Original Article

HEALTH SCIENCES MEDICINE

DOI: 10.32322/jhsm.1217542

J Health Sci Med 2023; 6(2): 289-293

Exploring the discoloration potential of *Propolis* extract and *Morus nigra* syrup on restorative dental composites: an in vitro study

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Cite this article as: Çelik ZC, Elbek Çubukçu Ç, Batmaz SG. Exploring the discoloration potential of *Propolis* extract and *Morus nigra* syrup on restorative dental composites: an in vitro study. J Health Sci Med 2023; 6(2): 289-293.

ABSTRACT

Aim: The aim of this study was to analyse color stability of dental composites immersed in *Propolis* extract and *Morus nigra* syrup at in-vitro conditions simulating clinical usage time intervals and to explore the relationship between discoloration potential and phenolic contents.

Material and Method: A total of 126 composite discs of nanohybrid (n=42), microfilled (n=42) and microhybrid (n=42) were prepared using a Teflon mold with a diameter/thickness of 6 mm/2 mm. After polishing procedures, composites were subdivided into three and immersed into %15 *Propolis* extract with total phenolic content=53 mg GAE/ml (B'eeo, İstanbul) and *Morus nigra* syrup with total phenolic content=25 mg GAE/100 g dw (Hünnap, İstanbul) and distilled water for 12 hours(T1) and 24 hours (T2) simulating 1-year and 2-year time spans respectively. Color measurements were performed by Vita easy Shade Compact (Vita Zahnfabrik, Bad Sackingen, Germany) prior to immersion and analysed by CIEDE2000 formula.

Result: Between T0 and T2, minimum/maximum color change values (ΔE) of nanohybrid, microfilled and microhybrid composites immersed in *Propolis* and *Morus nigra* syrup were 1,24/5,29 and 0,97/2,65 respectively. Nanohybrid composite discs were discolored within clinically acceptable limits in all test solutions. Microfilled and microhybrid composite discs showed clinically unacceptable discoloration at T1 and T2 periods in *Propolis* extract solution.

Conclusion: The phenolic and flavonoid components of herbal formulations can be considered as one of the major determinants in discoloring potential.

Keywords: *Morus nigra*, phenolic content, *Propolis*, discoloration

INTRODUCTION

Dental composites are widely used in dental restorations in daily clinical dental practice, consist of three major chemical content: organic polymeric matrix, inorganic fillers and silane/coupling agent to adhere the fillers to the organic resin. Composite resin materials, simulating the physical features of the hard dental tissues and have excellent aesthetic Of these features, color stability plays a major role in patient satisfaction and long-term survival (1). However multiple factors including inadequate polymerization, water absorption, chemical reactivity, oral hygiene, and surface roughness of the restoration result in discoloration on dental composites (2).

Color changes in composite resins may arise extrinsically or intrinsically. The intrinsic discoloration of resin composites is highly correlated with filler particle size, type, amount, polymeric properties, the presence of tertiary amines due to oxidation of the residual monomers, and the water permeability of the resin (3,4).

The etiology of discoloration associated with adsorption or dye absorption exogenous sources, especially discoloring agents/solutions. *Morus nigra* syrup and *Propolis* extracts are widely used in complementary supportive care of oral lesions due to anti-inflammatory, antimicrobial and antioxidant properties owing to their phenolic contents and flavonoid components (5,6).

Since they are over-the-counter (OTC) products and there is no restriction on the duration of use, the side effects of colorants should definitely be examined. Some

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Received: 11.12.2022 **Accepted:** 23.01.2023

beverages and oral hygiene products consisting phenolic contents, may affect negatively the color stability of composite resins because of their chemical and physical degrading elements (2, 7-10).

The present study has two hypothesis: (1) Nanohybrid aesthetic dental composite materials would show less discoloration beyond clinically acceptable limits after immersion into tested solution (2). *Morus nigra* syrup would cause more color change in all composites due to its darker and viscous nature.

