ABSTRACT

Background and Aims: Calcium phosphate is most of the inorganic content of dental calculus. Therefore, knowing or controlling the precipitation mechanism of calcium phosphate is very important for the inhibition of dental calculus formation at the beginning. Plants have been known to be excellent sources of many nutritional and phytochemical content. The aim of this study is to investigate the effects of *Petroselinum crispum*, *Eruca vesicaria ssp. sativa*, *Beta vulgaris* L.var *cicla*, *Rumex crispatus DC.* and *Cotinus coggyria Scop.* aqueous extracts on calcium phosphate precipitation, which is thought to reflect the onset of dental calculus formation in vitro.

Methods: The optical density (OD) increases first with the calcium phosphate nucleation and when the balance is reached, the optical density decreases gradually when the nuclei begin to aggregate and precipitate. The OD change was monitored by recording the absorbance at 620 nm.

Results: The effect on the calcium phosphate precipitation varied differently among the 5 types of aqueous extracts. The smoke tree (*Cotinus coggyria Scop.*) extract activated calcium phosphate precipitation while all others inhibited precipitation.

Conclusion: These results suggest that some types of plant aqueous extracts may have protective potential against dental calculus initially and, therefore they may be used in toothpastes or in mouthwashes.

Keywords: Dental calculus, calcium phosphate precipitation, plant aqueous extract

INTRODUCTION

Among oral and dental health problems, dental calculus has an increasing prevalence due to changes in the diet and nutritional habits of societies (Akar, 2014) and causes many oral problems, such as noneaesthetic appearance and bad breath. Although dental calculus is not directly responsible for the occurrence of health problems such as diabetes, urinary stone formation, and cardiovascular disease, it is a secondary factor in disease progression (Batool et al., 2018, Clarke, 2015).

Dental calculus is a mineralized bacterial plaque which is a hard and calcified deposit with a bacterial plaque layer clustered on natural teeth and restorations (Moolya et al., 2010). It is composed of various inorganic components, mainly calcium phosphate.
compound, and organic matrix (Jin & Yip, 2002; White, 1997). Many factors such as diet, especially alkaline foods and sugars, saliva pH, composition and bacterial load, age, sex, race, gender, tobacco use, presence of systemic diseases, drugs used, oral hygiene practices and socioeconomic status affect dental calculus formation (Akçalı & Lang, 2018). Bacterial endotoxins damage the gum and periodontal tissue (Hidaka, Nishimura, Nakajima, & Liu, 1996). Knowing and controlling the factors affecting calcium phosphate precipitation is very important for the inhibition of initial dental calculus formation (Tarașevič, Chusuei, & Alloro, 2003). The use of plants has also become widespread in dental and oral health, with secondary metabolites and a wide range of biological activities (Gulfraz, Sadig, Tanrı, Imran, & Qureshi, 2011).

Petroselinum crispum (parsley, Apiaceae) has an antithrombinic, anti-inflammatory, hepatoprotective, and plasma calcium-enhancing effect (Irazim, Al-Daraj, Al-Mashadani, Al-Hassani, & Mirza, 2012). It contains high amounts of apigenin, ascorbic acid, eugenol, carotenoids, flavonoids, coumarins, phenylpropanoids, phthalates, furano coumarins and tocopherol components (Ajmera, Kalani, & Sharma, 2019; Pápay, Kállai-Szabó, Lóduányi, Klebovich, & Antal, 2016; Tunali et al., 1999). Eruca vesicaria ssp. sativa (garden rocket) belongs to the cabbage Brassicaceae family. It is known as a diuretic, antiulcer, anti-inflammatory, antitumour, anticoagulant, antiallergic and antifungal activity (Taviano et al., 2018 and 2017; Gulfraz et al., 2011; Sacan, Orak, & Yanardag, 2008). It contains important secondary metabolites such as flavonoids, alkaloids, tannins, phenoaxides, saponins and ascorbic acid. Its essential oils contain high concentrations of an especially antibacterially effective erucic acid (Farwar Alam, Kaur, Jabbar, Javed, & Athar, 2007). Beta vulgaris L. var. cicla (chard) is from the family Chenopodiaceae and is a vegetable rich in vitamins A, B, C, calcium, iron, and phosphorus. In its structure, it contains fatty acids such as palmitic acid, citric acid, oleic acid, linoleic acid, and phospholipids, glycolipids, polysaccharides, saponin, pectin, and flavonoids (Tunali, 2020; Mzoughi et al., 2019). It has antidiabetic, antioxidant, antitumor, antimicrobial, hepatoprotective, anti-inflammatory, and anti-acyclovirinesterase activity (Sacan & Yanardag, 2010; Bolkent, Yanardağ, Tabakoğlu-Oğuz, & Oszay-Saçan, 2000; Mzoughi et al., 2019). Rumex crispus (curled dock) Polygonaceae is a plant rich in anthracene derivatives from the black grain family. It also contains tannins, flavonoids, and naphthalene derivatives. It has antioxidant and antimicrobial effects (Itis, Wintola, & Afolayan, 2019; Demir, Bozkurt, Onur, Kay, & Somer, 2017; Coruh, Gormez, Er, & Sengil, 2008). Cotinus coggygria (smoke tree) is a plant belonging to the Anacardiaceae family, generally known as “smoke tree”. It has been shown by in vivo and in vitro studies that it has many activities such as antioxidant, antibacterial, antifungal, antiviral, hepatoprotective, and anti-inflammatory (Matić, Stanić, Mihačič, & Bogojević, 2016). It has been reported that the Cotinus coggygria, which has a high flavonoid content, has cytotoxic effects on bacteria and shows inhibitory properties against the common components of dental plaque, S. mutans and S. sanguinis (Ferrazzano et al., 2013; Wang, Wang, Du, Fei, & To, 2016). In this study, the effects of five plant aqueous extracts (Petroselinum crispum, Eruca vesicaria ssp. sativa, Beta vulgaris L.var.cicla, Rumex crisatus DC, and Cotinus coggygria Scop.) on the mechanism of nucleation and aggregation of calcium phosphate precipitation which is thought to reflect initial dental calculus formation in vitro, was evaluated.

