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# Antimicrobial Potential of Grape Seed Extract and MTA: A Comparative Experimental Study

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**Abstract**

**AIM:** This study aims to evaluate and compare the antibacterial potential of mineral trioxide aggregate (MTA) and grape seed extract (GSE) against *Streptococcus mutans*, *Enterococcus faecalis*, *Staph aureus*, *Candida albicans*, *Lactobacillus* spp., and *Streptococcus sobrinus*.

**HYPOTHESIS:** We hypothesized that GSE could have potent antimicrobial effect against oral pathogens when compared to MTA.

**MATERIALS AND METHODS:** MTA and GSE were utilized to determine the antibacterial effectiveness against *S. mutans*, *E. faecalis*, *S. aureus*, *C. albicans*, *Lactobacillus* spp. and *S. sobrinus* using the agar well diffusion test. The tested materials were used according to the manufacturer's instructions and put into the prepared wells of agar plates; diluted inocula ($10^5$ and $10^6$ CFU/ml) of the tested microorganism strains were also used. For bacteria, all plates were incubated at 37°C in anaerobic conditions, and for *C. albicans*, at 30°C. The inhibitory zones were determined after 3 days. A digital caliper was used to measure the diameter of bacterial inhibition zones surrounding each well to the nearest size in mm.

**RESULTS:** GSE-created inhibition zones against all tested microorganisms except *C. albicans*, zones were significantly larger than MTA-created zones ($p < 0.05$).

**CONCLUSION:** Both MTA and GSE showed antimicrobial effect against all tested microorganisms except *C. albicans*, which will pave the route to use GSE as a natural herbal substitute of MTA.

## Introduction

Treatment of exposed vital pulp by direct pulp capping necessitates the covering of the exposed area with a capping material to promote the formation of a protective barrier and retain pulp vitality [1], [2]. One of the major goals in dentistry is to provide new pulp capping materials that have remineralizing properties and adhere to dentin via a mineral-rich interface [3]. The ideal pulp capping material should provide a suitable media for dentin bio mineralization while also having antibacterial properties to prevent further pulp injury caused by bacteria present in the dental pulp tissue. However, due to the survival and proliferation of some microorganisms within dentin, pulp, and/or periapical tissues, the treatment's long-term success may be in doubt [4].

*E. faecalis*, a difficult Gram-positive bacterium with a substantially greater risk of endodontic treatment failure, has received special attention. Invading and developing within dentinal tubules, colonizing the root canal, and reinfecting root-filled teeth, this microbe has the ability to survive in extremely alkaline pH conditions with scant nutrition [5]. Fungi are identified in the primary infection of root canals on rare occasions, but they are more common in teeth obturated with treatment-resistant lesions. *Candida albicans* is the most common fungus species, a microbe with a preference for dentin and resistance to several intracanal medicaments, such as calcium hydroxide-based ones [6]. Streptococci, Staphylococci, Lactobacilli, and filamentous germs are among the bacteria that enter the dentin prior to cavitation, and they are predominantly facultative anaerobes linked to persistent infections [7].

Since the introduction of "Mineral Trioxide Aggregate" MTA in 1993 by Torabinejad, who demonstrated reparative dentin production by odontoblast-like cells, MTA has gained widespread acceptance in dentistry [8]. Researchers confirmed that the primary ions in MTA were calcium and phosphorus, and that tiny hydrophilic particles such as tricalcium silicate, tricalcium aluminate, tricalcium oxide, and silicate oxide were the key components [9]. Although the potential antimicrobial effect against *Pseudomonas aeruginosa, Enterococcus faecalis, Staphylococcus aureus,* and *Escherichia coli*, its shortcomings employed a great demand for material development. High cost, long setting time, and discoloration are considered the main drawbacks of MTA [10]. Recently, several herbal products

were introduced with great antimicrobial potential. Grape seed extract [GSE] is one among these herbal products which inhibits a wide range of Gram-negative and Gram-positive bacteria, but it is particularly efficient against Gram-positive bacteria [11]. GSE has been shown to have antibacterial activity against a variety of foodborne bacterial infections, including *Campylobacter jejuni*, *Staphylococcus aureus*, *Helicobacter pylori*, *Aeromonas hydrophila*, *Enterococcus* spp., and others [12]. However, GSE is widely used in dentistry; there is no evidence for using it as pulp capping material.

Thus we hypothesized that (GSE) is expected to be natural herbal substitute to MTA.

So this study aimed to evaluate in vitro the antimicrobial effect of Grape seed extract (GSE) as pulp capping material compared to MTA as gold standard pulp capping material against *S. mutans, E. faecalis, S. aureus, C. albicans, L.spp, and S. sobrinus* which are routinely identified after routine endodontic treatment of an infected root with periapical disease [13] through Agar Diffusion Test.

