



## Original Research Article

# Comparison of the antibacterial activity of six medicinal plants, sodium hypochlorite, and chlorhexidine against enterococcus faecalis (In vitro study)

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## ABSTRACT

**Background:** Enterococcus faecalis is a gram-positive, facultative anaerobic bacterium recognized for its resistance to various antimicrobial agents. This organism is associated with the failure of endodontic treatments, even when potent antimicrobial irrigants are employed. Numerous medicinal plants have demonstrated antimicrobial properties that could be potentially effective against this bacterium.

**Objectives:** To evaluate the antibacterial properties of six medicinal plants in comparison to sodium hypochlorite and chlorhexidine against E. faecalis.

**Materials and Methods:** Antibacterial susceptibility tests against E. faecalis (ATCC 29212) were performed for 200 mg/ml ethanolic extracts of Acacia Senegal, Capparis decidua, Capparis micracantha, Acacia nilotica/ Adansonia, Dobera glabra, and Moringa oleifera by Agar Disc Diffusion method. Chlorhexidine 0.2% and Sodium hypochlorite 1% were used as positive controls, and ethanol 20% as a negative control. The diameters of the inhibition zones were measured.

**Results:** Acacia nilotica/adansonii leaves showed the largest inhibition zone diameter against E. faecalis. It displayed a significantly greater inhibitory effect against E. faecalis than Acacia Senegal (p-value =0.005), Capparis decidua (p-value =0.02), Capparis micracantha branches (p-value =0.000), Dobera glabra leaves (p-value =0.008), and Moringa oleifera leaves (p-value =0.000).

Acacia nilotica leaves displayed a similar inhibition zone diameter against E. faecalis as chlorhexidine gluconate 0.2%, but it showed no statistical significance.

Acacia nilotica leaves (L) and pods (P), as well as Dobera glabra branches (B), displayed a larger inhibition zone diameter against E. faecalis than Sodium hypochlorite 1%, but with no statistical significance.

**Conclusion:** The leaves of Acacia nilotica exhibit the most potent antibacterial properties against E. faecalis when compared to other plants parts. They outperform the antibacterial effectiveness of 1% sodium hypochlorite and demonstrate a similar antibacterial impact to that of 0.2% chlorhexidine. Therefore, Acacia nilotica leaves could serve as an ideal irrigant potentially substituting the chemical options.

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## 1. Introduction

Enterococcus faecalis is a type of gram-positive, facultative anaerobic coccus bacteria. It is capable of enduring extreme environments, such as elevated salt levels and temperature more than 45°C.<sup>1,2</sup> It is a normal component of the

microbial community found in the mouth, human gut, and female reproductive system. Enterococcus species are identified as possible human pathogens, accounting for 12% of all infections acquired in hospitals. E. faecalis is a well-known contributor to the failure of root canal treatments and is associated with various systemic conditions, including urinary tract infections, infections from surgical wounds,

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bacteremia, and bacterial endocarditis. Additionally, they have shown significant resistance to antimicrobial drugs.<sup>1</sup>

In dentistry, it is most frequently observed in teeth with failed endodontic treatment. But it is also found in infected teeth that had not been previously endodontically treated.<sup>1</sup> During endodontic treatment, sodium hypochlorite and chlorhexidine, the main antimicrobial irrigants, showed low ability to eradicate *E. faecalis* when evaluated by either PCR or culture method.<sup>3</sup>

Several plants have shown antimicrobial activity.<sup>4,5</sup> Phytochemical screening showed active ingredients and secondary metabolites responsible for their antimicrobial effect.<sup>4</sup>

Literature has displayed the antimicrobial potency of many plants against *Enterococcus faecalis*.<sup>6</sup> Furthermore, a blend of several plants has been investigated for their antibacterial properties against *E. faecalis*. One such formulation is *Morinda citrifolia*, an Indian formulation made by drying and grinding the fruits of three medicinal plants: *Terminalia bellerica*, *Terminalia chebula*, and *Emblica officinalis* has well-known antibacterial activity against *E. faecalis*.<sup>6</sup>

