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Influence of 17% EDTA and *Sapindus mukorossi* on the Surface Property of Protaper Gold Rotary Endodontic Instruments

Riaz Ahmed [1], Ziaullah Choudhry [2], Fazal Ur-Rehman Qazi [1], Sofia Malik [3], Shahbaz Ahmed [1], Sohail Saadat [4], Ahmed A. AlMokhatieb [5], Abdulaziz Abdulwahed [5], Mazen F. Alkahtany [6], Muhammad Adeel Ahmed [7], Khulud A. Al-Aali [8], Fahim Vohra [9,*] and Tariq Abduljabbar [9]

1 Department of Operative Dentistry, Ishrat-Ul-Ebad Khan Institute of Oral Health Sciences, Dow University of Health Sciences, Karachi 74200, Pakistan; drkhanjee@yahoo.com (R.A.); qazi.rehman@duhs.edu.pk (F.U.-R.Q.); saj119@hotmail.com (S.A.)
2 Department of Prosthodontics, Ishrat-Ul-Ebad Khan Institute of Oral Health Sciences, Dow University of Health Sciences, Karachi 74200, Pakistan; ziaullah.choudhry@duhs.edu.pk
3 Department of Dental Materials, Ishrat-Ul-Ebad Khan Institute of Oral Health Sciences, Dow University of Health Sciences, Karachi 74200, Pakistan; sofia.malik@duhs.edu.pk
4 Department of Community Dentistry, Ishrat-Ul-Ebad Khan Institute of Oral Health Sciences, Dow University of Health Sciences, Karachi 74200, Pakistan; sohail.saadat@duhs.edu.pk
5 Department of Conservative Dental Sciences, College of Dentistry, Prince Sattam Bin Abdulaziz University, Al-Kharj 11942, Saudi Arabia; A.almokhatieb@psau.edu.sa (A.A.A.); a.abdulwahed@psau.edu.sa (A.A.)
6 Department of Restorative Dental Science, Division of Endodontics, College of Dentistry, King Saud University, Riyadh 11451, Saudi Arabia; malkahtany@ksu.edu.sa
7 Department of Restorative Dental Sciences, College of Dentistry, King Faisal University, Al Ahsa 31982, Saudi Arabia; mshakeel@kfu.edu.sa
8 Department of Clinical Dental Sciences, College of Dentistry, Princess Nourah Bint Abdulrahman University, Riyadh 11564, Saudi Arabia; kaalaali@pnu.edu.sa
9 Department of Prosthetic Dental Science, College of Dentistry, King Saud University, Riyadh 11545, Saudi Arabia; tajabbar@ksu.edu.sa
* Correspondence: fvohra@ksu.edu.sa; Tel.: +966-1467-8639

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Abstract: The aim of the study was to evaluate the influence of 17% ethylenediaminetetraacetic acid (EDTA) and ethanolic extract of *Sapindus mukorossi* (*S. mukorossi*) in combination with canal shaping on surface properties of ProTaper Gold rotary endodontic file. Sixteen F1 ProTaper Gold rotary files underwent the standard protocol for root canal treatment using two irrigants (*S. mukorossi* and 17% EDTA) in single-rooted decoronated teeth. Eight unused files were used as a control. All files were examined under an atomic force microscope (AFM) to evaluate surface roughness (Ra) and root mean square (RMS). Data were analyzed using the *t*-test. RMS and Ra values of the control file were 1.37 and 0.607, respectively. The mean RMS (3.70 $\pm$ 1.41) and mean Ra (2.89 $\pm$ 1.41) in EDTA group were significantly higher than the control file ($p < 0.05$). The mean RMS in the *S. mukorossi* group (1.77 $\pm$ 0.66) did not show any significant difference with the control ($p > 0.05$). The Ra of *S. mukorossi* group (1.09 $\pm$ 0.05) was significantly higher than the control file ($p < 0.05$). Moreover, the mean RMS and Ra were found to be significantly higher in the EDTA group compared to the *S. mukorossi* group ($p < 0.05$). The mean Ra and RMS values of the ProTaper Gold files used to prepare canals using 17% EDTA and *S. mukorossi* irrigation were significantly higher than the control files. The EDTA-treated ProTaper Gold rotary files demonstrated higher surface Ra and RMS than the *S. mukorossi* files.