## Materials and Methods

### *Pulp capping agents*

In the current study, the selected materials for antimicrobial activity were MTA (Angelus, Londrina, PR, Brazil) and GSE (Nu Sci, HerbStore, USA).

### *Preparation of MTA and GSE*

MTA: 0.33 g of water per gram of cement was mixed until the chosen consistency is obtained.

GSE: 6.5 g powder of GSE was collected from capsules containing this powder (Puritans Pride Inc., Oakdale, NY, USA) and dissolved in 100 ml distilled water [14].

The samples were divided into two groups

- Group 1 MTA (control group)
- Group 2 GSE (experimental group)

### *Microorganisms preparation and agar diffusion test*

The tested microorganisms used in this study were *S. mutans* ATCC 25175, *E. faecalis* ATCC 2367, *S. aureus* ATCC 6538, *C. albicans* ATCC 10231, *L. spp* ATCC 4356, and *S. sobrinus* ATCC 27351.

### *Method: Agar well diffusion*

The tested capping agents were used according to the manufacturer's instructions and poured into the wells of agar plates that had been prepared. The antibacterial properties of the materials were investigated using the Agar diffusion technique on Mueller–Hinton agar plates against the tested microorganisms. Mueller–Hinton agar plates seeded with $1.8 \times 10^8$ cfu/mL ($0.5\ OD^{600}$) of the test bacteria. Plates were inspected for the presence of inhibitory zones after a 24-h incubation period at 37°C. The inhibition zones surrounding the wells were measured (mm) considering only halos > 6 mm Inhibition zones obtained are the mean of three replicates for each experiment [15].

### *Statistical analysis*

Data were coded and entered using the Statistical Package for the Social Sciences (SPSS) version 28 (IBM Corp., Armonk, NY, USA). Data were summarized using mean and standard deviation for quantitative variables and frequencies (number of cases) and relative frequencies (percentages) for categorical variables. Comparisons between groups were done using unpaired t test [16]. $p < 0.05$ was considered as statistically significant.

## Results

The antimicrobial efficacy of each material was measured by the diameter of the inhibition zone around each well filled by a material for each kind of bacterium and fungus (Figure 1).

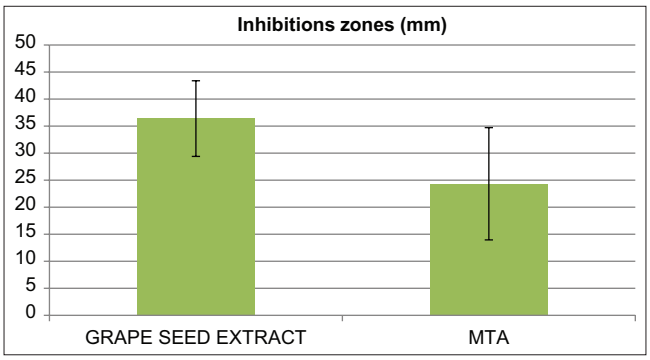


*Figure 1: Bar chart presenting inhibitions zones (mm) of MTA and GSE*

The antimicrobial efficacy was found to be best for Group II (GSE) followed by Group I (MTA) (Table 1) except for *C. albicans* (Figure 1), only group I (MTA) demonstrated the presence of inhibition zone.

**Table 1: Comparison between inhibition zones (mm of MTA and GSE)**

| | Grape seed extract | | MTA | | |
|---|---|---|---|---|---|
| | Mean | Standard deviation | Mean | Standard deviation | p value |
| Inhibitions zones (mm) | 36.40 | 6.99 | 24.33 | 10.39 | 0.049 |

There was a statistically significant difference between antibacterial and antifungal efficacy among two groups *as shown in* (Table 1 and Figures 2 and 3).

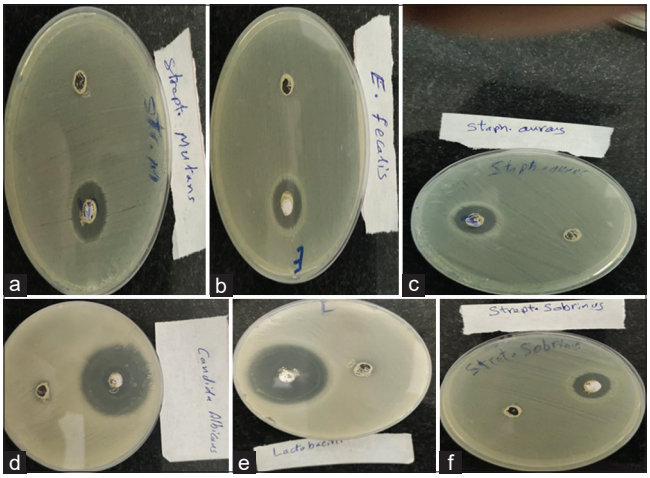


*Figure 2: Zone of inhibition produced by test filling materials, MTA against. (a) S. mutans, (b) E. faecalis, (c) S. aureus, (d) C. albicans, (e) Lactobacillus spp., (f) S. sobrinus*