With the increasing number of plants showing antibacterial activity, it is time to compare the antibacterial effects of different promising plants. Dental researchers focused their efforts on identifying a natural antibacterial agent that could effectively combat *Enterococcus faecalis*, surpassing the antimicrobial effectiveness of the current chemical solutions. This study aimed to investigate the antibacterial effectiveness of *Acacia senegal*, *Capparis decidua*, *Capparis micracantha*, *Acacia nilotica* (*Adansonii*), *Dobera glabra* and *Moringa oleifera* against *Enterococcus faecalis*, while also comparing their efficacy to the traditional endodontic antimicrobial irrigant, such as sodium hypochlorite and chlorhexidine.

## 2. Materials and Methods

### 2.1. Collection of the plants and preparation for the extracts

Gum nodules of *Acacia Senegal*, twigs of *Capparis decidua*, leaves and branches of *Capparis micracantha*, leaves and pods of *Acacia nilotica* (*Adansonii*), leaves and branches of *Dobera glabra* and leaves of *Moringa oleifera* were gathered and verified at the herbarium of Medicinal and Aromatic plants & Traditional Medicine Research Institute, assigned the code G-1983-1-MAPTRI-H.

Seventy grams of each crunched plant was soaked in ethanol (70%) for three days with daily filtration. The solvent was evaporated from each extract, and the extracts were subsequently placed in a freeze dryer until fully dried.

### 2.2. Antibacterial susceptibility test

Antibacterial susceptibility tests for the plants extracts were performed using Agar disc diffusion<sup>7-9</sup> according to the following steps:

#### 2.2.1. Preparation of the media

Muller-Hinton Agar (HIMEDIA, REF. M173-500g Mueller Hinton Agar) was prepared according to the manufacturer instructions. A total of 240 ml of the freshly autoclaved Muller-Hinton agar was distributed into sterile Petri dishes (20 ml for each dish) and allowed to cool at room temperature. The plates were then incubated at 35°C for 48 hours, and sterility was verified prior to use.

#### 2.2.2. Preparation of discs

Discs measuring 6 mm in diameter were created using Whatman filter paper number.<sup>1</sup> The discs were sterilized in a hot air oven at 160°C for a duration of two hours.

#### 2.2.3. Formulation of extracts solutions

Two hundreds milligrams of each extract was dissolved in 1 milliliter of 20% ethanol (Reagents Duksan, Ethyl Alcohol, absolute, product No.6923, 2.5L) and thoroughly mixed using a vortex to ensure complete dissolution.

To eliminate any potential synergistic effect of the solvent (20% ethanol) on the antibacterial properties of the extract, the following steps were taken:

1. A disc soaked in 20% ethanol was placed on a plate inoculated with *E. faecalis* and incubated at 37°C for 24 hours in anaerobic conditions. The solvent was only used after confirming that there was no inhibition zone around the disc.
2. 2. Ethanol 20% itself served as a negative control. In line with the disc diffusion technique protocol, the solvent used to dissolve the test material should be utilized as a negative control.<sup>8,9</sup>

#### 2.2.4. Reactivation and standardization of *Enterococcus faecalis* inoculum suspension

To reactivate the standard strain *Enterococcus faecalis* (ATCC 29212), it was added to 5ml of brain heart infusion (BHI) broth (HIMEDIA, REF. M210-500G) and maintained in anaerobic conditions for 48 hours. Next, a sterile swab was used to transfer *E. faecalis* from the BHI broth onto a Muller Hinton Agar plate. The plate was subsequently incubated at 37°C under anaerobic conditions for 24 hours. Three to five well-isolated colonies of the same morphological type were selected from a Muller Hinton Agar plate culture and transferred with a sterile loop into a tube containing 5 ml of BHI broth. To standardize this suspension, the broth was incubated anaerobically at 37 °C for 24 hours. After that, the turbidity of this suspension was adjusted visually to match that of 0.5 McFarland standard

( $1.5 \times 10^8$  CFU/ml) by diluting it with BHI broth.