The aim of this study was to investigate the discoloration potential of *Propolis* extract and *Morus nigra* syrup on dental restorative composites.

MATERIAL AND METHOD

This study does not require ethics committee approval as it is not within the scope of clinical and experimental studies on humans and animals (including data& material).

Sample Preparation

A total of 126 adhesive restorative resins consists of nanohybrid (n=42), microfilled (n=42) and microhybrid (n=42) composite (**Table 1**) discs of shade A1 (manufacturer prescription) were prepared with a diameter/thickness of 6 mm/ 2 mm. Composite samples were placed in Teflon molds as a single layer, and their upper and lower surfaces were stripped and polymerized with a LED light device for 20 sec. (VALO Ultradent , South Jordan, UT) in standard mode (1000 mW /cm 2). After sample preparation, they were kept at 37°C for 24 hours, then polishing with polishing discs 600-, 800- and 1200-grit size (Sof-Lex , 3M ESPE Dental Products, St Paul, MN, USA) for surface standardization. The samples were then washed with distilled water and gently dried.

Immersion into Solutions

Each composite group were subdivided into three subgroups of 14 discs according to immersing solutions (*Propolis* extract / *Morus nigra* syrup) given in **Table 1**. and distilled water. Every subgroup of composite resin was immersed in discoloring and control solutions at room temperature for 12 hours (T1) and 24 hours (T2). Reference time intervals were considered according to Ertürk-Avunduk et al. (11), keeping the samples in solutions for 12 hours in vitro would be simulate 1 year in vivo (2 times a day-1 minute), and keeping them in solution for 24 hours was accepted as a cumulative indicator of exposure for 2 years (11).

Spectrophotometric Analysis

Composite color change in resins were measured by Vita easy Shade Compact (Vita Zahnfabrik, Bad Sackingen, Germany) before and after immersions. Before the measurements, the spectrophotometer was calibrated according to the manufacturer's instructions. Measurements were performed under D65 standard light illumination on a standard white background, with the probe tip standardized perpendicular to the tooth sample surfaces, and 3 measurements were taken from each sample. Following the measurements, color changes calculated according to the CIEDE2000 formula (12):

$$\Delta E_{00} = \left[\left(\frac{\Delta L'}{K_L S_L}\right)^2 + \left(\frac{\Delta C'}{K_C S_C}\right)^2 + \left(\frac{\Delta H'}{K_H S_H}\right)^2 + R_T\left(\frac{\Delta C'}{K_C S_C}\right)\left(\frac{\Delta H'}{K_H S_H}\right)\right]^{1/2}$$

In this study, each of KL, KC, KH were set into 1.0. The value of ΔE00 < 2.25 was considered as a clinically acceptable threshold level for color changes (13).

Statistical Analysis

Statistical analysis of data was performed using IBM SPSS 28.0 statistical package program (IBM SPSS Statistics for Windows 2021, Armonk, NY: IBM Corp). The data showed normal distribution confirmed by the Shapiro-Wilk test. The inter-group comparisons by solutions were analyzed using one-way Anova and Bonferroni Post-Hoc tests. Intra-group comparisons by time interval were analyzed using the paired t-test. Significance level was determined as α= 0.05.

Table 1. Materials tested in this study and their contents

	Characterization	Total phenolic content	Total flavonoid	Manufacturer
Solutions				
*Propolis**	%15 aqueous extract	53 mg GAE/ml	35 mg CE/ml	B'eeo, İstanbul
*Morus nigra***	Cold infusion / syrup	25 mg GAE/100 g dw	4.7 mg CE/100 g dw	Hünnap, İstanbul
Composite resins				
Type/Shade	**Matrix**	**Filler**	**Total filler content w/w**	**Manufacturer**
Nanohybrid/A1	Bis - GMA, hydrophobic aromatic aliphatic dimethacrylate	Silane barium glass filler	78%	Kuraray, Japan
Micro-filled hybrid/A1	UDMA, dimethacrylate co-monomers	Silica glass filler	76%	GC Corporation, Japan
Microhybrid /A1	Bis-GMA, Bis-EMA, TEGDMA, UDMA	Silica-Zirconia filler	78%	3M ESPE, USA

