Title: In vitro investigation of antibacterial activity of Gum Arabic prepared by two different processing methods against Enterococcus faecalis

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Abstract:

Background

E. faecalis is a known cause of endodontic treatment failure. Synthetic drugs have been preferred for over decades but recently many plants have been reported for their antibacterial activity. The aim of this study is to investigate the antibacterial effect of mechanically ground and spray dried Acacia senegal against Enterococcus faecalis.

Method:

Antibacterial susceptibility test against Enterococcus faecalis (ATCC 29212) has been performed for 200mg of ethanolic extracts of spray dried & mechanically ground Gum Arabic. Sodium Hypochlorite (1%), Chlorhexidine (0.2%) and Antibiotic multi disc for gram positive bacteria have been used as positive controls and ethanol as a negative control. The diameter of the inhibition zones have been measured.

Results: spray dried Gum Arabic (14.67 mm) was significantly higher than mechanically ground (8.67mm). (p-value=0.02)

Gum Arabic, with both processing methods, exhibited lower antibacterial activity against E. faecalis than chlorhexidine (0.2%).The result was significant with

mechanically ground variant (P-value=.005), but the spray dried Gum showed no significance.

Spray dried Gum Arabic displayed significantly higher antibacterial activity against E. faecalis than (Tetracycline 300mcg) (p-value=.0049). While mechanically ground showed lower antibacterial activity than Tetracycline with no statistical significance.

Conclusion: Method of Gum Arabic processing affected its antibacterial potency. Spray-dried Gum Arabic is considered an active antibacterial agent against E. faecalis, while mechanically ground type is considered a non-active.

46 **Importance:**

47 Enterococcus faecalis has the ability to colonize and hide within the dentinal
48 tubules in the tooth. Dentinal tubules are not accessible by instrumentation during
49 endodontic treatment, they can only be reached and cleaned by irrigation. The
50 problem faced by dentists is that E. faecalis is resistant to sodium hypochlorite, the
51 standard irrigant for endodontic microbiota.

52 The possibility of using Gum Arabic as a natural antibacterial agent against E.
53 faecalis will help to improve the oral health with low cost and with no side effects
54 commonly associated with synthetics antibacterial agents.

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58 **Main Text:**

59 **Introduction:**

60 Enterococcus faecalis is a gram-positive, facultative anaerobic coccus bacteria. It
61 can survive under harsh conditions (1, 2). They are part of the habitual flora in the
62 mouth, human gastrointestinal tract, and female genital tract. E. faecalis is a known
63 cause of endodontic treatment failure as well as some systemic diseases. They have
64 developed high resistance to antimicrobial agents (1).

65 Isolates from oral infections differ from hospital-derived isolates as they do not
66 present many mobile genetic elements. However, oral isolates have virulence
67 factors related to adhesion and biofilm formation, which help the bacteria to
68 colonize different oral sites. Moreover, oral strains may also carry specific
69 antibiotic resistance determinants that have the potential to be transferred to other
70 pathogenic bacteria in biofilm communities (2).

71 Synthetic drugs have been preferred for decades, but now herbal medicines are
72 being used. Recently, several plants have been investigated for their ability to
73 inhibit the growth of microorganisms(3). Screening of promising medicinal plants
74 is ongoing now and then.
75 Gum Arabic "Acacia Senegal" has been considered one of 10 promising medicinal
76 plants in Africa (4). It is a naturally occurring gum that exudes from Acacia trees
77 (5). Sudan is the leading producer of Acacia Gums worldwide, followed by

Nigeria, Chad, Mali, and Senegal (6). Phytochemical screening of Acacia senegal has displayed the presence of tannins, glycosides, and flavonoids, in addition to oxidases, peroxidases, and pectinases, which have antimicrobial properties (7-10).

Many studies have proved the antibacterial efficacy of Gum Arabic against various bacterial species (7, 8, 11-15).

A considerable number of medicinal plants have been investigated for their antimicrobial potency against Enterococcus faecalis (16). No studies consider the effect of Gum Arabic against Enterococcus faecalis.