### 2.2.5. Antibacterial susceptibility test

A sterile cotton swab was dipped into the adjusted bacterial suspension and then used to streak the surface of Muller Hinton Agar Plates.

Three sterile discs were immersed in 10  $\mu$ L of the following extracts solutions:

Plant name	Part of the plant	Concentration & type of extract
Acacia senegal (Gum Arabic)	Gum nodules	200mg/ml ethanolic extract
Capparis decidua	Twigs	200mg/ml ethanolic extract
Capparis micracantha	Leaves	200mg/ml ethanolic extract
Capparis micracantha	Branches	200mg/ml ethanolic extract
Acacia nilotica (adansoni)	Leaves	200mg/ml ethanolic extract
Acacia nilotica (adansoni)	Pods	200mg/ml ethanolic extract
Dobera glabra	Leaves	200mg/ml ethanolic extract
Dobera glabra	Branches	200mg/ml ethanolic extract
Moringa olifera	Leaves	200mg/ml ethanolic extract

Chlorhexidine 0.2% (Clenora mouthwash, Chlorhexidine Gluconate BP 0.2%w/v) and Sodium hypochlorite 1% (Prevost Denpro Hyposol, 3% Sodium hypochlorite 500 ml) were used as positive controls And Ethanol (20%) as a negative control.

Once the discs were saturated with extracts solutions, they were transferred to plates that had already been inoculated with *E. faecalis*. These plates were incubated at 37°C for 24 hours in anaerobic environment. The plates were observed for the emergence of a clear zone around each disc, which is known as the inhibition zone. The diameter of the inhibition zone was recorded.

### 3. Results

The investigated plants displayed different degrees of antibacterial effect against *Enterococcus faecalis* varying from very low antibacterial activity in *Moringa olifera* to high in *Acacia nilotica* (Table 1).

The data were analyzed by SPSS 20, with ANOVA used to test the differences in means of inhibition zones for the plants under investigation. The F-statistics ( $F=8.812$ ) and P-value (P-Value  $<0.001$ ) indicated a significant difference between at least two means. Multiple comparisons were then conducted using the Least Significant Difference (LSD) to compare the investigated plants, with a level of significance of  $\leq 0.05$ . P-values for all comparisons were

presented on (table 2).

Out of all the plants studied, the leaves of *Acacia nilotica/adansoni* had the largest diameter of inhibition zone against *E. faecalis*. It displayed significantly greater inhibitory effect against *E. faecalis* than *Acacia Senegal* (p-value =0.005), *Capparis decidua* (p-value =0.02), *Capparis micracantha* branches (p-value =0.000), *Dobera glabra* leaves (p-value =0.008), and *Moringa olifera* leaves (p-value =0.000).

The diameter of the inhibition zone of *Acacia nilotica* leaves against *E. faecalis* (figure 1) was similar to chlorhexidine gluconate 0.2% (figure 4), but this result was not statistically significant (P-value =1.00).

The inhibition zone diameter for *Acacia nilotica* pods against *E. faecalis* (figure 2) was comparable to that of chlorhexidine gluconate 0.2% (figure 4), but the result was not statistically significant (p-value=0.682).

Chlorhexidine gluconate 0.2% displayed significantly higher antibacterial activity against *E. faecalis* than *Acacia senegal* Gum nodules (p-value=0.005), *Capparis decidua* twigs (p-value=0.02), *Capparis micracantha* branches (p-value=0.000), *Dobera glabra* leaves (p-value=0.008), and *Moringa olifera* leaves (p-value=0.000).

*Acacia nilotica* leaves (L) and pods (P), as well as *Dobera glabra* branches (B), displayed superior antibacterial activity against *E. faecalis* than Sodium hypochlorite 1%, but the differences were statistically not significant with P-values (L=0.110), (P=0.225), (B=0.785).

A 1% solution of sodium hypochlorite demonstrated superior antibacterial efficacy against *E. faecalis* compared to the gum nodules of *Acacia senegal*, the twigs of *Capparis decidua*, the leaves and branches of *Capparis micracantha*, the leaves of *Dobera glabra*, and the leaves of *Moringa olifera*. Notably, only the findings for the leaves of *Moringa olifera* and the branches of *Capparis micracantha* were statistically significant, yielding p-values of 0.00 and 0.02, respectively.

