EGYPTIAN DENTAL JOURNAL

Vol. 67, 1453:1462, April, 2021

Print ISSN 0070-9484 • Online ISSN 2090-2360

Fixed Prosthodontics and Dental Materials

www.eda-egypt.org • Codex : 48/21.04 • DOI : 10.21608/edj.2021.53752.1411

CHEMICAL CHARACTERIZATION OF BLEACHED TURMERIC HYDRO-ALCOHOLIC EXTRACT AND ITS EFFECT ON DENTIN MICROHARDNESS VERSUS SODIUM HYPOCHLORITE AS AN ENDODONTIC IRRIGANT: IN VITRO STUDY

Walaa H. Salem* , Taheya A. Moussa** *and* Nehal L. Abou Raya***

ABSTRACT

Aim: Chemical characterization of bleached turmeric hydro-alcoholic extract regarding amount of its curcuminoids, total phenols percent and antioxidant properties compared to unbleached turmeric hydro-alcoholic extract. Moreover, evaluate the effect of the bleached turmeric extract on dentin microhardness compared to sodium hypochlorite as an endodontic irrigant.

Methods: Quantification of curcuminoids was done by HPLC/MS test, evaluation of total phenols percentage was done using Folin-Ciocalteu reagent while antioxidant properties were evaluated using DPPH free scavenging ability. For the evaluation of microhardness, a total of 14 teeth were used. Mechanical preparation with intervening irrigation according to the corresponding group was done. Each tooth was then sectioned vertically into two halves and equally divided into two groups to be immersed in the corresponding irrigant solution. VHN was recorded before and after immersion. Statistical analysis of data obtained from each test was performed on basis of p-value<0.05 for significance.

Results: Quantification by HPLC/MS test showed that the amount of the curcuminoid was lower in the bleached turmeric extract than the unbleached turmeric extract. Also, total phenols percent in the bleached turmeric extract was the least among the test samples while the antioxidant properties of the bleached turmeric extract was the highest among the tested samples. On the other hand, results of microhardness test revealed that both groups showed significant reduction in microhardness of root dentin after treatment.

Conclusion: Bleaching of turmeric affected its chemical properties and lowered its active agents and polyphenols content. Also, bleached turmeric extract had comparable effect to sodium hypochlorite on dentin microhardness.

* Teacher Assistant, Biomaterials Department, Faculty of Dentistry, Nahda University in Beni Suef, Egypt.
** Professor, Biomaterials Department, Faculty of Dentistry, Cairo University, Egypt.
*** Lecturer, Biomaterials Department, Faculty of Dentistry, Cairo University, Egypt.

INTRODUCTION

Endodontic therapy aims to clean and disinfect the root canal system from all affected vital or necrotic pulp tissues, microorganisms and microbial by-products. The root canal system is highly complex and variable and thus limits the ability to clean and disinfect it predictably [1]. Thus, the use of various instrumentation techniques alone is not effective in producing bacteria-free root canal spaces. Therefore, the use of an endodontic irrigant along with mechanical instrumentation is mandatory to aid in disinfecting and lubricating the root canal, flushing out debris from the canal system, and dissolving organic and inorganic tissues [2].

To date, sodium hypochlorite (NaOCl) is considered the gold standard for endodontic irrigants due to its potent antimicrobial activity and its ability to dissolve the organic tissues [3]. However, NaOCl has major disadvantage; that it is being highly cytotoxic, especially at higher concentrations. In case of its extrusion through the apical foramen, NaOCl may cause hemolysis, skin ulceration and marked cell injury in endothelial cells and fibroblasts [4]. Moreover, NaOCl reduces the mechanical resistance of dentin by causing deterioration of collagen and proteoglycans which will in turn reduce the microhardness of dentin. These drawbacks created a continuous need to find a safer alternative to NaOCl for endodontic irrigation.

Herbal products are considered the best alternative for their lower side effects and less microbial resistance, provided that they can fulfil the major requirements to be used as endodontic irrigants.