Gum Arabic "Acacia Senegal" is processed differently according to the required quality. Mechanical milled Gum Arabic, on which the dried Gum nodules are crushed mechanically to produce a granular powder. Spray dried Gum Arabic, on which solution of Gum Arabic is pasteurized and sprayed into a stream of hot air to completely evaporate the water leaving the dry powder of Gum Arabic. Gum Arabic produced by spray drying has an advantage over raw gum as it is free of microbial contamination and dissolves much faster. (10)

The aim of this study is to investigate the antibacterial effect of mechanically ground and spray-dried Gum Arabic "Acacia senegal" against Enterococcus faecalis.

97 **Material and method:**

98 **Collection of the Plant:**

99 Gum nodules have been collected and authenticated at the Herbarium of Medicinal
100 and Aromatic Plants & Traditional Medicine research institute with code number
101 G-1983-1- MAPTRI-H. After that, nodules have air-dried at room temperature
102 away from the sun and processed in two different processing methods, spray
103 drying and mechanically grounding with a pestle and mortar.

104 **Preparation of extract:**

105 One hundred and fifty grams of each type of Gum Arabic have soaked in ethanol
106 (70%) for three days with daily filtration. The solvent has been evaporated under
107 reduced pressure using rotary evaporator apparatus. The extracts of both types of
108 Gum Arabic have been inserted in a freeze dryer till complete dryness.

109 **Antibacterial susceptibility test:**

110 Antibacterial susceptibility test has performed by Agar disc diffusion
111 according to the method of Elkhateeb 2017 (17) with the following steps:

112 **1. Preparation of the media:** Muller-Hinton Agar (HIMEDIA, REF. M173-500G)
113 has been prepared according to instructions. Each 20 mL of the freshly prepared
114 and autoclaved Muller-Hinton agar has been poured into a sterile petri dish and
115 maintained at room temperature to cool down. Before use, the plates were
116 incubated at 35 °C for 48 hours, and the sterility was checked.

117 **2. Preparation of discs:** Filter paper discs of 6 mm diameter have been
118 prepared from Whatman filter paper No. 1, placed in a petri dish, and sterilized
119 in a hot air oven at 160 °C for two hours.

120 **3. Preparation of extracts solutions:** 200 mg of each extract has been dissolved
121 in 1ml of solvent (ethanol 70%) and mixed in a vortex to ensure complete
122 dissolution.

123 **4. Reactivation and inoculation of Enterococcus faecalis:** E. faecalis (ATCC
124 29212) has reactivated into 5ml of brain heart infusion (BHI) broth (HIMEDIA,
125 REF. M210-500G) and left under anaerobic conditions for 48 hours. A sterile
126 cotton swab has been used to inoculate E. faecalis from BHI broth into a
127 Muller Hinton Agar plate. The plate was incubated under anaerobic conditions
128 at 37◦ for 24 hours.

129 **5. Preparation and standardization of inoculum suspension:** Three to five
130 well-isolated colonies of the same morphological type have been selected from
131 a Muller Hinton Agar plate culture and transferred with a sterile loop into a
132 tube containing 5 ml of BHI broth. The broth was incubated anaerobically at
133 37 °C for 24 hours. The turbidity of E. faecalis suspension has been adjusted
134 equal to that of 0.5 McFarland standard (1.5×10^8 CFU/ml) by using BHI
135 broth to dilute of E. faecalis suspension.

136 **6. Antibacterial susceptibility test:**

137 Sterile cotton swab was rolled in the adjusted bacterial suspension to streak the
138 five plates' surface of Muller Hinton Agar.

139 Each three sterile discs have immersed in 10 µL of the following extracts
140 solutions:

141 - Spray-dried Gum Arabic
142 - Mechanically ground Gum Arabic

143 Ethanol (70%) (Reagents Duksan, Ethyl Alcohol, absolute, product No.6923, 2.5L)
144 has been used as a negative control.

145 Chlorhexidine 0.2% (Clenora mouthwash, BP 0.2%w/v), Sodium hypochlorite 1%
146 (Prevest Denpro Hyposol, 3% Sodium hypochlorite 500 ml), and Readymade
147 multidisc for Gram-positive isolates (Axiom laboratories, Code No. 001, Mantola
148 Pahar Ganj- New Delhi-110055) have used as positive controls.

149 The antibiotics included in the multidisc for Gram-positive have displayed in
150 (Figure 1).

151 The saturated discs have transferred to their coded plates which inoculated with E.
152 faecalis. The plates have been incubated at 37°C for 24 hours under anaerobic
153 conditions. The plates have been observed for the formation of a clear zone

154 around each disc (inhibition zones). The diameter of the inhibition zone has been

155 measured.

