Original Article

# Effect of phytopigments on discoloration of nanohybrid composite: An *in vitro* study

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## Abstract

**Objective:** The purpose of this study is to comparatively evaluate the effect of discoloration of nanohybrid composite by four different phytopigments.

**Materials and Methods:** Fifty disk-shaped samples of nanohybrid (3M Filtek Z350) resin composites were prepared using an acrylic template of dimension 5 mm × 3 mm. They were randomly divided into five groups and immersed in solutions of tomato powder, beetroot powder, java plum powder, and turmeric powder. Distilled water was used as the control group. The samples were placed in respective solutions for 3 h daily and stored in artificial saliva for the rest of the day for 28 days. Color values (L*, a*, b*) were measured by colorimeter using the CIE L*a*b* system at the end of the 7th and 28th days of immersion. Color differences ΔE*ab were statistically analyzed.

**Results:** All the samples showed a change in color of nanohybrid composite resin to varying degrees. The mean ΔE*ab value obtained with beetroot solution was the highest among all the groups at the end of the 7th and 28th days, depicting that beetroot solution showed maximum mean color variation, followed by java plum solution, turmeric solution, and tomato solution.

**Conclusion:** All the phytopigments used in this study have the potential to discolor the nanohybrid composite resin, with beetroot causing the most severe discoloration.

**Keywords:** Colorimeter; discoloration; nanohybrid composite resin; phytopigments

## INTRODUCTION

Composite resin is extensively utilized as an esthetic restorative material for both anterior and posterior teeth primarily because of its enhanced physical characteristics and adhesive properties.[1,2] It has the capability to replicate the natural color and shade of teeth, facilitating esthetically pleasing dental restorations. Composite discoloration may be attributed due to various reasons such as type of resin, fillers, and types of food intake. The adherence of exogenous colorants to the surface and their infiltration into the resin matrices may induce chromatic alterations and compromise the esthetic integrity of the outcome.[3,4] The surface roughening due to wearing and degradation of chemical can impact gloss levels, potentially leading to an increase in extrinsic staining.[5] In composite resins, inadequate curing and the presence of unconverted camphorquinone are potential causing of discoloration.[6]

Nanohybrid composites usually consists of milled glass fillers (40 to 50 nm) that acts as dispersed phase. They provide better mechanical strength and surface finish than hybrid composites.[7]

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Date of submission : 01.04.2024
Review completed : 13.04.2024
Date of acceptance : 15.04.2024
Published : 10.05.2024

Access this article online
Quick Response Code:
Website: https://journals.lww.com/jcde
DOI: 10.4103/JCDE.JCDE_169_24



**How to cite this article:** Geethanjali R, Krishnamurthy M, Natanasabapathy V, Kumar VN, Leburu A, Elangovan SK. Effect of phytopigments on discoloration of nanohybrid composite: An *in vitro* study. J Conserv Dent Endod 2024;27:552-5.

Carotenoids, a class of tetraterpene pigments, manifest a spectrum of colors including yellow, orange, red, and purple. They are categorized into two groups: carotenes and xanthophylls. Carotenes, exemplified by α-carotene, β-carotene, β,ψ-carotene, and lycopene, are hydrocarbons. Conversely, xanthophylls are carotenoids containing oxygen atoms. Although many studies on evaluating discoloration of coffee, tea, and other beverages on composites are present, it is important to know the effect seen in organic food consumed by the present generation and its influence on composite restorative material.

The aim of the study is to assess and evaluate four different phytopigments on discoloration of nanohybrid composite resin, and the objective is to evaluate the discoloration using two hydrophobic phytopigments, namely curcumin and lycopene found in turmeric and tomato, respectively, and two hydrophilic phytopigments, namely anthocyanins and betalains found in java plum and beetroot.

## MATERIALS AND METHODS

### Sample preparation

Fifty disk-shaped samples of the nanohybrid resin composites (3M ESPE, Filtek Z350 XT, Universal Restorative syringe, CE 0123, Lot 1017 St. Paul, MN 551441000, USA) were prepared with 5 mm × 3 mm acrylic template. In order to attain a smooth and plane surface while preventing the formation of air bubbles, the sample was positioned within a template placed between two glass plates lined with mylar matrices, LED light-curing unit (Ivoclar Bluephase N MC) with a light intensity of 800 mW/cm2 was cured for 40 s on both upper and lower surfaces. The curing unit's tip was precisely positioned directly above the 2 mm thick glass plate. Prior to conducting baseline color value assessments using a colorimeter (Genist International digital colorimeter), the disks were immersed in distilled water. To implement blinding procedures, the samples underwent randomization using a "free online random generator tool."

- Group 1 – Turmeric powder ($n = 10$)
- Group 2 – Beetroot powder ($n = 10$)
- Group 3 – Java plum powder ($n = 10$)
- Group 4 – Tomato powder ($n = 10$)
- Group 5 – Distilled water ($n = 10$).