Keywords: atomic force microscopy; ProTaper Gold; *Sapindus mukorossi*; EDTA; roughness

1. Introduction

Nickel–titanium (NiTi) alloys were developed in the early 1960s by W. F. Buehler, a metallurgist at the Naval Ordnance Laboratory in Silver Springs, Maryland, USA [1]. NiTi rotary instruments have become the gold standard in the field of dentistry in the last two decades [2]. They have superiority in terms of flexibility and torsional fracture resistance compared to conventional stainless steel (SS) files [3]. Apart from having various benefits, unexpected fractures of NiTi rotary instruments can occur during clinical use due to flexural fatigue and torsional stress [4]. Among various NiTi systems used, Protaper NiTi rotary instruments have been used most commonly in clinical dentistry [5]. The files of the Protaper NiTi rotary system possess different tapers and file lengths, along with passive tips and a convex triangular design. The geometry of the ProTaper Universal system (Dentsply, Sirona, York, PA, USA) and that of the ProTaper Gold rotary system (Dentsply, Sirona, York, PA, USA) are the same with advancement in the metallurgy in the layer system [6]. ProTaper Gold allows greater flexibility and cyclic fatigue, thus respecting the initial anatomy of the S-shaped canals [6].

The aim of root canal treatment (RCT) is to clean and shape the root canals by considering mechanical, chemical and biological objectives [7]. Microorganisms are the key contributing factors in periapical and pulpal diseases. In order to eliminate bacteria from root canal system, the mechanical instrumentation is required which leads to production of layer of debris called the "smear layer" [8]. The literature has reported that the smear layer is critical to root canal treatment failure [9,10]. The presence of a smear layer creates a barrier for the disinfectants to penetrate within dentine chips leading to root canal treatment failure [11].

During canal shaping and cleaning, endodontic files are in continuous contact with the root canal irrigants. However, short-term interaction with these irrigants can influence the surface properties of the NiTi rotary instruments used [12]. Various therapeutic and chemical products for endodontic irrigation include QMix, MTAD, 17% ethylene diamine tetra acetic acid (EDTA), calcium hydroxide, chlorhexidine gluconate, sodium hypochlorite (NaOCl), and saline [13,14]. EDTA acts as a decalcifying agent and removes the inorganic component of the smear layer. However, it does not remove the organic debris attached to the canal surface [15]. Although EDTA is effective in removing the smear layer, there are concerns about dentin erosion. Moreover, the previous literature has revealed that EDTA can cause damaging effects on the NiTi rotary files used for canal preparation due to its low pH, resulting in surface roughness (Ra) and change in root mean square (RMS) [12,16,17]. Sodium hypochlorite (NaOCl) is commonly used as a root canal irrigant; however, it is corrosive towards metals and causes micro-pitting due to removal of nickel. Similarly use of erosive and acidic irrigants can cause 81 microstructural defects leading to crack formation and stress concentrations [18].

In order to overcome this problem, herbal alternatives, i.e., *Sapindus mukorossi* (*S. mukorossi*), have gained the researchers attention for their antifungal, antimicrobial, and anti-inflammatory properties. The major ingredients of the pericarp of its fruits are sugars (10%), saponins (10–11.5%), and mucilage [19]. The surfactant action is because of the higher content of saponins. The detergent-like properties of *S. mukorossi* fruit reduces the surface tension of the irrigant, which leads to the dissolution of water-insoluble substances [20]. When used as an irrigant, it helps eliminate debris, which is components of the smear layer, pulp canal tissues, as well as microbes. Sonawane S et al. and Dhar J et al. reported its effectiveness for dental caries management [21,22]. *S. mukorossi* can be a potential root canal irrigant due to its emulsification and surfactant properties in removing and dissolving debris and smear layers [23]. However, its role as a final irrigant on the surface properties of the NiTi rotary file is still not known and needs investigation. Atomic force microscopy (AFM) has been employed in assessment of metallic alloy–based instruments in endodontics [12]. AFM is based on the principle that a probe scans the investigated surface and its motion is captured and used to create a three-dimensional image. AFM offers versatility as most surfaces, including ceramics, composites, polymers, and biological samples can be assessed [24].