156

157 **Results:**

158 Gum Arabic with different processing method inhibited the growth of E. faecalis
159 with variant degrees. The inhibition zone measurements reflect the degree of
160 antibacterial activity (Table 1) (Fig. 2-7). Data has been analyzed using SPSS 20.

161 Gum Arabic, with both processing methods, displayed antibacterial activity against
162 E. faecalis. However, the inhibition zone of the spray-dried Gum Arabic (14.67
163 mm) was significantly higher than mechanically ground (8.67mm) (level of
164 significance≤0.05, P-value =0.02).

165 Gum Arabic, with both processing methods, exhibited lower antibacterial activity
166 against E. faecalis than chlorhexidine (0.2%). The result was significant with the
167 mechanically ground variant, but the spray dried Gum showed no significance (P-
168 Values in Table 2).

169 Spray-dried Gum Arabic displayed a greater diameter of the inhibition zone
170 (14.67mm) than 1% Sodium hypochlorite (12mm), but the mechanically ground
171 type showed lower activity (8.67mm). However, both results showed no statistical
172 significance (P-values in Table 2).

173 Only Ciprofloxacin 5mcg, Levofloxacin 5mcg, Cotrimoxazole 25mcg &
174 Tetracycline 300mcg of antibiotics in the multidisc for Gram-positive bacteria
175 exhibited antibacterial activity against E. faecalis (Table 1).

One sample t-test was used to check the significant difference when comparing Gum Arabic to antibiotic multidisc (Tables 3, 4, and 5).

Gum Arabic, with both processing methods, displayed significantly lower antibacterial activity to E. faecalis than (Ciprofloxacin 5mcg) & (Levofloxacin 5mcg) (P-values in Table 3).

Spray-dried Gum Arabic (14.6 mm) showed almost the same antibacterial effect against E. faecalis as Cotrimoxazole 25 mcg (15mm), but the result was statistically not significant. However, mechanically ground Gum Arabic was significantly lower than Cotrimoxazole (P-values in Table 4).

Spray-dried Gum Arabic displayed significantly higher antibacterial activity against E. faecalis than (Tetracycline 300mcg). In contrast, mechanically ground showed lower antibacterial activity than Tetracycline with no statistical significance (P-values in Table 5).

Chlorhexidine (0.2%) showed a greater inhibition zone diameter (16 mm) against E. faecalis than sodium hypochlorite (1%) (12mm). However, the result was statistically insignificant (level of significance≤0.05, P-value=0.11).

Discussion:

Spray-dried Gum Arabic was found to be active antibacterial against Enterococcus faecalis. In contrast, mechanically ground Gum was found to be inactive antibacterial. The activity scale used in the study referred to Alves et al.(18), and Mukhtar & Ghori (19), who regarded an inhibition zone less than 9mm (inactive product), 9-12mm (partially active product), 13-18mm (active product) , and more than 18mm(very active product).

The chemical composition of Gum Arabic and its pharmacological properties may not always be consistent or stable. It may vary depending on several climatic factors, edaphic conditions, season, age of the tree from which the gum is collected, and other variables (20).

The current study has displayed better antibacterial activity of spray-dried Gum Arabic against E. faecalis over the mechanically ground type. This result clearly reflects the effect of the processing method on the antibacterial activity of Gum Arabic against E. faecalis. However, variation in the processing method does not necessarily affect the antibacterial activity of Gum Arabic against all bacterial specie. It has been found that variation in the processing method did not affect the antibacterial activity of Gum Arabic against Streptococcus mutans.(N. Elmubarak, Y. Ibrahim, A. Gareeballah, and N. Sanhouri submitted for publication)

Based on the current study, the authors agreed with Ali and his colleagues (20), who recommended using of a single source of Gum Arabic for research work to achieve standardization for different studies.

Antibacterial activity of spray dried Gum Arabic and mechanically ground is also differ in their statistical significance when compare them to chlorhexidine and sodium hypochlorite

This study has displayed that Chlorhexidine (0.2%) has greater antibacterial activity against E. faecalis than Sodium hypochlorite (1%). This result come in corroborates with Ferraz et al. (21) and Echeverri & Alderete (22)

Conclusion:

The method of Gum Arabic processing affected its antibacterial potency. Spray-dried Gum Arabic is considered an active antibacterial agent against E. faecalis, while mechanically ground type is regarded as non-active.