Curcuma longa, commonly called as turmeric belongs to ginger family. It is a native of Southeast Asia and cultivated mainly in India. It has been shown to have a wide spectrum of actions like anti-inflammatory, antioxidant, antibacterial, antifungal, antiprotozoal, and antiviral activities. Components of turmeric are named curcuminoids [curcumin (diferuloyl methane), demethoxycurcumin, and bisdemethoxycurcumin]. These components are polyphenols with a strong antioxidant function. Curcumin, the most important fraction is responsible for the biological activities of turmeric [5]. However, the major problem of curcumin is its yellow color that can possibly stain dental restorations and the surrounding oral tissues [6].

Therefore, in this study, bleaching of turmeric powder was performed. The bleaching of turmeric was previously described to be used as a topical acne cream [7]. The bleaching process implies contacting turmeric powder with a strong bleaching agent such as NaOCl for sufficient time followed by thorough washing with distilled water.

The aim of this research was to evaluate the chemical characteristics of the bleached turmeric hydro-alcoholic extract solution and compare it to non-bleached turmeric hydro-alcoholic extract solution. Moreover, evaluate the effect of the bleached turmeric hydro-alcoholic extract solution on radicular dentin microhardness compared to NaOCl for being the gold standard of endodontic irrigants. Chemical characterization was done by high performance liquid chromatography/mass spectrometry (HPLC/MS) test, evaluation of total phenols percent and evaluation of antioxidant properties in terms of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free scavenging ability.

The null hypotheses of the present study were that the chemical properties of the bleached and un-bleached turmeric hydro-alcoholic extracts would not be different; in addition, there would be no difference in dentin microhardness after immersion in bleached turmeric hydro-alcoholic extract and NaOCl.

MATERIAL AND METHODS

Commercially available turmeric powder, NaOCl solution and ethanol were used in the present study. The materials and reagents used are listed in table (1).

TABLE (1) Materials and reagents used in this study

Material/Reagent	Manufacturer	Lot No.
Turmeric powder	Puritan's Pride, USA	198239
Household bleach	Clorox	1336
99.9 % Ethanol	Sphinx chemicals, Egypt	19006
Distilled water	Prepared in Research lab, faculty of pharmacy, Nahda University in BeniSuef	
Folin-Ciocalteu phenol reagent	Loba Chemie PVT. LTD, India	LM3272

METHODS

Preparation of turmeric extract

a) Bleaching of turmeric powder:

Bleaching of turmeric powder was done according to the method described by ***Nguyen, 2000*** [7]. One gram of turmeric powder (Puritan's Pride, USA) was added to 10 ml of household bleach (Clorox) in a sealed test tube, then vortexed. After that, the solution was centrifuged at 4000 rpm for 2 minutes to separate the liquid from the powder. Bleaching time of 3 minutes was found to be the optimum duration to produce properly bleached turmeric powder.

After the bleaching process, turmeric powder was washed to remove the residues of the bleaching agent. Distilled water was added to the bleached powder, then vortexed to ensure all powder particles were contacted with water to ensure proper washing. After that, the suspension was centrifuged at 4000 rpm for 2 minutes. The previous process was repeated several times until the pH value of the washing water became neutral (= 7) measured using digital pH meter (Jenway, Cole-parmer Ltd, UK).

b) Preparation of hydro-alcoholic extract of bleached turmeric:

Preparation of the extract was done as described by ***Hegde et al, 2012*** [9]. Fifty grams of bleached turmeric powder were placed in a glass container, then 70 ml of distilled water and 30 ml of ethanol were added to the powder to obtain hydro-alcoholic extract of bleached turmeric (70:30). The glass container was properly sealed to prevent the evaporation of the solution, then was kept to stand for 7 days with frequent stirring at room temperature (24 ± 1). Afterwards, the suspension was filtered to separate the powder from the liquid. After complete filtration, the liquid solution was stored in sealed test tubes and kept refrigerated.

