Evaluating The Effect of Some Medicinal Plants (\textit{Mentha piperita}, \textit{Ocimum basilicum}, \textit{Rosmarinus officinalis}, \textit{Salvia officinalis}) on Whitening of the Permanent Teeth

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\textbf{A R T I C L E  I N F O}

Research Article

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\textbf{A B S T R A C T}

Nowadays, whitening of stained teeth has become the most popular topic in aesthetic and cosmetic dentistry. Because of the side effects of materials that were used for bleeding, in this study the effects of some plants which were used in Anatolian folk medicine on the treatment of tooth staining were examined. In this study, upper central incisors which were extracted for periodontal reasons were used. The colour values of numbered teeth were obtained and the teeth were immersed into three different essential oils of medicinal plants (\textit{Mentha piperita}, \textit{Ocimum basilicum}, \textit{Rosmarinus officinalis}, \textit{Salvia officinalis}) for different time periods (1 day, 1 week, 1 month). At the end of the immersion periods, colour measurements of all samples were made and the colour changes were analysed. Obtained data were statistically analysed by using ANOVA and Duncan test. As a result of the variance analysis, plant species and the duration of immersion was found to be statistically significant. Within the limits of this study, we can indicate that tested medicinal plants has a whitening effect by resulting significant change in tooth colour.

\textbf{Introduction}

Colour of healthy teeth is determined by factors such as colour of crown enamel, colour tone of the dentin, enamel thickness increasing towards the occlusal and incisal edges, and decreasing towards the cervical edge, and enamel transparency varying according to the degree of calcification. Enamel colour normally varies between blue-white, yellow and gray-white. Teeth covered with transparent enamel reflect the colour of the underlying dentin and appear brown-yellow, while teeth covered with thick opaque enamel appear gray. Dental colour physiologically darkens with age due to infiltration of ions and minerals to the enamel over time, thinning of enamel due to abrasion, leakage of coloured materials from dentin channels, formation of secondary and tertiary dentin, pulp stones, and denticles (Önal, 2004). Other than physiological colouring, many factors can also cause tooth discoloration. Depending on the localization and ethology, colourings can occur due to exogenous or endogenous sources or in combination of both (Haywood and Heymann, 1989; Hattab et al., 1999; Esener, 2003). Exogenous discoloration occurs as a result of accumulation of factors such as tea, coffee, fruit juice, cigarette, and various mouthwashes, etc. (Goldstein et al., 1994; Esener, 2003). Endogenous discoloration is examined under two headings: systemic and local factors. Drugs (such as tetracycline), dystrophic calcifications, fluorosis, hyperbilirubinemia, amelogenesis imperfecta, and dentinogenesis imperfecta are among the systemic factors. Pulp necrosis, intrapulpal hemorrhage, residual pulp tissues after endodontic treatment, endodontic materials, coronal restoration materials, root resorption and aging can be considered as local factors of endogenous discoloration (Cimilli, 2000).
In recent years, "whitening practices" have been widely preferred in aesthetic dentistry because they are more conservative and less expensive than prosthetic approaches in the treatment of tooth discoloration (Haywood and Heymann, 1989; Goldstein et al., 1994; Cimilli, 2000).

Whitening treatment options vary depending on the type, localization and degree of tooth discoloration. It is of utmost importance for treatment whether discoloration is exogenous or endogenous (Yamanel and Çağlar, 2011).

Based on the application method, there are three different vital tooth whitening methods, including office-type whitening, home-type whitening, and whitening with over-the-counter products developed along with a growing interest in recent years (Matis et al., 2009). In-home-type whitening, low-concentration carbamide peroxide (10-20%) or hydrogen peroxide (3-8%) is applied for 2-4 hours daily for 2 to 3 weeks (Jointer, 2007). In office-type whitening, high-concentration hydrogen peroxide (15-37%) or carbamide peroxide (35-37%) is used by the dentist (Bernardon et al., 2010). In addition to these whitening methods conducted by professionals, there is a growing interest in over-the-counter products that were introduced in the United States in the early 2000s and are a cheaper alternative to traditional whitening methods (Donley et al., 2007). Among these whitening products that can be easily found in markets, pharmacies and even on the internet, there are toothpastes, gels, mouthwashes, gums, and whitening tapes. All these products contain carbide or hydrogen peroxide at low concentrations (Matis et al., 2009). In the treatment of nonvital teeth, thermocatalytic method, walking bleaching technique, home and office-type whitening or combined techniques can be used (Donley et al., 2007; Bernardon et al., 2010)). In the thermocatalytic technique, 30-35% hydrogen peroxide is heat activated after being placed in the pulp chamber (Rosentritt et al., 2005). However, this method is thought to cause external cervical root resorption after the whitening procedure (Hannig et al., 2007).