Spray-dried Gum Arabic has higher antibacterial activity against E. faecalis than Tetracycline 300mcg.

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281

Figures

Figure 1: Multiple antibiotic disc (Axiom laboratories, multidisc for Gram positive isolates, Code No. 001, Mantola Pahar Ganj- New Delhi-110055)

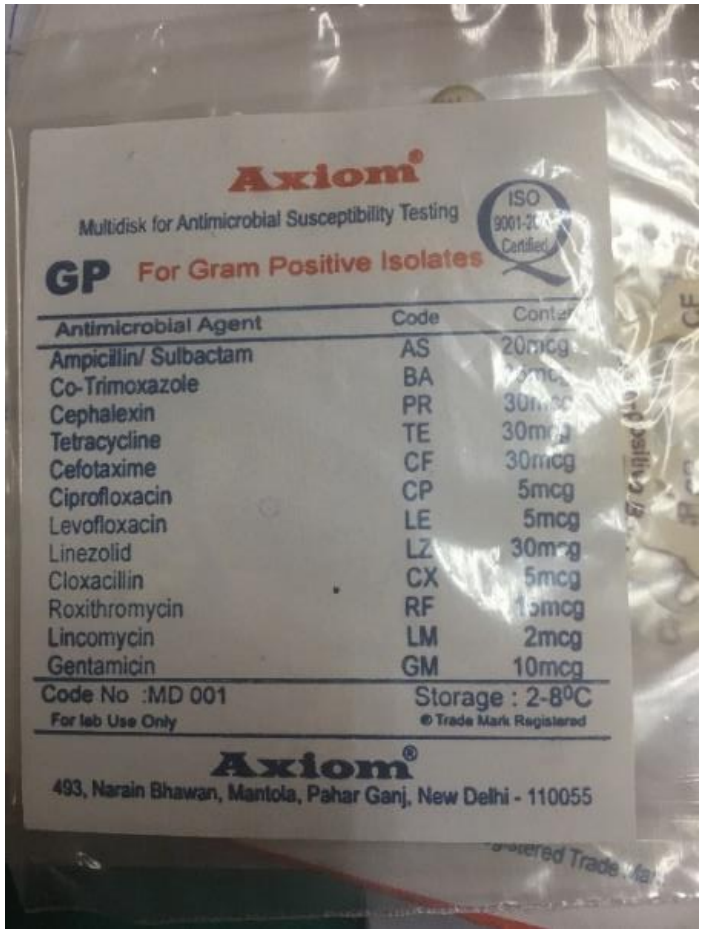

Figure 2: Inhibition zones around a disc saturated with spray dried Gum Arabic on plates inoculated with E. faecalis

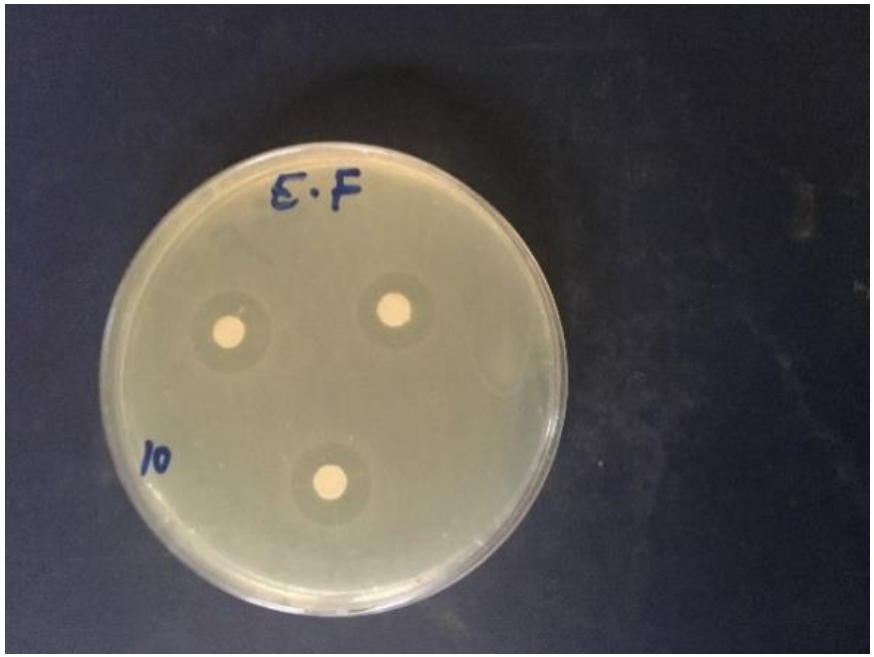

Fig. 3: Inhibition zones around a disc saturated with mechanically ground Gum Arabic on plates inoculated with E. faecalis