Chemical characterization

Chemical characterization was done to determine the effect of bleaching process on the active constituents of turmeric. Therefore, characterization was done for three groups; standard turmeric powder (Puritan's Pride, USA), turmeric hydro-alcoholic extract before bleaching and turmeric hydro-alcoholic extract after bleaching.

a) High performance liquid chromatography/mass spectrometry (HPLC/MS):

Turmeric was analyzed according to a method previously described by ***Ashraf et al, 2015*** (10). The used instrument was LC 1260 infinity and MS 6460 triple quadrupole LC/MS systems (Agilent technologies, USA). Stock standard solution was prepared by dissolving one gram of non-bleached turmeric powder in one liter solvent (70% distilled water and 30% absolute ethanol) to be equivalent to 1000 part per million (ppm). The solution was further diluted to get 500 ppm concentration and 100 ppm concentration. A calibration curve was then obtained from the previous solutions. Tables (2 and 3) show liquid chromatographic (LC) and mass spectrometric (MS) instrument conditions respectively.

TABLE (2) Liquid chromatography (LC) conditions

Analytical column	Eclipse plus C_{18} (4.6x100 mm), 3.2 μm
Column temperature	40˚C
Injection volume	10 μL
Mobile phase	A: Water + 0.1% formic acid (98%) B: Methanol (2%)
Run time	12 minutes
Flow rate	0.7 mL/min

TABLE (3) Mass spectrometric (MS) conditions:

Gas temperature	350˚C
Gas flow	10 L/min
Nebulizer pressure	40 psi
Capillary voltage (V_{cap})	3500 V

b) Determination of total phenols content

Total phenols content was determined following the method described by ***Singleton and Rossi*** using Folin-Ciocalteu method (11). One ml of the natural extract was mixed with 1 ml of Folin Ciocalteu reagent. After three minutes, one ml of saturated sodium carbonate solution (20%) was added to the mixture and the volume was adjusted to 10 ml by addition of distilled water. The reaction mixture was kept in dark place for one hour with intermittent shaking. The absorbance was measured at 750 nm using a spectrophotometer (Jenway 6305 UV/Vis, UK). Phenolic contents were calculated on the basis of the standard curve for gallic acid. The results were expressed as mg of gallic acid equivalent per gram of dry extract.

c) Determination of antioxidant properties

The antioxidant activity of the natural extracts was determined based on their radical scavenging ability to react with a stable DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical according to ***Blois 2002*** [12]. The purple colored stable free radicals DPPH changes to the yellow color upon reduction. A solution of 0.1 mM (m Molar) of DPPH in methanol was prepared and considered as the blank solution. One ml of this solution was added to 3 ml of the natural extract. The mixture was vigorously shaken and allowed to stand at room temperature (24 ± 1) for thirty minutes in a dark place. Then, the absorbance of both the blank solution and the solution containing the natural extract was measured using a spectrophotometer at 517 nm. The radical scavenging activities of butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) were considered as positive controls. The corresponding blank readings (A0) were taken and the capability to scavenge the DPPH radical was calculated using the following equation:

$$\textbf{DPPH scavenging effect (\%)} = \frac{\textbf{A0 - A1}}{\textbf{A0}} \textbf{ x100}$$

Where A0 is the absorbance of the blank solution containing all the reagents except the test compounds and A1 is the absorbance in the presence of the tested extracts.

Microhardness test

a) Sample size calculation

Sample size calculation for microhardness test was done according to ***Ulusoy et al (2013)*** [8], the effect size was found to be (1.21366). Sample size was calculated using G*Power version 3.1.9.2 for sample size analysis at α=0.05 and 80% power, which yielded a sample size of 12 samples per group. Fourteen samples per group were prepared to gain extra power.

b) Teeth preparation

The test was done according to the method described by ***Saghiri et al, 2013*** [13]. A total number of 14 extracted single-rooted teeth were assigned for measurement of dentin microhardness test. The selected teeth were assorted according to

the following criteria: complete root formation, non-carious, no signs of root resorption and non-endodontically treated. Teeth were then equally divided into two groups according to the assigned irrigating solution. The root canals were enlarged up to master apical file number 50 K- file using step back technique. The use of each file was followed by 1 ml of irrigation solution using 30-G needle according to the assigned group. After instrumentation, all root canals were finally irrigated with 2 ml of one of the irrigating solutions according to the group for 30 seconds. The intervening irrigating solutions were used in each group as follows:

Group 1 (intervention): 50% hydro-alcoholic bleached turmeric solution.