Chlorhexidine 0.2% resulted in a larger zone of inhibition (16 mm) against *E. faecalis* (figure 4) compared to sodium hypochlorite 1% (12 mm) (figure 3), but the difference was not statistically significant (p-value = 0.11).

Multiple comparisons between of *A. senegal*, *C. decidua*, *C. micracantha*, *A. nilotica*, *D. glabra* & *M. olifera* for their anti-bacterial activity against *E. faecalis* using least significant difference (LSD) and level of significance 0.05. (P-values are presented on the table).

### 4. Discussion

The antibacterial activity of six medicinal plants, sodium hypochlorite, and chlorhexidine against *E. faecalis* was compared in the study. According to Alves et al.<sup>10</sup>, and Mukhtar & Ghori<sup>11</sup>, an inhibition zone measuring less than 9mm signifies an inactive product, a measurement between 9mm and 12mm denotes a partially active product,

**Table 1:** Dimention of the inhibition zones expressed in millimeters for Acacia Senegal, Capparis decidua, Capparis micracantha, Acacia nilotica/adansoni, Dobera glabra, and Moringa oleifera on plates inoculated with *E. faecalis*.

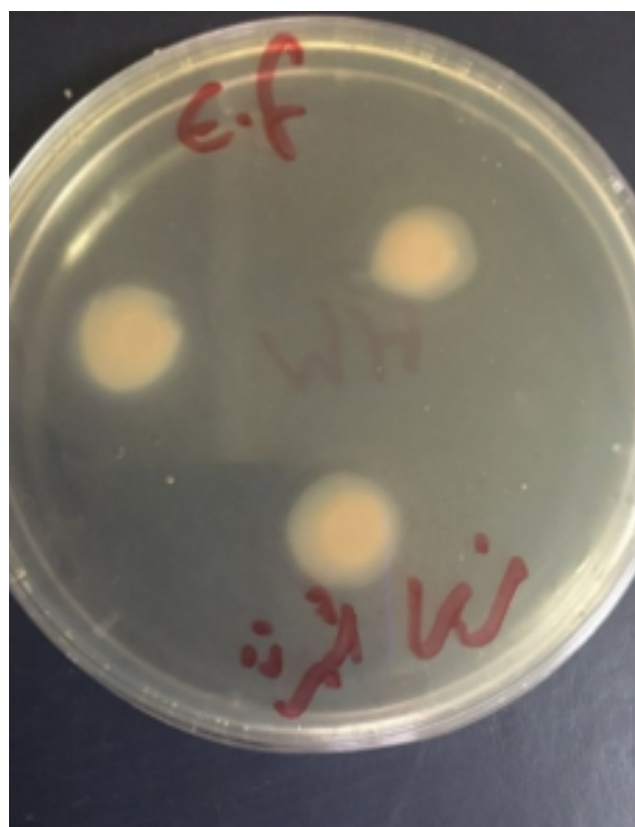
Plant extract with concentration 200mg/ml	Dimensions of inhibition zones in millimeters					
	D1	D 2	D 3	n	mean	SD.
Acacia Senegal (Gum nodules)	9	8	9	3	8.67	0.58
Capparis decidua (twigs)	10	10	10	3	10	0.00
Capparis micracantha (leaves)	12	10	12	3	11.33	1.15
Capparis micracantha (branches)	8	10	0	3	6	5.29
Accacia nilotica (adansonia) (leaves)	17	15	16	3	16	1.00
Accacia nilotica (adansonia)(pods)	14	15	16	3	15	1.00
Dobera glabra (leaves)	15	12	0	3	9	7.94
Dobera glabra (branches)	12	14	12	3	12.67	1.15
Moringa oleifera (leaves)	7	0	0	3	2.33	4.04
Ethanol 20%	0	0	0	3	0	0.00
Sodium hypochlorite (1%)	12	12	12	3	12	0.00
Chlorhexidine 0.2%)	15	16	17	3	16	1.00