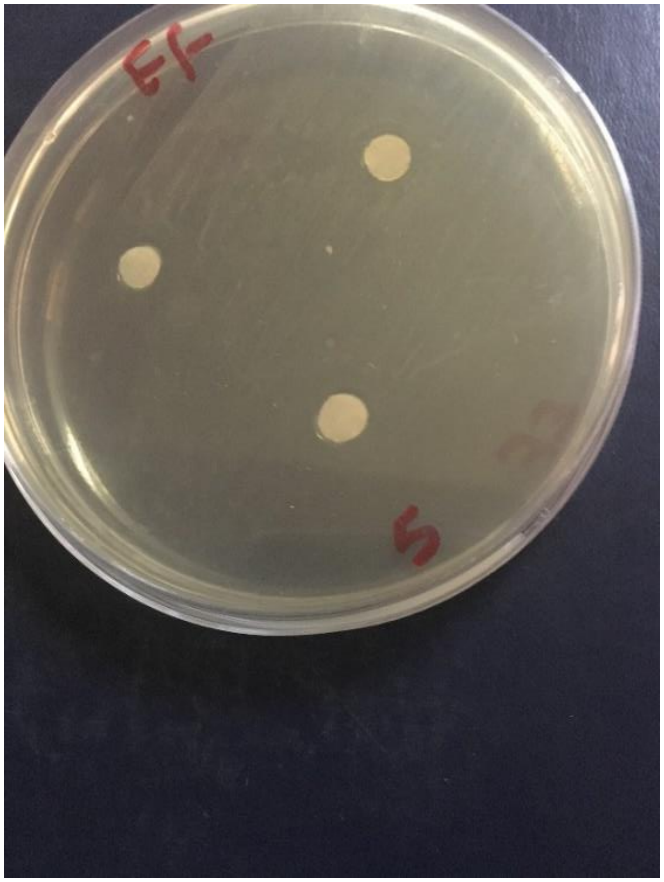

Fig. 4: Inhibition zones around a disc saturated with Sodium hypochlorite 1% (Hyposol) wash on plates inoculated with E. faecalis