### Immersion in test solution

The baseline color values were measured with a colorimeter according to the CIE $L^*a^*b^*$ color scale. 0.1% weight solution of turmeric powder (Healthworks Turmeric Powder), beetroot powder (Hudetex Beetroot Powder Organic), java plum powder (Bixa Botanical Jamun Powder), and tomato powder (Eastanbul Tomato Powder) ($n = 10$) were made with distilled water. The samples were submerged in the test solutions for a duration of 3 h and stored in artificial saliva for the rest of the day for 28 days. The solutions were replaced daily, and it was periodically agitated.

### Coloration analysis

On the 7$^{th}$ and 28$^{th}$ days, the samples underwent testing with colorimeter to assess changes in color values. The color difference before and after submersion was determined with the equation $\Delta E^*ab = ([\Delta L]^2 + [a]^2 + [b]^2)\ 1/2$.

### Statistical analysis

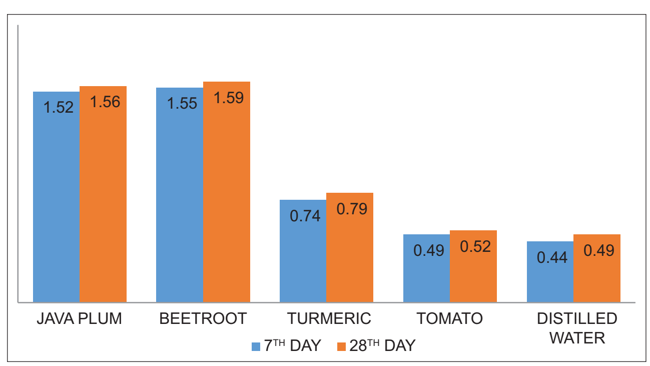
IBM SPSS Statistics for Windows, version 20.0., Armonk, NY, USA: IBM Corp, USA, was used, and the overall discoloration comparison between the groups was analyzed using two-way ANOVA test, followed by *post hoc* Tukey's (honest significant difference) test at the end of the 7$^{th}$ and 28$^{th}$ days at $P < 0.00$.

## RESULTS

Table 1 shows the mean and standard deviation in discoloration at the end of the 7$^{th}$ day and 28$^{th}$ days after immersion in the test solution. Graph 1 shows the mean comparison between groups. The difference between the color changes of all the groups was statistically different. The mean $\Delta E^*ab$ value obtained with beetroot solution was the highest among all the groups at the end of the 7$^{th}$ and 28$^{th}$ days. Beetroot solution showed the maximum discoloration with a mean value of 1.55 at the end of the 7$^{th}$ day and 1.59 at the end of the 28$^{th}$ day, followed by java plum with 1.52 and 1.56, respectively, as both were hydrophilic in nature, followed by the hydrophobic phytopigments turmeric and tomato.

## DISCUSSION

This research evaluated the ability of plant-based phytopigments from food to discolor the nanohybrid composite resin. Composites containing Bis-GMA demonstrated greater hydrophilicity and higher water sorption rate compared to those that contain urethane dimethacrylate.[7] Incorporation of small quantity of triethylene glycol dimethacrylate (TEGDMA) into a Bis-GMA-based resin matrix can lead to a notable




**Graph 1:** The mean comparison between groups

**Table 1: Comparison between average color changes ΔE in each group at the end of the 7th and 28th days**

| Groups | *n* | $\Delta E_1$ mean (end of 7th day) | $\Delta E_2$ mean (end of 28th day) | SD (end of 7th day) | SD (end of 28th day) | *P* |
|---|---|---|---|---|---|---|
| Distilled water | 10 | 0.65 | 0.68 | 0.78 | 0.79 | 0.001** |
| Turmeric | 10 | 0.74 | 0.77 | 0.09 | 1.1 | |
| Beetroot | 10 | 1.55 | 1.89 | 0.06 | 0.08 | |
| Tomato | 10 | 0.42 | 0.67 | 0.04 | 0.06 | |
| Java plum | 10 | 1.52 | 1.58 | 0.09 | 0.12 | |

*$P<0.05$. SD: Standard deviation

augmentation in the water sorption of composite material.[8] TEGDMA exhibits a strong affinity with water molecules, leading to increased surface hydrophilicity.[9] Composite resins characterized by elevated high water sorption and hydrophilicity are prone to discoloration due to the absorption of colorants along with water into the resin matrices. Excessive water sorption has the potential to diminish the longevity of resin composite.[10]

The incorporation of pigments into composite relies on how well the pigment blends into the matrix. Polyphenols are hydrogen-releasing acidic compounds. The discoloration of composite resins causes composite discoloration.

Turmeric is known as "golden spice of India." Curcumin, the yellow color pigment of turmeric,[11] is the major curcuminoid/polyphenolic compound which is of 9%.[12] They are hydrophobic in nature. The increased quantity of polyphenols and pigments causes staining ability in turmeric.