The ProTaper Gold is comparatively a newer rotary system and enough evidence is available related to the effect of irrigants such as EDTA on the surface characteristics of ProTaper Gold [24]. However, the effect of the new irrigant (*S. mukorossi*) on ProTaper Gold rotary system surface properties is still not determined. Therefore, it is hypothesized that there will be no significant differences in Ra and RMS of the ProTaper Gold file after using ethanolic extract of *S. mukorossi* and 17% EDTA solution as compared to unused control files. Moreover, it was also hypothesized that 17% EDTA and ethanolic extract of *S. mukorossi*-treated files will not demonstrate any significant difference in Ra and RMS of ProTaper Gold files. Therefore, the aim of the present study is to evaluate the surface roughness (Ra and RMS) of ProTaper Gold file after interaction with 17% EDTA and ethanolic extract of *S. mukorossi* in combination with root canal shaping in root canal treatment.

2. Materials and Methods

In the present study ProTaper Gold rotary files (F1) (Dentsply, Sirona, York, PA, USA) were included. After examination all the distorted files and files with manufacturing faults were excluded. Sample size was calculated using open epi sample size calculator using means and standard deviations, and the estimated sample size required 8 ProTaper rotary files in each group. Eight untreated files were used as a control. The research and ethics committee at Dr ishrat-ul-Ibad khan Institute of Oral Health sciences approved the research project (IRB-1593/DUHS/Approval/2020).

2.1. Preparation of Ethanolic Extract of S. mukorossi

Desiccated fruit of *S. mukorossi* was used for the formulation of experimental root canal irrigant. The dry pericarps of *S. mukorossi* (1.0 kg), which were present around seed nut, were separated with a sharp blade. These pericarps were blended to produce fine particles in a sterilized home blender (Enviro 3 in 1 juicer blender ENJ 301, Karachi, Pakistan). The resultant 10 grams of powder was soaked in 100 mL of absolute ethanol (99%) for 24 h at room temperature. This solvent was filtered and removed with the help of rotary evaporator and the extract was kept in clean screw-capped vials (Premium vials B4702-12 G with screw cap) at room temperature until use. To form operational concentration of 5mg/mL, the extract was re-dissolved in distilled water.

2.2. Sample Preparation

Single-rooted premolars with straight canals (Schneider method) [25], extracted for orthodontic reasons with developed apices and free of root canal treatments were collected. Attached deposits and plaque were removed with an ultrasonic scaler (Woodpecker, Unicorn DenMart Limited, Delhi, India) and specimen teeth were disinfected with Chloramine T trihydrate (A.B. Enterprises, Mumbai, India) for 48 h at 4 °C. Prior to use, teeth were stored in saline at room temperature. Teeth were decoronated using malleable disc (A-DS Diamond disc 19 mm, thickness 0.17 mm) in order to attain a standardized 15 mm root length. All the specimens were randomly allocated in to two groups on the basis of irrigant received during root canal preparation. Teeth in group A and group B were exposed to *S. mukorossi* (experimental irrigant) and 17% EDTA (MD-Cleanser by Meta Biomed, Eagan, MN, USA), respectively.

The patency and the canal working length was evaluated by #10 K file (Mani Inc., Utsunomiya, Tochigi Ken, Japan) until it was noticeable at the apical foramen (observed under magnifying loupes, 3.5 X-R) length was reduced by 1mm from this point. The canals were cleaned and shaped using ProTaper Gold Rotary System (Dentsply Sirona, Long Island City, NY, USA) at a rotation speed of 300 rpm with a moderate in-and-out motion in a crown-down technique. Initial flaring and shaping was performed with SX and S1 files, followed by ProTaper finishing files (F1). Both irrigating solutions (17% EDTA and *S. mukorossi*) were introduced into the canals of respected roots for approximately 1 min of irrigation using 5 cc irrigants in a disposable syringe having a stainless steel 27-G beveled needle while finishing was performed (F1 files). The needle was placed within 1 to 2 mm of the working length in each canal. Rotary files were placed for finishing procedure in the canals three times (each root) in the presence of irrigants. After root canal preparation, control files and files in the respective irrigant groups, were taken as samples to be examined under atomic force microscope.