Group 2 (Control): 2.5% NaOCl solution.

After final irrigation, each root canal was dried using paper points then finally flushed and copiously irrigated with 10 ml distilled water and dried with absorbent paper points. The canals orifices were sealed with a small cotton pellet to prevent contamination of the root canal space during sectioning procedures.

c) Root sectioning

Teeth were decoronated, then two longitudinal grooves were made on the buccal and lingual external surfaces of the root samples of the different test groups then the roots were bisected longitudinally into two halves using Isomet 4000 saw (Beuhler, Germany) at speed 2500 rpm (figure 1.A). The specimens were then embedded on plastic rings of uniform diameter filled with freshly mixed auto polymerized resin with the polished surface facing outwards (figure 1.B).

d) Vicker's hardness indentation testing

The Vicker's hardness number was recorded using Wilson hardness machine (Beuhler, Germany) by weight 50 gm for 10 seconds to get baseline data for each sample. The samples were then immersed in the corresponding irrigant for each group for 15 minutes. After that, the samples were rinsed with distilled water and dried. After that, the micro-hardness values of each test group were recorded using the same machine and previously mentioned method. The obtained data were tabulated and statistically analyzed.

Statistical Analysis Method

Data were coded and entered using the statistical package for the Social Sciences (SPSS) version 26 (IBM Corp., Armonk, NY, USA). Data was summarized using mean and standard deviation and were explored for normality using Kolmogrov-Smirnov test.

Comparisons between groups were done using unpaired t-test ***(Chan, 2003)*** [14]. P-values less than 0.05 were considered as statistically significant.

Fig. (1.A) Root bisected longitudinally into two halves

Fig. (1.B) Specimen embedded in auto polymerized resin

RESULTS

Chemical characterization

a) High performance liquid chromatography/ mass spectrometry (HPLC/MS)

i) Qualitative analysis

Qualitative data showed that the three curcuminoids (curcumin, desmethoxycurcumin and bisdemethoxycurcumin existed in both samples (bleached and unbleached turmeric hydro-alcoholic extracts). These curcuminoids are the active components which are polyphenols in nature and are responsible for the antimicrobial and anti-oxidant properties of turmeric. Table (4) shows precursors and products ions spectra for the curcuminoids. Results are expressed as mass/charge (m/z).

TABLE (4): Precursors and products ion spectra for turmeric analytes:

Compound name	Precursor ion spectra	Product ion spectra
Curcumin	m/z 369.5	m/z 176.95
Desmethoxycurcumin	m/z 337.35	m/z 175
Bisdemethoxycurcumin	m/z 307.0344	m/z 145.1024

ii- Quantitative data

Quantification could be done only for bisdemethoxycurcumin because it had the most suitable "Limit of Detection" (LOD) and "Limit of Quantification" (LOQ). The analysis data by HPLC/MS method showed that the concentration of bisdemethoxycurcumin in the unbleached hydro-alcoholic extract was 325 mg/l, while in the bleached hydro-alcoholic extract it was 42.7 mg/l. The linear calibration curve is shown in figure (2) where the arrow denotes the blank solvent (70% water and 30% ethanol), then the dots are 100 ppm, 500 ppm, 1000 ppm_ascendingly. The chromatograms of bisdemethoxycurcumin in the unbleached and bleached turmeric extracts are shown in figures (3.A) and (3.B) respectively.

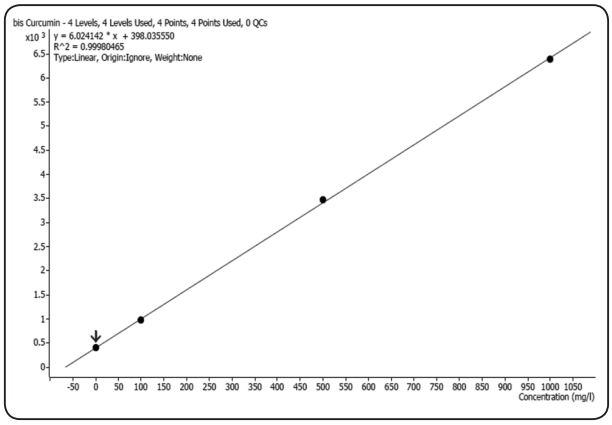

Fig. (2) Calibration curve for the prepared standard solutions.

