This suggests that triple combined extracts are the most effective. Also, variations observed in the susceptibility of Gram-positive and Gram-negative bacteria could have resulted from the relative composition of cell wall components. Gram-positive bacteria have a thicker peptidoglycan layer, while Gram-negative have thicker lipopolysaccharides layer. Some bioactive components could be very selective on the group of microorganisms they destroy.

Maldonado-Celis *et al.* [19] screened the fruit of *M. indica* for the phytochemical constituents and discovered the following compounds: phenolic acids, flavonoids, carotenoids, monoterpenes, sesquiterpenes, esters, lactones aldehydes and ketones [20]. Further screening for the antimicrobial activity of individual or combined bioactive components of these extracts is necessary to exert components that are responsible for the actions noted. This study supports the overwhelming evidence that herbal medicine could serve as an alternative source

to orthodox medicine to curb the pandemic of resistance seen in bacteria. However, there are other areas of research that are necessary to cement the proof of the efficacy of this plant as an antimicrobial agent. First, the bactericidal and bacteriostatic nature of this extract needs a comprehensive elucidation. This could be studied through computational studies of molecular docking and molecular dynamics of the extracts to assess the antibacterial potential. Also, the extract efficacy could be standardized by using different extraction solvents under various temperature and pH conditions.

## 5. Conclusion

This study suggests that the tripartite combination of fresh leaf, bark, and root extracts of *M. indica* could be utilized for the treatment of infections arising from *E. coli* and *S. aureus* especially on the body surfaces. It has also shown that the double combined dry ethanolic extracts of *M. indica* possess better efficacy for *S. aureus* and *E. coli* compared to fresh extracts.

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## Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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