(D1), (D2), and (D3): Dimentions of inhibition zones around first, second and third discs and (n): number of measurements for each extract SD.: Standard deviation

**Figure 1:** Zones of inhibition which observed surrounding the three discs which saturated with ethanolic extract of Acacia nilotica/adansonia (leaves) and situated on a plate inoculated with *E. faecalis*

a range of 13mm to 18mm indicates an active product, and a measurement exceeding 18mm reflects a very active product. Based on this activity scale, Acacia Senegal, Capparis micracantha (branches), and Moringa oleifera (leaves) are inactive against *E. faecalis*. However, Capparis decidua (twigs), Capparis micracantha (leaves), and Dobera glabra (leaves, branches) are partially active, while Acacia nilotica leaves and pods are effective antibacterial agents against *E. faecalis*.

Otto et al. mentioned that active ingredients vary within different parts of a plant.<sup>12</sup> This explains why Capparis micracantha branches in the present study are partially active against *E. faecalis*, while the leaves are inactive.

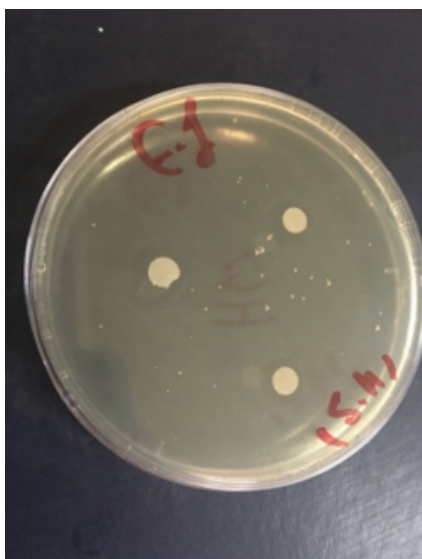
**Figure 2:** Zones of inhibition which observed surrounding the three discs which saturated with ethanolic extract of Acacia nilotica/adansonia (pods) and situated on a plate inoculated with *E. faecalis*

**Table 2:** Multiple comparisons between of *A. senegal*, *C. decidua*, *C. micracantha*, *A. nilotica*, *D. glabra* & *M. olifera* for their anti-bacterial activity against *E. faecalis* using least significant difference (LSD) and level of significance 0.05.