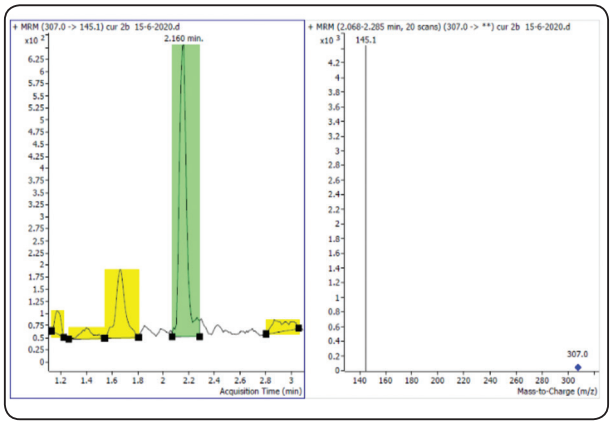

Fig (3.A) Chromatogram for bisdemethoxycurcumin in the unbleached hydro-alcoholic extract

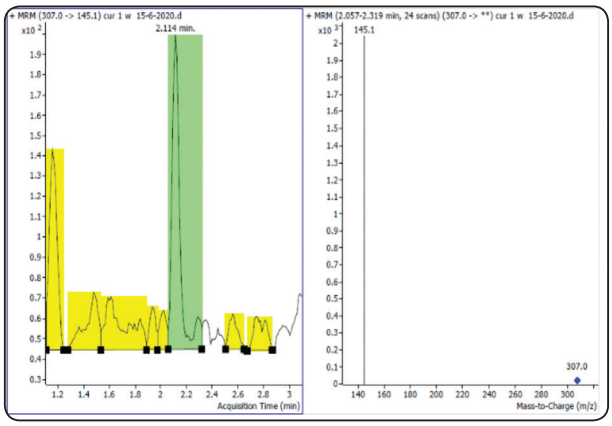

Fig. (3.B) Chromatogram of bisdemethoxycurcumin in the bleached hydro-alcoholic extract

b) Total phenols content

Turmeric powder showed the most polyphenols content (1.988%) followed by unbleached turmeric hydro-alcoholic extract (0.23%), while bleached turmeric hydro-alcoholic extract showed the least polyphenols content (0.036%).

c) Anti-oxidant properties

Bleached turmeric hydro-alcoholic extract showed the highest anti-oxidant properties (75.985%), followed by turmeric powder (69.841%), while unbleached turmeric hydro-alcoholic extract showed the least percent (62.139%).

Microhardness test

Tables (5 and 6) show the difference in microhardness before and after treatment for intervention and control respectively. Both groups showed significant reduction in dentin microhardness after treatment. There was no significance between the mean differences of groups before and after treatment (P-value > 0.05) (table 7).

TABLE (5): Mean microhardness values before and after treatment for group 1 (Intervention)

	Group 1		P value
	Mean	SD	
Microhardness (VHN) Baseline	37.683	2.431	<0.001
Microhardness (VHN) After treatment	32.724	4.851	
Microhardness (VHN) difference (after-baseline)	-4.959	3.864	

TABLE (6): Mean microhardness values before and after treatment for group 2 (Control)

	Group 2		P value
	Mean	SD	
Microhardness (VHN) Baseline	38.209	9.155	0.005
Microhardness (VHN) After treatment	32.791	5.986	
Microhardness (VHN) difference (after-baseline)	-5.417	6.074	

TABLE (7): Relation between mean differences in microhardness of the both groups

	Group 1		Group 2		
	Mean	Standard Deviation	Mean	Standard Deviation	P value
Microhardness (VHN) difference (after-baseline)	-4.959	3.864	-5.417	6.074	0.804

DISCUSSION

In the present study, we used a method for bleaching of the turmeric powder to avoid the staining effect of its yellow color. To our knowledge, no previous studies analyzed the chemical properties of bleached turmeric extract or evaluated the effect of bleached turmeric extract on dentin microhardness compared to NaOCl as endodontic irrigants.