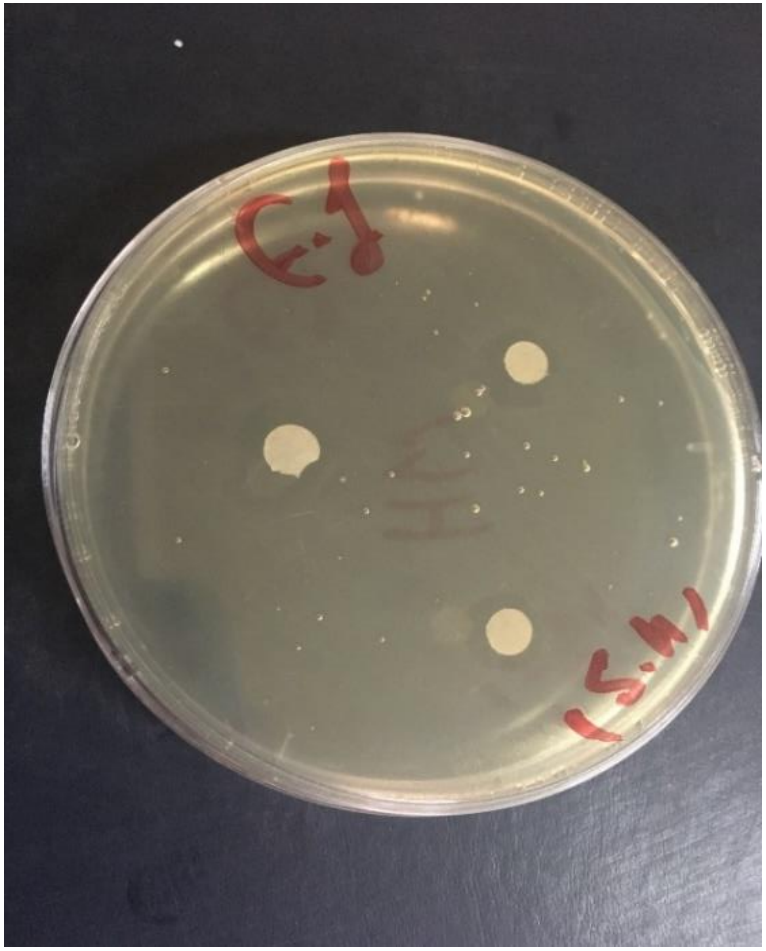

Fig. 5: Inhibition zones around a disc saturated with Chlorhexidine 0.2% (positive control) on plates inoculated with E. faecalis

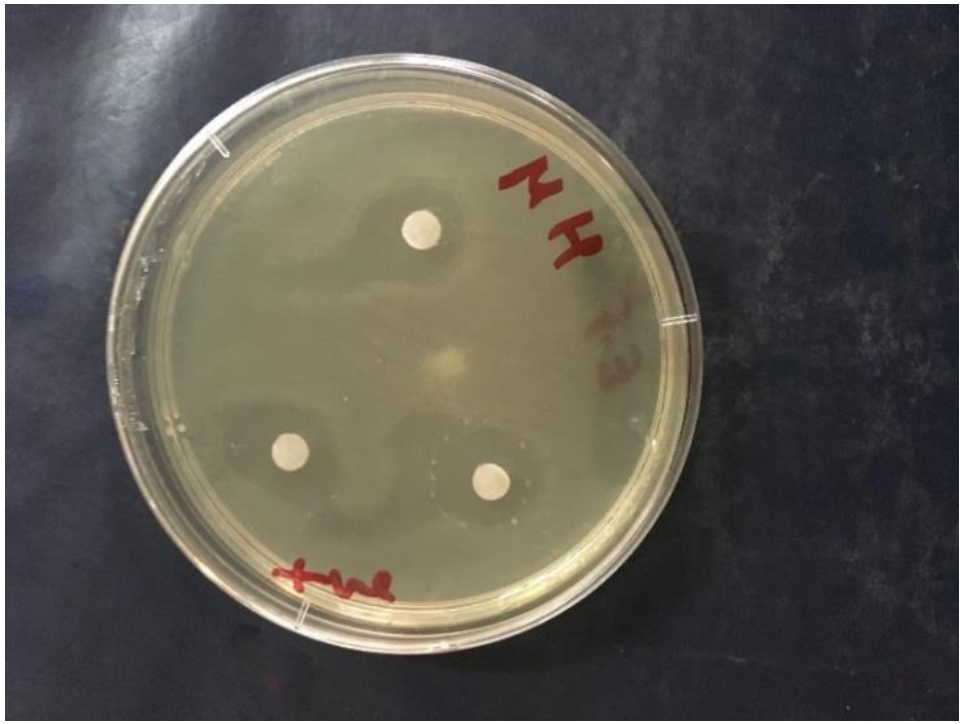

Fig. 6: Inhibition zones around a disc multidisc antibiotic for Gram positive bacteria (positive control) on plates inoculated with E. faecalis

Fig. 7: Inhibition zones around a disc saturated with Ethanol 70% (negative control) on plates inoculated with E. faecalis