Medicinal and aromatic plants are plants that are used as medicines to maintain health, to cure or prevent diseases; and their therapeutic use goes back a long time in human history. Some stone inscriptions and tablets contain recommendations and the average $L^*$, $a^*$, $b^*$ values were recorded three times. Colour was measured again after teeth were placed in medicinal plant essential oils (Mentha piperita, Ocimum basilicum, Rosmarinus officinalis, Salvia officinalis) for different durations (24 hours, 1 week, 1 month), and the colour changes ($\Delta E^*$) were calculated by the following formula (Ferrari et al., 2004):

$$\Delta L^* = L^*_{\text{final}} - L^*_{\text{initial}}$$

$$\Delta a^* = a^*_{\text{final}} - a^*_{\text{initial}}$$

$$\Delta b^* = b^*_{\text{final}} - b^*_{\text{initial}}$$

$$\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$$

All statistical analyses were performed using SPSS Statistics 20.0 (SPSS Inc. Chicago, IL, USA) computer program at 95% confidence interval and P<0.05 significance level. The obtained data were analysed by two-way analysis of variance (ANOVA) and inter-group comparisons were subjected to Duncan test.

Results

Results of the variance analysis used to compare $\Delta L$ values are shown in Table 1. It was determined that the type of essential oil (P<0.001), the waiting time (P<0.05) and the interactions (P<0.001) were statistically significant.

Mean $\Delta L$ values and standard deviations of the samples kept in different plant essential oils are shown in Table 2.
Maximum colour change was observed in samples kept in *Salvia officinalis* essential oil for 24 hours (-13.08), while minimum color change was observed in samples kept in *Mentha piperita* essential oil for 30 days (1.98).

Multiple comparison test (Duncan test) results revealed that:

- Color change values obtained in samples kept in *Ocimum basilicum* essential oil (-0.60) and *Rosmarinus officinalis* essential oil (-0.61) were similar, whereas color change values obtained in samples kept in *Salvia officinalis* essential oil (-0.86) and *Mentha piperita* essential oil (-4.18) were different from each other and the remaining values,
- Color change values obtained in 7 days (-2.07) and 30 days (-2.14) were similar, whereas these were different from values obtained in 24 hours (-4.07).

As seen in the variance analysis table, waiting time and type of plant essential oil had a significant effect on the Δa value (P<0.05), whereas the interactions were not statistically significant (P>0.05) (Table 3).

Mean Δa values and standard deviations of the samples kept in different plant essential oils are shown in Table 4. a values were found to decrease most in *Salvia officinalis* essential oil after 7 days (-0.46).

Multiple comparison test (Duncan test) results revealed that:

- Color change values obtained after 7 days (-0.23) and 30 days (-0.05) were not different from each other, whereas these were different from the values obtained after 24 hours (0.34),
- Samples kept in *Mentha piperita* essential oil (-0.36) and *Rosmarinus officinalis* essential oil (-0.11), *Rosmarinus officinalis* essential oil (-0.11) and *Salvia officinalis* essential oil (0.17) and *Ocimum basilicum* essential oil (0.28) showed similar results, whereas these groups were different than samples kept in *Mentha piperita* essential oil.
Variance analysis results used for the comparison of Δb values are shown in Table 5. It was found that type of plant essential oil (P<0.001) and waiting time (P<0.05) were significant, whereas the interactions were not statistically significant (P>0.05).

Mean Δb values and standard deviations of the samples kept in different plant essential oils are shown in Table 6. b values were found to decrease most in Mentha piperita essential oil after 30 days (-2.50).

Multiple comparison test (Duncan test) results revealed that:
- Values obtained after 7 days (0.25) and 30 days (-0.23), and values obtained after 7 days (0.25) and 24 hours (1.68) were not significantly different.
- Samples kept in Mentha piperita essential oil (-2.30) and Rosmarinus officinalis essential oil (-0.91), and Rosmarinus officinalis essential oil (-0.91) and Salvia officinalis essential oil (-0.73) showed similar results, whereas the values obtained from samples kept in Ocimum basilicum essential oil (5.57) were significantly different than other groups.

Variance analysis results used for the comparison of ΔΕ values are shown in Table 7. It was found that type of plant essential oil and the interactions were statistically significant (P<0.01), whereas waiting time is not statistically significant (P>0.05). Mean ΔΕ values and standard deviations of the samples kept in different plant essential oils are shown in Table 8. Maximum color change was observed in Salvia officinalis essential oil (14.61), whereas minimum color change was observed in Mentha piperita essential oil (5.36) after 24 hours.