*Folin-Ciocalteu method by Scientific Bio Solutions Laboratory, **Kamiloglu et al. (14)
Bis-GMA: bisphenol A diglycidylmethacrylate; TEGDMA: triethyleneglycol dimethacrylate, UDMA: urethane dimethacrylate; BIS-EMA: Bisphenol-A-Ethyl methacrylate

Table 2. The means (± SD) of ΔE00 values of restorative materials at T1 and T2 time intervals in the study.

	Propolis extract		*Morus nigra* syrup		Distilled water	
	T1 (mean±SD)	T2 (mean±SD)	T1 (mean±SD)	T2 (mean±SD)	T1 (mean±SD)	T2 (mean±SD)
Nanohybrid	1.24±0.65	2.04±1.29	1.32±0.63	1.11±0.45	0.98±0.14	1.01±0.3
Microfilled	3.59±0.47	5.29±0.58	0.97±0.49	1.72±0.25	1.07±0.29	1.28±0.73
Microhybrid	4.24±0.65	4.25±0.65	1.23±0.78	2.65±0.95	0.91±0.34	1.93±0.71

RESULTS

The means and standard deviations of ΔE00 values at T1 and T2 time intervals are shown in **Table 2**.

Between T0 and T2, minimum/maximum color change values (ΔE00) of nanohybrid, microfilled and microhybrid composites immersed in *Propolis* and *Morus nigra* syrup were 1,24/5,29 and 0,97/2,65 respectively. Nanohybrid composite discs were discolored within clinically acceptable limits (ΔE00<2.25) in all test solutions (**Figure 1**). Microfilled and microhybrid composite discs showed clinically unacceptable discoloration at the end of T1 and T2 periods in *Propolis* solution. Microhybrid composite discs immersed in *Morus nigra* solution showed clinically unacceptable discoloration only at T2.

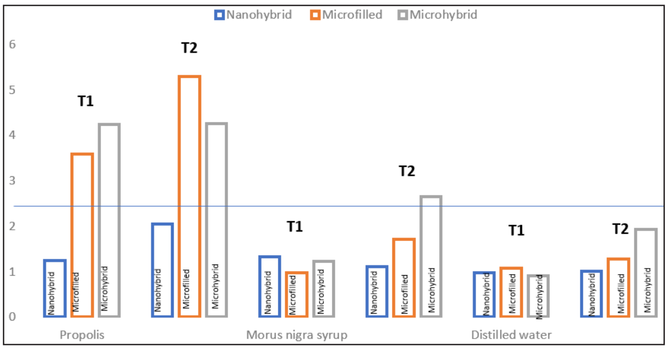

Figure 1. Discoloration of dental composites immersed in test/control solutions regarding clinical acceptable limit.

There was a statistically significant difference between T 1 and T 2 time periods in samples of microfilled composites kept in *Propolis* solutions (p<0.001). More color changes were observed in the T2 time frame for all composites immersed into *Propolis* extract (Figure 1). The greatest discoloration was observed in the end of T1 and T2 time periods in microhybrid (4,24±0,65) and microfilled (5.29 ±0,58) composite samples. There was no statistical difference between T1 and T2 for *Morus nigra* syrup and distilled water (p>0,05). No significant correlation was between filler content of composite materials and degree of a discoloration (p=0.899)

DISCUSSION

Propolis extracts (15) and *Morus nigra* syrup (16) have significant contribution on supportive care of oral lesions. However, there is no study investigating the discoloring effects of these OTC agents on composite resins. The present study is the first to investigate discoloring effects of the commercial products of these OTC solutions on anterior composites. The first hypothesis of this study was confirmed as the nanohybrid composites were more resistant to discoloration for both *Propolis* extract and *Morus nigra* syrup. The second hypothesis of this study was not confirmed that *Morus nigra* syrup would cause more color change in all composites due to its darker more viscous nature as it showed less color changes compared to *Propolis* extract groups.