2.3. Atomic Force Microscope (AFM) Assessment

All the F1 files were examined for surface changes under AFM. Pre-operative evaluation of new (unused) F1 file was performed for comparison. Instruments were evaluated in the apical 5 mm at 2 points with 2 mm section. AFM probes (curvature < 10 mm) mounted on cantilevers (250 lm), with spring constant of 0.1 N^{-1}m were used. Scanned areas were perfect squares of 1 × 1 μm. Three-dimensional AFM images (400 × 400 lines) were processed with Scala Pro 5.0 software (Omicron NanoTechnology GmbH, Taunusstein, Germany). The surface roughness (Ra) and root mean square (RMS) of the scanned surface profiles were recorded. All the samples (F1 files) were compared at similar sites on the files among the study groups.

2.4. Statistical Analysis

Data were analyzed by Statistical Program for the Social Sciences (SPSS version 25.0, IBM, Armonk, NY, USA). Shapiro–Wilkes test was applied for assessment of normality. Mean and standard deviation (SD) were reported for Ra and RMS. Independent *t*-test was applied to compare mean Ra and RMS between the groups. One-sample *t*-test was applied to compare control file Ra and RMS with the Ra and RMS of both groups (17% EDTA and *S. mukorossi*). ANOVA was applied to compare the three groups overall (control, *S. mukorossi*, and 17% EDTA). A *p*-value of <0.05 was considered significant.

3. Results

The mean, standard deviations, and minimum and maximum values of Ra and RMS measurements among the two test groups are presented in Table 1. The obtained data had normal distribution. RMS and Ra values of the unused control file were 1.37 and 0.607, respectively. Mean RMS in EDTA group (3.70 ± 1.41) (Figure 1) were significantly higher from the control group (Figure 2) ($p < 0.05$). Similarly, mean Ra in EDTA group (2.89 ± 1.41) also demonstrated significantly higher values than the control group ($p < 0.05$). Mean RMS in *S. mukorossi* group (1.77 ± 0.66) did not show any significant difference with the control group ($p > 0.05$). Ra of *S. mukorossi* group (1.09 ± 0.05) (Figure 3) exhibited significantly higher values than the control group ($p < 0.05$) (Table 1). The comparison of Ra and RMS among the control, *S. mukorossi*, and 17% EDTA specimens showed significant differences ($p < 0.001$) (Table 2).

Table 1. Means and standard deviations of RMS and Ra among the study groups.

Group	Surface Assessment	Minimum	Maximum	Mean	SD	*p*-Value *
17% EDTA	RMS	1.06	5.90	3.76	1.42	0.817 **
	Ra	1.14	5.42	2.89	1.41	0.804 **
Sapindus mukorrossi	RMS	1.14	3.06	1.77	0.66	0.209 **
	Ra	1.02	1.16	1.09	0.05	0.368 **

* *t*-test, RMS, root mean square, Ra, roughness. ** Non-significant ($p > 0.05$).

Table 2. Comparison of RMS and Ra among control and test groups.

Surface Assessment	Control (Mean ± SD)	17% EDTA (Mean ± SD)	*Sapindus mukorrossi* (Mean ± SD)	*p* Value §
RMS	1.37 ± 0.51 *p*-value *	3.76 ± 1.41 0.002 **	1.77 ± 0.66 0.132	<0.0001
Ra	0.607 ± 0.15 *p*-value *	2.89 ± 1.41 0.003 **	1.09 ± 0.05 0.001 **	<0.0001

* *t*-test, ** Significant difference ($p \leq 0.05$), § ANOVA.