Multiple comparison test (Duncan test) results revealed that:
- Values obtained after 24 hours (8.17), 7 days (7.68) and 30 days (8.08) were not significantly different.
- Samples kept in Mentha piperita essential oil (7.24) and Rosmarinus officinalis essential oil (6.14), and Mentha piperita essential oil (7.24) and Ocimum basilicum essential oil (8.18) showed similar results, whereas color changes obtained in samples kept in Salvia officinalis essential oil (10.36) were significantly different from other groups.

Table 6. Mean Δb values and standard deviations of samples kept in different plant essential oils (N= 20).

| Time     | Medicinal Plants          | Mean  | Std  | Mean  | Std  | Mean  | Std  | Mean  | Std  |
|----------|---------------------------|-------|------|-------|------|-------|------|-------|------|
|          | Mentha piperita           | -2.41 | 4.28 | 5.52  | 4.43 | 0.14  | 4.13 | 3.49  | 5.15 |
| 24 Hours | Ocimum basilicum          | 5.37  | 5.17 | 5.37  | 5.17 | -1.44 | 4.96 | -2.35 | 7.10 |
|          | Rosmarinus officinalis    | 4.14  | 0.12 | 4.14  | 0.12 | 3.25  | 1.36 | -1.36 | 6.18 |
|          | Salvia officinalis        | 3.25  | 1.36 | 3.25  | 1.36 | 4.13  | 1.36 | -1.36 | 6.18 |

Table 7. Variance analysis results of ΔΕ values.

| Source                | Type III Sum of Squares | df | Mean Square | F    | Sig.  |
|-----------------------|-------------------------|----|-------------|------|-------|
| Time (T)              | 10.959                  | 2  | 5.480       | 0.270| 0.763 |
| Plant Essential Oil (PEO) | 578.146            | 3  | 192.715     | 9.502| 0.000 |
| T*PEO                | 735.830                 | 6  | 122.638     | 6.047| 0.000 |
| Error                | 4624.289                | 228| 20.282      |      |       |
| Total                | 21226.776              | 240|             |      |       |

Table 8. Mean ΔΕ values and standard deviations of samples kept in different plant essential oils (N= 20).

| Time     | Medicinal Plants          | Mean  | Std  | Mean  | Std  | Mean  | Std  | Mean  | Std  |
|----------|---------------------------|-------|------|-------|------|-------|------|-------|------|
|          | Mentha piperita           | 5.36  | 3.34 | 6.66  | 4.25 | 6.05  | 3.22 | 14.61 | 5.29 |
| 24 Hours | Ocimum basilicum          | 8.00  | 4.70 | 9.15  | 4.72 | 6.13  | 4.06 | 7.44  | 5.81 |
|          | Rosmarinus officinalis    | 8.36  | 3.84 | 8.74  | 4.44 | 6.23  | 3.37 | 9.01  | 5.95 |
|          | Salvia officinalis        | 8.36  | 3.84 | 8.74  | 4.44 | 6.23  | 3.37 | 9.01  | 5.95 |

Discussion

Different chemical agents are used to treat tooth discoloration (Alaçam, 2000; Haywood and Heymann, 1989). Whitening agents used today are either hydrogen peroxide (H₂O₂) containing or hydrogen peroxide producing chemical substances (Çalışkan, 2006).

Although the mechanism of whitening is not yet fully explained, it is stated to be based on oxidation. Whitening agents used in dentistry generally consist of different forms of hydrogen peroxide such as hydrogen peroxide, sodium perborate, and carbamide peroxide (MacIsaac and Hoen, 1994; Haywood, 1992; Perrine et al., 1999). Carbamide peroxide-containing preparations have equal oxidative capabilities as hydrogen peroxide or sodium perborate (Haywood et al., 1990; Haywood, 1992). When carbamide peroxide comes into contact with tissues, oxygen is released at different levels depending on the concentration of urea, ammonia, carbon dioxide, hydrogen peroxide and preprare (Haywood et al., 1990; Haywood, 1992; Perrine et al., 1999).