Phenolic compounds are divided into two groups as phenolic acids and flavonoids. Besides flavonoids and phenolic contents are polyphenolic antioxidants showing antimicrobial activities in oral cavity (17) that also responsible from the sour flavor of fruits and vegetables, and some provide yellow, yellow-brown, red-blue color tones (18).

Keskin and Kolaylı (19) in 2019 reported that the total phenolic substance amount of Anatolian *Propolis* ranged between 16.13-178.34 mg GAE /g. The total phenolic content of the commercial sample of *Propolis* containing %15 aqueous extract used in our study was 53 mg GAE/ml as indicated in manufacturer catalog. In addition, Ozgen et al. (20) stated that the average TP content was 2737 µg GAE/g dw in Türkiye, which was compatible with Kamiloglu et al. (14) ,the reference study for our study. Percentile proportion of an extract, the character of solvent (aqua, ethanol, methanol etc.) and the type of an infusion (cold or hot) closely affect the total phenolic content of a solution. In a present study, *Morus nigra* syrup was obtained by cold infusion which means evaporation process at low temperatures by lowering the boiling point under vacuum and might be the reason low content of phenoic compound. Gonçalves et al. (21) showed the phenolic content of hot infusions were significantly greater than that of cold infusions. Comparing the TPC and TF of test solutions in out study the characterization of *Morus nigra* syrup might be responsible from lower ΔE00 values detected for all types of composites.

Discoloration of composite resins might be multifactorial. Yazici et al. (22) stated that the effect of staining solutions such as tea and coffee on the color changes in composites depends on the immersion time and the content of the resin material. Besides, some studies emphasized the effect polishing procedures ,on color stability of nanocomposites. (23,24). The type of staining solution might also be very decisive in the coloring of the composite: beverages such as tea, coffee, coke, which are often consumed in daily life, oral hygiene products such as chlorhexidine are investigated by studies (1-3, 10,11,22-25,27) and their discoloring effects on the composites are clearly stated.

Regardless of the solution, the structural properties organic matrix of composites may act in higher susceptibility to water absorption and material disintegration. As indicated on literature (26,27) which are in accordance with our study, the greater proportion of TEGDMA in microhybrid composite might be resulted in an increase in water uptake, herewith increased discoloration (4,24±0,65) compared to nanohybrid (1,24±0,65) and microfilled (3,59±0,47) composite group at the end of the T1 period. In a recent study (28), it was stated that hydrophilic organic matrix elements such as Bis-EMA tends to react less favorably to pigment incorporation comparing UDMA or Bis-GMA. Therefore, hydrophobic aromatic/aliphatic dimethacrylate content of nanohybrid composite tested in this study may be closely associated with the reason lower color changes compared other composite groups.

The amount of inorganic filler might be one of the possible reasons on susceptibility for color changes as the spaces between the filler particles favor pigment deposition (29). However, there were no significant correlation between the degree of a color change and the percentile filler content of composite in our study (p>0,05).

The staining susceptibility of a composite resin may also be attributed to its filler type. Unlike to Poggio et al. (30) which stated nanohybrid absorbs staining substances more easily than microhybrid, the less discolored composite discs were nano-hybrids in our study. On the other hand, Reddy et al. (23) showed that nano-hybrid composites undergo less color change compared to microhybrid composites when exposed to beverages with different phenolic contents, similarly to our study.

Within the limitations of the present study, it can be concluded that the ratio of phenolic and flavonoid components in any herbal formulation played a more important role in its discoloration potential than color or viscosity alone.

ETHICAL DECLARATIONS

Ethics Committee Approval: This study does not require ethics committee approval as it is not within the scope of clinical and experimental studies on humans and animals (including data& material).

Informed Consent: This study does not require informed consent.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

Financial Disclosure: The authors declared that this study has received no financial support.

Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper and that they have approved the final version.

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