

The objectives of this study were to evaluate the inhibitory effect on plaque and the tooth discoloration associated with agents used in the chemical plaque control of oil pulling in comparison with chlorhexidine-containing mouthwash and water in a four-day plaque regrowth model.

### **Study Design**

At the beginning of a two-week preparation period, plaque, calculus and dental stains will be thoroughly removed, both hand and by ultrasonic scaling and polishing, and oral hygiene instruction will be provided for self-administered plaque control (Fig. 1). At the end of the preparation period, all participants are expected to have clinically healthy gingivae. The participants will randomly divided into five groups, and the coded allocated product will be provided. The instructions for use of the allocated products will be given to the individuals with a printed program by a dentist who will not involved in the study. On day 1 of the study, after the plaque has been stained with a plaque disclosure tablet (Curaprox, Kriens, Switzerland), all participants will undergo scaling and polishing to remove plaque and tooth staining, and teeth will be redisclose with a tablet to ensure that all the participants will be provided with a zero plaque score at the start of the trial. Then, the tooth color will be determined with a digital spectrophotometer (VITA Easyshade® V; VITA Zahnfabrik, Bad Säckingen, Germany). Afterwards, individuals will asked to avoid all types of dental cleaning and to follow the study protocol. The participants will be instructed to avoid other mouthwashes, chewing gum, or the use of toothpaste during the four-day period. They will be instructed to control the application time with a timer and will be reminded twice a day to use the allocated products for a four-day period by the dentist who assigned the product. At the end of the fourth day, tooth color will be redetermined with the VITA Easyshade® V. The teeth will then disclosed with a Curaprox® plaque staining tablet, and plaque scores will record using the Quigley-Hein and Turesky plaque index (Q-H) (Quigley & Hein, 1962; Turesky et al., 1970). Finally, the gingival index (GI) will be recorded. All clinical examinations will be performed by a blinded observer. After the clinical parameters will be recorded, the teeth will polish to remove all plaque and any tooth staining.

### **Clinical Examinations**

The Quigley-Hein and Turesky plaque indexes (Quigley & Hein, 1962; Turesky et al., 1970) will be used to measure dental plaque formation and the degree of deposition, and gingival index (Loe & Silness, 1963) to evaluate gingival inflammation. To assess tooth color change, tooth color determination will be performed at the beginning and end of the study with the VITA Easyshade® V digital spectrophotometer and will record as a CIE L\* a\* b\* value. Measurements will make under D65 standard lighting conditions corresponding to daylight, and the device will be calibrated before each measurement. Color measurements will make from the middle third of each participant's right maxillary central incisor tooth. The color of the tooth will be determined three times, and the average CIE L\* a\* b\* value will be obtained.

The color difference ( $\Delta E$ ) values of the teeth at the beginning ( $L_0, a_0, b_0$ ) and the end ( $L^*, a^*, b^*$ ) of the study will be calculated by using the following CIELAB color difference formula:

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

( $\Delta L = L^* - L_0$ ,  $\Delta a = a^* - a_0$  ve  $\Delta b = b^* - b_0$ )

The  $L^*$ ,  $a^*$  and  $b^*$  values represent the color values measured at the end of four days, while the  $L_0$ ,  $a_0$  and  $b_0$  values represent the color values measured at the beginning of the study.

### **Statistical Analysis**

The data obtained as a result of the measurements made by the participants after using different products will be analyzed using a statistical software program (IBM SPSS Statistics, v23.0®; IBM Corp., Chicago, IL, USA). Because the group number is more than three, (CHX, DW, coconut oil, turmeric oil, black cumin oil) the parametric method (ANOVA) will be used in the analysis of normally distributed data. Also, homogeneity of variance, another assumption of the ANOVA test, will also be tested during the analysis. For the multiple comparison tests, the Tukey method will be used in homogeneous groups, and the Games-Howell method will be used in non-homogeneous groups, depending on the number of samples in the groups (Tabachnick et al., 2007). Analyses will be performed at a 95% reliance interval ( $\alpha=0.05$ ).

**Figure 1** Flow chart of the study.