In the thermocatalytic method combined with hydrogen peroxide, which is one of the various techniques used for whitening, caustic substances that are released cause pH to drop in the cervical crown of the tooth, resulting in cervical root resorption (Friedman et al., 1980; Gimlin and
Sodium perborate or carbamide peroxide is used to prevent cervical root resorption caused by pH changes that occur during the whitening treatment (Caughman et al., 1999). In a study investigating the diffusion rates of whitening agents outside the root, it was determined that 35% hydrogen peroxide resulted in highest, sodium perborate resulted in moderate, and carbamide peroxide resulted in lowest diffusion out of the root (Lee et al., 2004). Considering the low diffusion level of carbamide peroxide and its success in whitening, it can be said that it is safer to use than other preparations (Lim et al., 2004). Today, whitening therapy is frequently used on vital or nonvital discoloured teeth (Güler et al., 2015, İpek and Bayrak, 2010). The most important advantage of whitening treatments is that natural tooth structure is not disrupted. However, patients should be informed about possible cervical root resorption and complications such as rediscoloration of teeth and should be called for regular checkups (Güler et al., 2015).

Therefore, whitening effectiveness of different plant essential oils used in complementary and alternative treatment were investigated in this study. When ΔE values were evaluated, lowest color change values were observed in Mentha piperita essential oil (5.36), and highest color change values were observed in Salvia officinalis (14.61).

An increase in L value indicates that the transparency and therefore the whiteness of the teeth are increased. When ΔL values were evaluated, it was found that lowest color change values were observed in Mentha piperita volatile oil (-0.25), and highest values were obtained in Salvia officinalis volatile oil (-13.08), that is, the transparency of teeth kept in Salvia officinalis volatile oil increased.

In a study, it was determined that tooth colour was significantly improved by the walking-bleach whitening technique applied to remove dental discolorations caused by different root canal pastes; but in the examinations conducted after 3 months, it was found that most of the teeth displayed discolorations and the whitening effect disappeared (Zıraman and Aslan, 1995).

For this reason, this study was conducted over a long time period and it was found that the whitening effect of plant essential oils persisted after a period of one month. Basting et al. (2003) evaluated the effect of carbamide peroxide preparation at seven different concentrations on the micro hardness of enamel. As a result of the study, carbamide peroxide agents at different concentrations were found to decrease the micro hardness of enamel, but this effect was reduced and the agents were less harmful to the mineral structure of enamel when the products contained fluoride. Efeoğlu et al. (2005) evaluated 10% carbamide peroxide applied enamel tissue by micro-CT and found that 10% carbamide peroxide application for 2 weeks caused demineralization in enamel up to a depth of 50μm. The obtained results have shown that essential oils of these plants can be used for whitening.

Colour scales, digital photographs, spectrophotometers and colorimeters are used today to measure color changes during whitening treatments (Guan et al., 2005). The most common of these methods is to determine the colour of the teeth with a colour scale before and after treatment and compare the results (Analoui et al., 2004). In this subjective method, many factors such as the dentist’s experience, age, eye fatigue, patient’s make-up, light conditions in the room, and room decor can affect the evaluation of colour.

It is difficult to control and standardize these factors in clinical conditions (Joiner, 2006). Objectively measuring tooth colour ensures that the results are reliable and reproducible (Wiegand et al., 2005).

Colour is expressed by the \( L^*a^*b^* \) space system according to Commission Internationale de l’Eclairage (CIE). CIE is the acronym of the L’Eclairage in the Commission Internationale, and it is the International Commission on Illumination. CIE \( L^*a^*b^* \) color space system is an analysis system that allows the measurement of color in three dimensions (Zekonis et al., 2003). The axes in this three-dimensional color space system are \( L^* \), \( a^* \) and \( b^* \). The \( L^* \) value is the lightness measurement of an object and is a number between 0 and 100. 0 indicates black, and 100 indicates white color (Joiner, 2006). \( a^* \) and \( b^* \) take values between -128 and +127. In this study, \( L^*a^*b^* \) color space system was used so that color measurements and color change evaluations would be performed with a numerical analysis system and therefore provide more reliable results. When \( L^* \), \( a^* \) and \( b^* \) values were evaluated, it was found that \( L^* \) increased with time, indicating that teeth became brighter, and \( a^* \) and \( b^* \) decreased, indicating that redness and yellowness of teeth decreased.

In the study, a value decreased most when samples were kept in Mentha piperita essential oil for 24 hours, and \( b^* \) value decreased most when samples were kept in Mentha piperita essential oil for 30 days. These results suggest that redness is reduced after 24 hours, and yellowness is reduced after 30 days in samples kept in Mentha piperita essential oil.

Objective measurement of colour changes as a result of whitening treatments allows the determination of the degree of whitening in discoloured teeth due to different etiologies and allows comparison of different whitening methods and agent concentrations that are used. In addition, the facts that these results are reproducible and can be statistically analysed are important advantages of such colour analysis methods (Ermiş et al., 2007).

Within the limitations of this study conducted in \textit{in vitro} conditions, we observed that essential oils of all the medicinal plants that were used in the study had whitening effects, and we believe that these results should be supported by clinical studies.

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