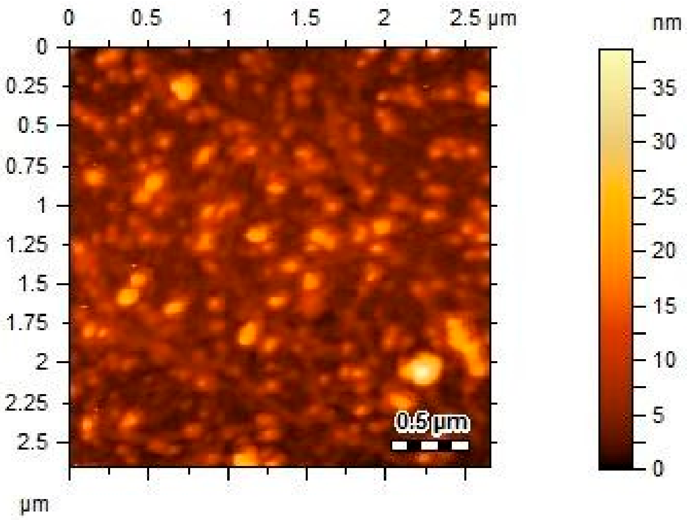

Figure 1. Atomic force microscopy image of F1 file treated with 17% EDTA.

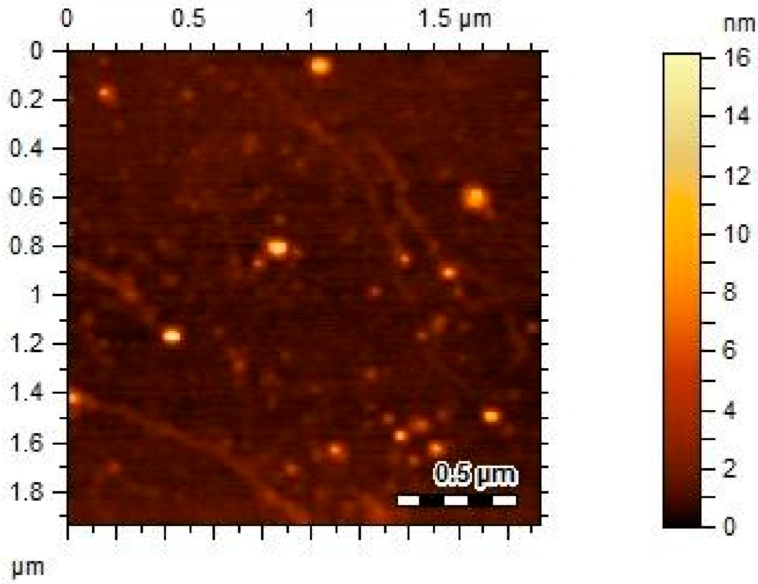

Figure 2. Atomic force microscopy image of control F1 file.

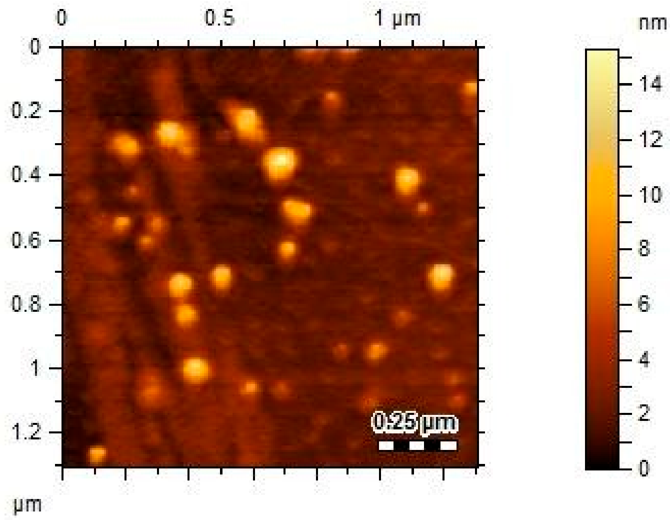

Figure 3. Atomic force microscopy image of F1 file treated with *Sapindus mukorossi*.

Mean RMS was found to be significantly higher in EDTA-treated files (3.76 ± 1.41 nm) compared to *S. mukorossi* files (1.77 ± 0.66 nm) ($p < 0.05$) (Figure 4).