## Discussion

The current study introduced the use of GSE as a direct pulp capping material. The findings revealed that the antibacterial efficacy was found to be best for Group II (GSE) followed by Group I (MTA) except for *C. albicans*. Our result is consistent with research conducted by Shrestha *et al*. [17] which showed that GSE has potential antimicrobial effects against *S. aureus* while GSE showed minimal or no reactivity against strains of *C. albicans.* This may be contributed to type and concentration of the used solvent to provide (GSE).On the contrary, Song *et al*. [18] reported that GSE has no effect on *S. aureus*.

In accordance with our result Estrela *et al*. [19] demonstrated that MTA revealed antimicrobial activity against *S. aureus, E. faecalis, P. aeruginosa, B. subtilis,* and *C. albicans*. MTA's antibacterial activity is derived from the primary components of MTA, tricalcium silicate, and dicalcium silicate. These constituents hydrate to form alkaline calcium silicate gel in a few hours when mixed with water [20] calcium hydroxide in a silicate matrix releases hydroxide ions, resulting in high alkalinity, which, in turn, creates an unfavorable environment for microbial growth. Fridland and Rosado [21], reported that MTA was able to maintain a high pH in the range of 11–12 for 78 days by releasing its soluble fraction to an aqueous environment over a long period at a decreasing rate. These features of MTA account for the antibacterial effect against the aforementioned bacteria: *S. mutans*, L. *rhamnosus, L.* paracasei, and *P. gingivalis* [22]. It could be speculated that *E. faecalis* would not survive in the vicinity of MTA due to the high alkalinity (pH 11–12) [23] which was in accordance with the results of this study. On the other hand, various investigations have shown that MTA has limited and dubious antibacterial activity [24].

As a result, it seemed reasonable to combine antibacterial agents with MTA in several studies to increase its antibacterial activity. Several studies have attempted to improve its antibacterial capabilities by combining it with different concentrations of NaOCl, K-Y jelly, and different concentrations of CaCl2 [25]. When 0.12% chlorohexidine (CHX) was mixed with MTA, it exhibited much better antibacterial activity than MTA mixed with distilled water [26], but the results also showed that mixing CHX with MTA had a considerable unfavorable impact on the production of reparative dentin after pulp capping [27].

So looking for novel alternative bioactive capping materials with antibacterial activity and lower cost could be a promising alternative for this clinical quandary. Oligomeric proanthocyanidin complexes (OPCs) and proanthocyanidin (grape seed extract) are primarily approved for their antioxidant activities. These chemicals, however, have antibacterial, antiviral, anticarcinogenic, anti-inflammatory, anti-allergic, and vasodilatory properties [28].

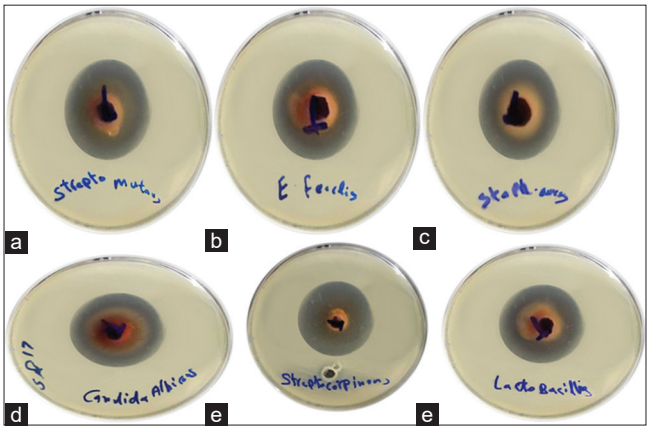


*Figure 3: Zone of inhibition produced by test filling materials, GSE against. (a) S. mutans, (b) E. faecalis, (c) S. aureus, (d) C. albicans, (e) Lactobacillus spp., (f) S. sobrinus*

## Conclusions

Based on the result of the research presented, it was concluded that GSE has a significant antimicrobial effect compared to MTA. The ability for GSE to suppress bacteria discovered in this study offers up new perspectives for its usage as pulp capping materials. Before GSE can be clearly suggested as a pulp capping materials, further preclinical and clinical investigations are required to assess recommended type of solvent with optimum concentration, biocompatibility, safety, and mechanical properties.

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