Lycopene is a red-colored pigment present in red fruits and vegetables. It was first discovered in tomato and later named as lycopene.[13] It is a carotenoid hydrocarbon which is hydrophobic in nature. The double-bond system of these compounds is responsible for its pigment as it forms chromophore.[14] As conjugated double bonds increase, there is an increase in the wavelength for maximum absorption.[15] A previous study conducted by Thaliyadeth *et al*.[11] compared nanohybrid (direct) resin based composite (G aenial) and indirect (Adoro) resin composites with Indian spices; turmeric powder, tamarind extract, paprika powder, and saffron extract and concluded that turmeric group caused more staining than other tested material with indirect resin based composite showing less stainability than nano hybrid (direct) resin composite.

Anthocyanins are hydrophilic phytopigments, are found in java plum, and are water-soluble pigments of phenolic group. Among these, cyanidin-3-glucoside is usually found in plant species.

Betalains are pigments containing nitrogen, are found in plant families 'caryophyllales order. They are hydrophilic and are seen in beetroot. Betalains, such as betacyanins and betaxanthins, are added as color additives in food.

Color perception is a psychological concept and is influenced by an observer and can be differently interpreted. Colorimeter quantifies the quantity of light reflected by specific colors, with color measurements relying on the CIE Lab system. It was developed by the Commission Internationale d'Eclairage, which is designed to characterize colors according to human perception. The CIE L*a*b* system was selected for assessing color variation (E*ab) due to ability to determine subtle color changes and has other advantages such as repeatability, sensitivity, and objectivity.[16] Another study done by Ceci *et al*. [17] evaluated microfilled flowable, nanofilled, nanohybrid, microfilled, and nanohybrid ormocer composites. Test group were immersed in cola and control group in physiological solution for 24 h and immersed in coffee or red wine for a period of 28 days. The study concluded that cola group caused more staining when compared to other. A study by Muhittin *et al*.[18] evaluated microhybrid and nanofilled composite resin color stability after immersion in red wine along with other caffeine beverages and concluded that red wine was the most significant.

Methods employed for removing composite discoloration encompass air polishing, bleaching or whitening, conventional repolishing, and in certain cases, replacement of existing highly stained composite. It is worth noting that air polishing might induce surface roughness. While repolishing restores composite color, this process entails partial removal of material from restoration surfaces, which typically does not result in any significant alterations. In scenarios with deep pigment penetration, it is impossible to revert back to its initial shade by polishing and bleaching is recommended in such cases.[19] Several investigations have examined the alteration in color of composites with concerning factors such as composition, depth and intensity of curing light, and surface texture.[20,21]

A study by Schroeder *et al*.[22] evaluated the color stability of two composites with unprotected/protected hydrosoluble gel, where it was subjected to different stains and brushing simulation, and concluded that hydrosoluble gel protection was able to minimize color change only on the 1st day. The results of this study offer valuable insights into the potential behavior of nanocomposites when exposed to various phytopigments present in foods, thus helping the clinician's choice of material and patient's control over their nutrition practices.

## Future perspectives

Tooth color is influenced by the characteristics of tooth itself. When light strikes, a portion of it is reflected back from the tooth's surface.[23] The human eye typically cannot perceive ΔE values below 1.5, but it is measurable with the spectrophotometer; therefore, further studies with the help of a spectrophotometer could provide more precise results.

### Limitations of the study

The findings should be interpreted with consideration of the constraints inherent in an *in vitro* study. Extrapolating these results to *in vivo* conditions may be challenging due to the differences. In this study, the discoloration was evaluated only at two different time intervals (7th and 28th days of immersion) and the concentration of the solution and duration of immersion had minimum relevance to the dietary habits of the population. Resins have the potential to absorb fluids, which can subsequently lead to discoloration.[24]

These results are subjected to variations under different conditions including variations in immersion media, immersion protocols, types of composite resins, and solution concentration used. Moreover, it is important to note that the oral environment is dynamic and differs significantly from laboratory conditions simulated *in vitro*. Further *ex vivo* research involving previously restored Class III and Ellis I and II is warranted. Future studies on comparing different types of composite resin are also required as this study evaluates only nanohybrid composite resin. Clinical trials are necessary to explore the impact of phytopigments on discoloration on nanohybrid composites.

## CONCLUSION

Within the constraints of this research, it is acknowledged that phytopigments present in food have the capacity to cause discoloration in composites, with beetroot demonstrating the most pronounced discoloration effect. Furthermore, contemporary composite resins available in the market like nanohybrid composites are still susceptible to discoloration by exogenous factors despite the advancement in field of resin-based restorative material.

### Acknowledgment

I would like to express my sincere gratitude to my HOD, Dr. Madhuram K MDS, and Dr. Velmurugan sir for the continuous support of my research work and for their patience, motivation, and immense knowledge. Their guidance helped me in the time of research and writing of this paper. I could not imagine having a better advisor and mentor for my study.

### Financial support and sponsorship

Nil.

### Conflicts of interest

There are no conflicts of interest.

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