Bleaching of the turmeric powder could be done by sodium hypochlorite or sodium perborate. In the present study we used sodium hypochlorite (Clorox) for the ease of availability. After the bleaching process, washing of the bleach was done by centrifugation because it was faster and more convenient than the other methods.

The solvent of the turmeric extract was hydro-alcoholic (70% water-30% ethanol) as the active

ingredient curcumin was proved not to lose its active potential when extracted in ethanolic solvent. Besides, the active component of turmeric is insoluble in water. Therefore, hydro-alcoholic extract was chosen according to various studies stated that ethanol is a good solvent for turmeric with better efficacy extracting the active components compared to aqueous extract [9,15,16]; in addition, the gradient percent of the solvent (70:30) and the concentration of turmeric in the solvent (50%) were chosen according to the method described by ***Chaitanya et al, 2016*** [15], which proved antibacterial effect against *E.faecalis* comparable to NaOCl. On the other hand, NaOCl was used in concentration 2.5% because this is the most commonly used concentration in the clinical situation to minimize its high toxic effect.

Chemical analysis was done to determine the effect of the solvent extraction method (hydro-alcoholic extract) on the constituents of turmeric before bleaching. Then determine the effect of bleaching on those constituents. Therefore, chemical analysis was done for the bleached turmeric hydro-alcoholic extract, unbleached turmeric hydro-alcoholic extract and the standard turmeric powder. Analysis was done by three methods; HPLC/MS test, total phenols content using Folin-Ciocalteu method and anti-oxidant activity in terms of DPPH free scavenging ability.

HPLC/MS test was reported to be a sensitive test for the quantification of curcuminoids in turmeric solution [10,17]. In spite of the qualitative findings of HPLC/MS which revealed that both samples contained the three curcuminoids, quantification could be done only for bisdemethoxycurcumin due to its suitable LOD and LOQ that enable it to be quantified in the solution. The concentration of bisdemethoxycurcumin in the bleached turmeric extract was much less than its concentration in the unbleached turmeric extract which could be due to the effect of NaOCl the bleaching process on turmeric components which may be considered as aggressive.

Total phenols percent in the bleached turmeric extract was the least. This could be attributed to the bleaching process by NaOCl which is an organic solvent. As explained in previous studies, NaOCl causes cleavage of the aromatic structure of phenols leading to the formation of unwanted chlorinated compounds [18]. On the other hand, anti-oxidant free scavenging ability of the bleached turmeric extract was the highest. This result could be attributed to the formed chlorinated products as a result of the bleaching process. These chlorine oxidants raised the free radical scavenging ability of the extract.

As regards dentin surface, when the endodontic irrigant come in contact with dentin during irrigation procedures, it might alter dentin and enamel surfaces by altering their mineral content. Such alterations will eventually affect the interactions of the tooth structure with obturation and future coronal restorative materials. For the previously mentioned reasons, the effect of endodontic irrigants on dentin should be evaluated [19].

Microhardness can be considered as an indirect evidence of mineral changes in root canal dentin. In the present study, root dentin microhardness was evaluated by Vickers microhardness test for its suitability and sensitivity to surface changes [20].

The results of the present study revealed that both groups showed significant reduction in dentin microhardness after immersion in the solutions with non-significant difference between them. The reduction in microhardness caused by the intervention group (bleached turmeric hydro-alcoholic extract) was probably due to the presence of ethanol in the extract that adversely affected the collagen fibers. It was previously explained that water is vital in developing and maintaining the structure of the molecules comprising the collagen fibrous network by forming a highly ordered inner hydration layer that creates hydrogen bonds along the underlying peptide chains. Moreover, it forms hydrogen-bonded bridges, which further contribute to the structure of collagen by forming

intra- and inter-chain links within its molecules. Ethanol, on the other hand is known to dehydrate dentin chemically by replacing the water bonded to the collagen. The dehydration will eventually lead to contraction of dentin collagen network diameters, thereby increasing interfibrillar spaces and decreased microhardness. [22,23]

These results are in disagreement with ***Prabhakar et al in 2013*** [24], where they reported that turmeric has no significant effect on dentin microhardness. This disagreement could be due to the use of aqueous turmeric extract in the mentioned study, while in the present study hydro-alcoholic extract was used.