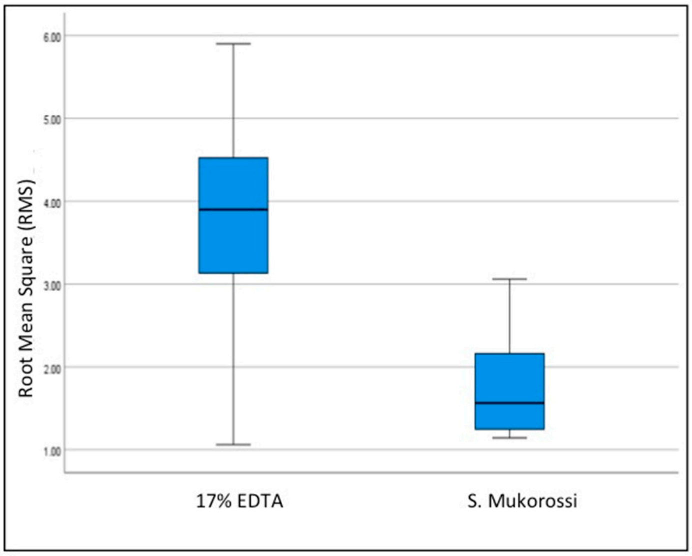

Figure 4. Comparison of RMS among test groups.

Mean Ra was found to be significantly higher in EDTA-treated files (2.89 ± 1.40 nm) compared to *S. mukorossi* files (1.09 ± 0.05 nm) ($p < 0.05$) (Figure 5).

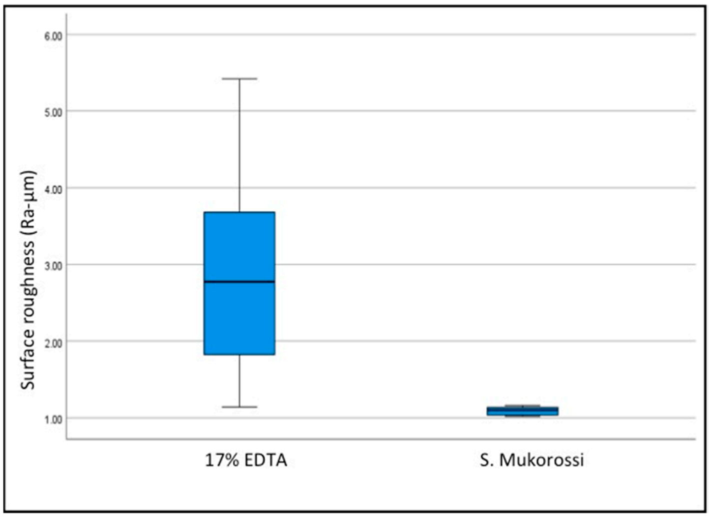

Figure 5. Comparison of surface roughness (Ra) among test groups.

4. Discussion

The present study was based on the hypothesis that there would be no significant difference in mean Ra and RMS of ProTaper Gold files after ethanolic extract of *S. mukorossi* and 17% EDTA solution compared to unused control files. Moreover, it was also hypothesized that 17% EDTA and ethanolic extract of *S. mukorossi*-treated files would not demonstrate any significant difference in Ra and RMS. Both the postulated hypothesis were rejected as both the irrigants demonstrated significant effect on Ra and RMS of tested files compared to the control group. Moreover, it was also found that EDTA causes more surface topographic changes on Protaper gold files compared to *S. mukorossi*.

The Ra and RMS characterization of endodontic nitinol rotary files offer valuable information about surface defects, performance, and associated limitations [25]. The evaluation of surface topography and biomaterial interfaces through atomic force microscopy (AFM) has gained popularity in recent years [26]. High spatial resolution and three-dimensional surface topography of specimens can be achieved with AFM. It is a highly sensitive and reliable technique that offers a suitable means of obtaining qualitative and quantitative information regarding the surface-related properties of rotary NiTi files [27].