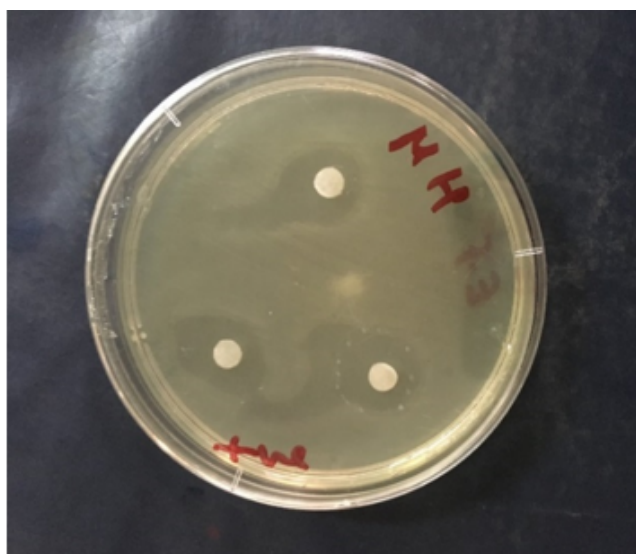
(I) Plant2 (J)		Mean Difference (I-J)	Std. Error	P-Value	95% Confidence Interval	
					Lower Bound	Upper Bound
Acacia senegal	C. decidua	-1.333	2.414	.585	-6.296	3.629
	C. micracantha (L)	-2.667	2.414	.279	-7.629	2.296
	C. micracantha (B)	2.667	2.414	.279	-2.296	7.629
	A. nilotica (L)	-7.333	2.414	.005	-12.296	-2.371
	A. nilotica (P)	-6.333	2.414	.014	-11.296	-1.371
	D. glabra (L)	-0.333	2.414	.891	-5.296	4.629
	D. glabra (B)	-4.000	2.414	.110	-8.963	0.963
	M. olifera	6.333	2.414	.014	1.371	11.296
	NaOCL (1%) (+ve)	-3.333	2.414	.179	-8.296	1.629
	CHX (0.2%) (+ve)	-7.333	2.414	.005	-12.296	-2.371
	C. micracantha (L)	-1.333	2.414	.585	-6.296	3.629
	C. micracantha (B)	4.000	2.414	.110	-0.963	8.963
	A. nilotica (L)	-6.000	2.414	.020	-10.963	-1.037
C. decidua (T)	A. nilotica (B)	-5.000	2.414	.048	-9.963	-0.037
	D. glabra (L)	1.000	2.414	.682	-3.963	5.963
	D. glabra (P)	-2.667	2.414	.279	-7.629	2.296
	M. olifera	7.667	2.414	.004	2.704	12.629
	NaOCL(1%) (+ve)	-2.000	2.414	.415	-6.963	2.963
	CHX (0.2%) (+ve)	-6.000	2.414	.020	-10.963	-1.037
	C. micracantha (B)	5.333	2.414	.036	0.371	10.296
	A. nilotica (L)	-4.667	2.414	.064	-9.629	0.296
	A. nilotica (P)	-3.667	2.414	.141	-8.629	1.296
	D. glabra (L)	2.333	2.414	.343	-2.629	7.296
C. micracantha (L)	D. glabra (B)	-1.333	2.414	.585	-6.296	3.629
	M. olifera	9.000	2.414	.001	4.037	13.963
	NaOCL (1%) (+ve)	-0.667	2.414	.785	-5.629	4.296
	CHX (0.2%) (+ve)	-4.667	2.414	.064	-9.629	0.296
	A. nilotica (L)	-10.000	2.414	.000	-14.963	-5.037
	A. nilotica (P)	-9.000	2.414	.001	-13.963	-4.037
	D. glabra (L)	-3.000	2.414	.225	-7.963	1.963
	D. glabra (B)	-6.667	2.414	.010	-11.629	-1.704
	M. olifera	3.667	2.414	.141	-1.296	8.629
	NaOCL (1%) (+ve)	-6.000	2.414	.020	-10.963	-1.037
C. micracantha (B)	CHX (0.2%) (+ve)	-10.000	2.414	.000	-14.963	-5.037
	A. nilotica (P)	1.000	2.414	.682	-3.963	5.963
	D. glabra (L)	7.000	2.414	.008	2.037	11.963
	D. glabra (B)	3.333	2.414	.179	-1.629	8.296
	M. olifera	13.667	2.414	.000	8.704	18.629
	NaOCL (1%) (+ve)	4.000	2.414	.110	-0.963	8.963
	CHX (0.2%) (+ve)	0.000	2.414	1.000	-4.963	4.963
	D. glabra (L)	6.000	2.414	.020	1.037	10.963
	D. glabra (B)	2.333	2.414	.343	-2.629	7.296
	M. olifera	12.667	2.414	.000	7.704	17.629
A. nilotica (L)	NaOCL (1%) (+ve)	3.000	2.414	.225	-1.963	7.963
	CHX (0.2%) (+ve)	-1.000	2.414	.682	-5.963	3.963
	D. glabra (B)	-3.667	2.414	.141	-8.629	1.296
	M. olifera	6.667	2.414	.010	1.704	11.629
	NaOCL(1%) (+ve)	-3.000	2.414	.225	-7.963	1.963
	CHX (0.2%) (+ve)	-7.000	2.414	.008	-11.963	-2.037
	M. olifera	10.333	2.414	.000	5.371	15.296
	NaOCL(1%) (+ve)	0.667	2.414	.785	-4.296	5.629
	CHX (0.2%) (+ve)	-3.333	2.414	.179	-8.296	1.629
A. nilotica (P)	NaOCL (1%) (+ve)	-9.667	2.414	.000	-14.629	-4.704
	CHX (0.2%) (+ve)	-13.667	2.414	.000	-18.629	-8.704
	NaOCL (1%) (+ve) CHX (0.2%)(+ve)	-4.000	2.414	.110	-8.96	0.96
D. glabra (L)						
D. glabra (B)						
M. olifera						

(P-values are presented on the table).