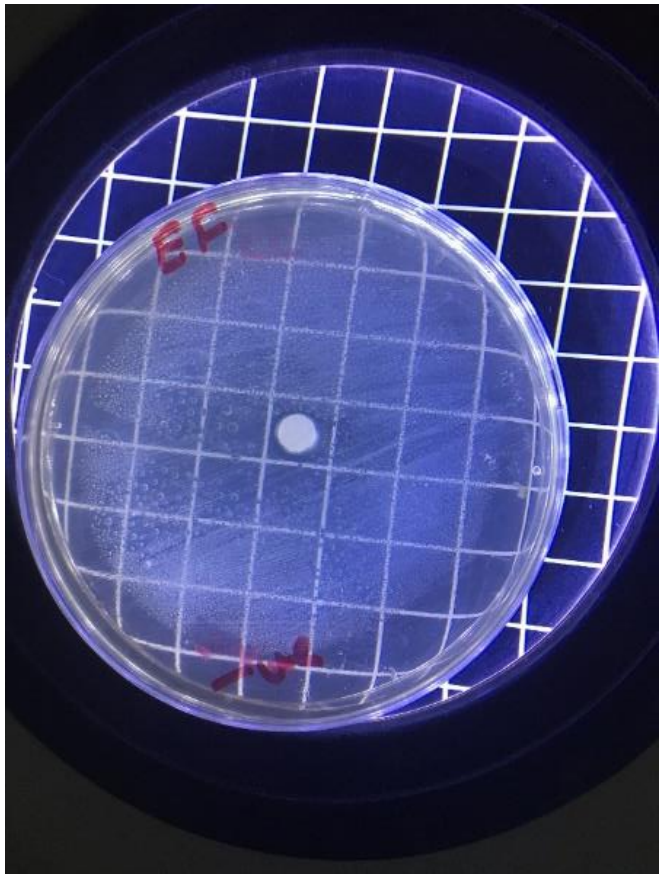

Table 1: Measurements of the inhibition zones of Gum Arabic against E. faecalis.

Plant extract (200mg/ml)	Inhibition zone diameter (mm)					
	M1	M 2	M3	N	mean	Std. dev.
Spray dried Gum Arabic	14	15	15	3	14.67	0.58
Mechanically Gum Arabic	9	8	9	3	8.67	0.58
Ethanol 70% (-ve)	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
CP (Ciprofloxacine 5mcg)	20	-	-	1		
LE (Levofloxacine 5mcg)	20	-	-	1		
BA (Co-Trimoxazole 25 mcg)	15			1		
TE (Tetracycline 300mcg)	10			1		

(M1), (M2), and (M3): measurements of inhibition zones around first, second and third discs

and (N): number of measurements for each extract

Table 2: Multiple comparisons between spray dried and mechanically milled Gum Arabic chlorhexidine, Sodium Hypochlorite and antibiotics for their antibacterial activity against E. faecalis using least significant difference (LSD). P-values are presented on the table and the level of significance is 0.05.

(I) Plant2	plant (J)	Mean Difference (I-J)	Std. Error	P-Value	95% Confidence Interval	
					Lower Bound	Upper Bound
Spray dried Gum	Mechanically ground Gum	6.000	2.414	.020	1.037	10.963
	Ethanol 70% (-ve)	14.667	2.414	.000	9.704	19.629
	NaOCL(1%) (+ve)	2.667	2.414	.279	-2.296	7.629
	CHX (0.2%) (+ve)	-1.333	2.414	.585	-6.296	3.629
Mechanically ground Gum	Ethanol 70% (-ve)	8.667	2.414	.001	3.704	13.629
	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

NaOCL: Sodium hypochlorite (Clorox),

CHX: Chlorhexidine

Table 3: One sample t-test statistics to compare the inhibition zone diameter (mm) of Gum Arabic against **E. faecalis** to the inhibition zone of (Ciprofloxacin 5mcg) and (Levofloxacin 5mcg). P-Values are presented on the table and the level of significance is 0.03. **(Test Value = 20)**

No.	Plant extract	Mean of Inhibition zone diameter (mm)	Std. Deviation	t	P-Value	Comment
(1)	Spray dried Gum Arabic	14.67	0.58	-16.000	.004	S
(2)	Mechanically milled Gum Arabic	8.67	0.58	-34.000	.001	S

Table 4: One sample t-test statistics to compare the inhibition zone diameter of Gum Arabic against E. faecalis to the inhibition zone of CoTrimoxazole 25 mcg. P- Values are presented in the table and the Level of significance is 0.03 **(Test Value = 15)**

No.	Plant extract	N	Mean of Inhibition zone diameter (mm)	Std. Deviation	P-Value	Comment
(1)	Spray dried Gum Arabic	3	14.6667	.57735	.423	NS
(2)	Mechanically milled Gum Arabic	3	8.6667	.57735	.003	S

NS: Not significant**, S:** Significant

Table 5: One sample t-test statistics to compare the inhibition zone diameter (mm) of Gum Arabic against E. faecalis to the inhibition zone of Tetracycline 300mcg. P-values are presented on the table and the level of significance is 0.03 **(Test value =10)**

No.	Plant extract	N	Mean	Std. Deviation	P-Value	Comment
(1)	Spray dried Gum Arabic	3	14.667	0.577	.0049	S
(2)	Mechanically milled Gum Arabic	3	8.667	0.577	.057	NS

NS: Not significant**, S:** Significant