On the hand, NaOCl caused reduction in dentin microhardness due to its dissolving action on the organic content of dentin [25]. Results of the present study are in agreement ***Oliveira et al in 2007*** [26], ***Garcia et al in 2013*** [27] where they reported that NaOCl caused significant reduction in dentin microhardness. Moreover, ***Tartari et al in 2016*** [28] demonstrated the effect of NaOCl on the chemical structure of dentin and explained it by collagen deproteination and tissue dissolution.

CONCLUSION

Based on the limitations of the current study, it could be concluded that:

1. Bleaching of the turmeric powder with NaOCl affected its chemical properties and lowered its active phenols components.
2. Bleached turmeric hydro-alcoholic extract had a comparable effect to NaOCl on the microhardness of root dentin.

RECOMMENDATIONS

1. Using other alternatives for bleaching of turmeric powder rather than NaOCl to avoid its effect on chemical composition.
2. Evaluation of the effect of bleached turmeric hydro-alcoholic extract on chemical structure and surface of dentin.
3. Evaluation of bleached turmeric hydro-alcoholic extract on other mechanical properties of radicular dentin, microleakage and antibacterial efficiency against other microorganisms.
4. Evaluation of the antibacterial properties and cytotoxic potential of bleached turmeric extract as an endodontic irrigant.

Conflict of Interest

The authors declare no conflict of interest

REFERENCES

1. Topbas C, Adiguzel O. Endodontic Irrigation Solutions : A Review Endodontic Irrigation Solutions : A Review. J Dent App J Dent App. 2017;7(January 2018):25–32.
2. Vineet AS, Rajesh M, Sonali, Mukesh. A Contemporary Overview of Endodontic Irrigants – A Review. J Dent App J Dent App. 2014;1(1):105–15.
3. Mohammadi Z, Abbott P V. Antimicrobial substantivity of root canal irrigants and medicaments: A review. Vol. 35, Australian Endodontic Journal. 2009. p. 131–9.
4. Bosch-Aranda ML, Canalda-Sahli C, Figueiredo R, Gay-Escoda C, Alkahtani A, Alkahtany SM, et al. Managing sodium hypochlorite accidents : the reality of toxicity. J Clin Exp Dent. 2012;72(6):271.
5. Sharma A. Turmeric - Its Applications in Dentistry. J Adv Pharm Technol Res. 2016;3(1):1–4.
6. Bindal P, Bindal U, Dabbagh A, Ramanathan A, Ginjupalli K. Comparative effects of turmeric, coffee, and chewable tobacco on the color stability of tooth-colored restorative materials. Br Dent J. 2015;219(12):570–570.
7. Nguyen VB. United States Patent (19 FIG-2. 2000; (19):233–5.
8. Ulusoy ÖIA, Görgül G. Effects of different irrigation solutions on root dentine microhardness, smear layer removal and erosion. Aust Endod J. 2013;39(2):66–72.
9. N Hegde P (Dr) M. Evaluation of Antimicrobial Activity of Aqueous and Hydro-Alcoholic Curcuma Longa Extracts against Endodontic Pathogens. IOSR J Pharm. 2012;2(2):192–8.
10. Ashraf K, Mujeeb M, Ahmad A, Ahmad N, Amir M. Determination of Curcuminoids in Curcuma longa Linn.

by UPLC/Q-TOF-MS: An Application in Turmeric Cultivation. J Chromatogr Sci. 2015;53(8):1346–52.