Canal preparation is one of the most critical steps in root canal treatment since it determines the outcome of the endodontic treatment. NiTi instruments come in contact with the irrigants during chemo-mechanical preparations. During root canal treatment, mechanical friction or chemical stimuli cause loss of surface structure of the files used thus leads to wear and micro-crack formation [28–30]. Moreover, composition and manufacturing variations also account for the topographic variability of the NiTi rotary files. In a study by Lopes et al., [31] fatigue resistance and number of cycles before fracture for rotary instruments and relation to surface roughness were observed. It was concluded that the smaller the groove depth on the rotary instrument surface, the greater the number of fatigue cycles before fracture of the instrument. However, it was also shown in a previous study that the use of EDTA irrigant did not affect the cyclic fatigue resistance of the NiTi-tested files when used alone or in combination with other irrigants [32]. In the present study, two root canal irrigants, i.e., ethanolic extract of *S. mukorossi* and 17% EDTA solution were used as a root canal irrigant in order to assess their effect on the surface characteristics of ProTaper Gold files with the help of AFM. It was found that there is an increase in Ra and RMS values of ProTaper Gold files after irrigating canals with both the irrigants compared to the unused files used as a control ($p < 0.05$). These findings are in line with the findings of the study conducted by Prasad et al., [12]. They assessed the effect of 17% EDTA and 5% NaOCl on iRaCe and ProTaper NiTi instruments and observed that both the irrigants significantly increased the Ra values as compared to untreated files. The authors predicted that the surface irregularities formed during the manufacturing process of NiTi rotary files makes them prone to corrosion and surface changes [12].

In the present study, we also compared the Ra and RMS of both the irrigation groups and discovered that the mean Ra and RMS in the EDTA group were considerably greater than in the *S. mukorossi* group. Similar findings were observed in the study by Prasad et al., which evaluated the effect of herbal irrigants, i.e., Triphala, EDTA, and NaOCl, on the structure of ProTaper rotary files [12]. In the study by Prasad et al., Triphala-irrigated ProTaper files had lower values of Ra and RMS compared to EDTA treated files [12]. Furthermore, unused files had lower values of Ra and RMS than Triphala-irrigated files [12]. Moreover, a study conducted by Ametrano et al. revealed that EDTA possesses potential to cause surface changes on NiTi rotaty files [33]. However, low Ra and RMS of *S. mukorossi*-treated files can be explained by the fact that *S. mukorossi* is a less reactive hydrocarbon compound compared to EDTA, hence showing low file surface degradation.

The increased surface Ra of the NiTi rotary instruments displays multiple consequences. Primarily, surface Ra is an important feature that affects the fracture mechanism of rotary instruments being used in the canal system [34]. Moreover, dentin particles also become clogged on the surface irregularities, thus making the sterilization process difficult for these instruments to become completely aseptic [35]. In addition, the canal preparation is also compromised, which affects the quality of root canal treatment, resulting in potential failures.

As there is little to no evidence regarding the influence of *S. mukorossi* as a root canal irrigant on surface properties of ProTaper Gold rotary files is available, the findings of the present study provide baseline evidence for *S. mukorossi* as a potentially safe root canal irrigant for rotary instruments compared to 17% EDTA; however, this needs to be further validated through future experiments. Due to the cleaning, antimicrobial, and surfactant effect of *S. mukorossi* [20–23], it has great potential as a root canal irrigant; however, the use of *S. mukorossi* for removal of smear layers requires decalcifying action, and comparative evidence related to the use of *S. mukorossi* and EDTA as a calcifying agent on root dentin is not available. Therefore, further studies in this regard are warranted. Primarily, the study was executed with an in vitro design; therefore, the outcomes can only be applied to the specific rotary files and irrigants involved in the study. Moreover, root canal irrigation is commonly performed with NaOCL and rotary instruments have less contact with EDTA than NaOCL. It is therefore recommended that further randomized, controlled trials assessing the influence of *S. mukorossi* as an irrigant on different NiTi file systems in comparison to NaOCL and their protocols are performed.

5. Conclusions

The mean Ra and RMS values of ProTaper Gold files exposed to root canal irrigants *S. mukorossi* and 17% EDTA were higher than for control files. EDTA-treated files demonstrated higher Ra and RMS values than *S. mukorossi* treated ProTaper Gold rotary files. *S. mukorossi* resulted in lower topographical changes of ProTaper Gold files than EDTA. Further studies investigating the influence of *S. mukorossi* on smear-layer removal and other NiTi file systems are recommended.

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Informed Consent Statement: Informed consent was obtained for removal of teeth and use of extracted teeth.

Data Availability Statement: The data are available on request from the corresponding author.

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