L: Leaves, B: Branches, P: Pods, NaOCL: Sodium hypochlorite (Clorox), CHX: Chlorhexidine



**Figure 3:** Zones of inhibition which observed surrounding the three discs which saturated with sodium hypochlorite (1%) and situated on a plate inoculated with *E. faecalis*



**Figure 4:** Zones of inhibition which observed surrounding the three discs which saturated with chlorhexidine (0.2%) and situated on a plate inoculated with *E. faecalis*

Although *Acacia senegal* was found to have a minimal inhibition zone and was considered inactive against *Enterococcus faecalis* according to the activity scale proposed by Alves et al.<sup>10</sup>, and Mukhtar & Ghor<sup>11</sup>, Nuha et al. discovered that by altering the processing method of *Acacia senegal*, it can be transformed from inactive to active antibacterial. (Nuha Elmubarak et al. unpublished data)

The leaves and pods of *Acacia nilotica* demonstrated remarkable efficacy against *E. faecalis*, with the leaves exhibiting the highest effectiveness among all the plants

studied. Their performance surpassed that of 1% sodium hypochlorite and was comparable to that of 0.2% chlorhexidine. Nevertheless, research conducted by Amita Yadav and colleagues indicated that the antibacterial properties of the ethanolic extract of *Acacia nilotica* leaves were inferior to those of ethyl acetate and methanolic extracts when assessing their activity against *Enterococcus faecalis* (ATCC 29212).<sup>13</sup> Consequently, the authors suggested that further investigations including various solvent polarity extracts of *Acacia nilotica* leaves should be conducted on teeth infected with *E. faecalis* to determine the most effective extract for application as an endodontic irrigant.

In the current study, it was observed that the ethanolic extract of *Moringa oleifera* leaves exhibited the least antibacterial activity when compared to other plants that were studied. In fact, it was found to be inactive against *E. faecalis* as per Alves et al.<sup>10</sup>, and Mukhtar & Ghor<sup>11</sup>. Even though the *Moringa oleifera* tree is often referred to as the miracle tree, this outcome was not surprising, given that Moyo Busani and colleagues discovered that the acetone and water extract, at a concentration of 5mg/ml, did not have any antibacterial activity against *E. faecalis*.<sup>14</sup>

The study found that Chlorhexidine (0.2%) had a larger inhibition zone diameter against *E. faecalis* than sodium hypochlorite (1%), which is consistent with previous studies by Ferraz et al.<sup>15</sup> and Echeverri & Alderete.<sup>16</sup>

Microbial infections have become a significant clinical threat due to the development of microbial resistance to existing antimicrobial agents. Thus, it is essential to conduct antimicrobial susceptibility testing and compare potential medicinal plants. The current research demonstrates that *Acacia nilotica/adansonii* represents the most potent alternative in combating *E. faecalis*. Nonetheless, the findings from the agar disc diffusion method remain ambiguous, and the study is subject to certain limitations, as the size of the inhibition zones produced by the plants may be influenced by the plant's capacity to diffuse through the agar rather than its actual antimicrobial efficacy. Consequently, the authors advocate for further investigation to substantiate these findings.

## 5. Conclusion

Out of all the plants studied, *Acacia nilotica/Adansonii* leaves have the most potent antibacterial effect against *E. faecalis*. Their antibacterial power is even greater than 1% sodium hypochlorite and is equivalent to 0.2% chlorhexidine. Accordingly, *Acacia nilotica* leaves can be a perfect endodontic irrigant to replace the gold standard. However, more investigations are needed to elucidate its suitability for clinical use.

On the other hand, *Moringa olifera* displayed the weakest antibacterial activity and is considered inactive against *E. faecalis*.



## 6. Conflict of Interest

None.


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