11. Singleton VL, Rossi JAJ. Colorometry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am J Enol Vitic. 1965;16:144–58.
12. Blois MS. Antioxidant determinations by the use of a stable free radical [10]. Nature. 2002. p. 1199–200.
13. Saghiri MA, García-Godoy F, Asgar K, Lotfi M. The effect of Morinda Citrifolia juice as an endodontic irrigant on smear layer and microhardness of root canal dentin. Oral Sci Int. 2013;10(2):53–7.
14. Chan Y. Biostatistics102: Quantitative Data – Parametric & Non-parametric Tests. singapore Med J. 2003;44(8):391–6.
15. Chaitanya BV, Somisetty KV, Diwan A, Pasha S, Shetty N, Reddy Y, et al. Comparison of antibacterial efficacy of turmeric extract, morinda citrifolia and 3% sodium hypochlorite on Enterococcus faecalis: An in-vitro study. J Clin Diagnostic Res. 2016;10(10):ZC55–7.
16. Joy Sinha D, D S Nandha K, Jaiswal N, Vasudeva A, Prabha Tyagi S, Pratap Singh U. Antibacterial Effect of Azadirachta indica (Neem) or Curcuma longa (Turmeric) against Enterococcus faecalis Compared with That of 5% Sodium Hypochlorite or 2% Chlorhexidine in vitro. Bull Tokyo Dent Coll. 2017;58(2):103–9.
17. Van Nong H, Hung LX, Thang PN, Chinh VD, Vu L Van, Dung PT, et al. Fabrication and vibration characterization of curcumin extracted from turmeric (Curcuma longa) rhizomes of the northern Vietnam. Springerplus. 2016;5(1).
18. Michałowicz J, Duda W, Stufka-Olczyk J. Transformation of phenol, catechol, guaiacol and syringol exposed to sodium hypochlorite. Chemosphere. 2007;66(4):457–663.
19. Anil S, Duvvi B, Adarsha MS, Usha HL, Ashwini P, Murthy CS, et al. Comparative Assessment of Different Concentrations of Sodium Hypochlorite and Calcium Hypochlorite on Microhardness of Root Canal Dentin - An In Vitro Study. Int J Oral Care Res. 2018;6(1):54–8.
20. Ari H, Erdemir A, Belli S. Evaluation of the Effect of Endodontic Irrigation Solutions on the Microhardness and the Roughness of Root Canal Dentin. J Endod [Internet]. 2004 Nov 1 [cited 2020 Mar 12];30(11):792–5. Available from: https://www.sciencedirect.com/science/article/pii/S0099239905600866.
21. Kara Tuncer A, Tuncer S, Siso SH. Effect of QMix irrigant on the microhardness of root canal dentine. Aust Dent J. 2015;60(2):163–8.
22. Nalla RK, Kinney JH, Tomsia AP, Ritchie RO. Role of alcohol in the fracture resistance of teeth. J Dent Res. 2006;85(11):1022–6.
23. Pantoja CA de MS, Silva DH da, Soares A de J, Ferraz CCR, Gomes BPF de A, Zaia AA, et al. Influence of ethanol on dentin roughness, surface free energy, and interaction between AH Plus and root dentin. Braz Oral Res. 2018;32:e33.
24. Sinha DJ, Vasudeva A, Gowhar O, Garg P, Sinha A, Prakash P, et al. Comparison of antibacterial efficacy of turmeric extract, morinda citrifolia and 3% sodium hypochlorite on Enterococcus faecalis: An in-vitro study. Acta Odontol Scand. 2013;5(2):1453–7.
25. Cecchin D, Soares Giaretta V, Granella Cadorin B, Albino Souza M, Vidal C de MP, Paula Farina A. Effect of synthetic and natural-derived novel endodontic irrigant solutions on mechanical properties of human dentin. J Mater Sci Mater Med. 2017;28(9).
26. Oliveira DP, Barbizam JVB, Trope M, Teixeira FB. In vitro antibacterial efficacy of endodontic irrigants against Enterococcus faecalis. Oral Surgery, Oral Med Oral Pathol Oral Radiol Endodontology. 2007;103(5):702–6.
27. Garcia AJA, Kuga MC, Palma-Dibb RG, Só MVR, Matsumoto MA, Faria G, et al. Effect of sodium hypochlorite under several formulations on root canal dentin microhardness. J Investig Clin Dent. 2013;4(4):229–32.
28. Tartari T, Bachmann L, Maliza AGA, de Andrade FB, Duarte MAH, Bramante CM. Tissue dissolution and modifications in dentin composition by different sodium hypochlorite concentrations. J Appl Oral Sci. 2016;24(3):291–8.