MATERIALS AND METHODS

Preparation of plant aqueous extracts

Plant extracts were prepared at the Department of Chemistry, Faculty of Engineering, Istanbul University-Cerrahpasa. Petroselinum crispum, Beta vulgaris L.var.cicla were identified by Prof. Dr.Neriman Ozhatay. Rumex crisatus DC., and Eruca sativa were identified by Prof. Dr. Kerim Alpinar. Cotinus coggygria was identified by Prof. Dr. Sukran Kultur, Faculty of Pharmacy, Istanbul University. The plant materials were washed with water and dried at room temperature. The dried plants were stored at -20°C until used. Dried leaves (50 g) were extracted by adding 500 mL of distilled water and boiling for 8 hours. The extracts were then filtered and lyophilized. Then, were kept at -20°C. When used, the extracts were dissolved in distilled water to obtain a saturated solution.

Screening of calcium phosphate precipitation

To obtain the standard curve for nucleation and aggregation of calcium phosphate precipitates were obtained by recording the change in optical density at 620 nm at two-minute intervals for approximately 30 minutes using the RT-9000 Semi-auto Chemistry Analyzer instrument, after mixing equal volumes of calcium chloride dihydrate (4 mM) and trisodium phosphate (10 mM) solutions at 37°C. Final concentrations of calcium and phosphate ions were about 2mM and 5 mM respectively. They are between physiologically physiological and salivary concentrations. The optical density increases first with the calcium phosphate nucleation, when the balance is reached, the optical density decreases gradually when the nuclei begin to aggregate and precipitate (Selmi Cepis, Akyuz, & Yarat, 2020).

The effects of plant aqueous extract on calcium phosphate precipitation were examined by the addition of 50 mL of an aqueous extract into the mixture of calcium and phosphate ions. Each sample was studied five times. At the end of the experiment, charts drawn between time and optical density (absorbance) and the effects of each aqueous extract on calcium phosphate precipitation were evaluated statistically.

Statistics

The Statistical Program for the Social Sciences (SPSS) (16.0, Windows) was used in the statistical evaluation of the data obtained as a result of the study and p<0.05 was considered statistically significant. The consistency of continuous variables to normal distribution was investigated using the Kolmogorov-Smirnov test. Student’s t test was used in two independent group comparisons for normally distributed variables. Mann Whitney U test was used in comparison of two independent groups for non-normally distributed variables. Values are given as mean ± standard deviation (SD).
RESULTS

**The standard curve for precipitation of calcium phosphate:** Time-course measurements of optic density obtained by 2 mM of calcium and 5 mM of phosphate solutions at 620 nm were illustrated in Figure 1 (Selmi Cepis, Akyuz, & Yarat, 2020).

**Effects of plants aqueous extracts in calcium phosphate precipitation:** The effects of *Petroselinum crispum*, *Eruca vesicaria ssp. sativa*, *Beta vulgaris* L.var.cicla, *Rumex cristatus DC.* and *Cotinus coggygria Scop.* aqueous extracts are shown in Figure 2. Compared to a standard curve, all extracts except *Cotinus coggygria* prolonged nucleation time, decreased the nucleation rate significantly. *Cotinus coggygria* and *Rumex crispus* shortened aggregation time significantly. All extracts increased aggregation rate significantly compared to a standard curve. The increase in the aggregation rate for *Cotinus coggygria* was significantly more than those of other extracts (Table 1 and Figure 2).

DISCUSSION

Physiologically, normal Ca$^{2+}$ ion concentration in saliva has been reported to be 1.03–3.6 mM and PO$_4^{3-}$ ion concentration 4.5-6 mM. Any imbalance in these concentrations bring various problems in terms of dental and oral health. The most important of these is dental calculus formation. Calcium phosphate supersaturation, which is realized by increasing the concentration of Ca$^{2+}$and PO$_4^{3-}$ ions in saliva, is a thermodynamic driving factor for dental calculus formation (Morta, Mante, Rasa, & Gintaras, 2018). Therefore, to obtain a standard curve, which nucleation and aggregation phases of calcium phosphate precipitation can be monitored in a short time interval and in a good manner, preliminary experiments were carried out with different concentrations of these two ions. As a result, for final concentration in the mixture, 2 mM calcium and 5 mM phosphate ion concentrations, which are in the interval of salivary physiologic concentration, were found suitable to obtain a standard curve of calcium phosphate precipitation. To examine the effect of plant aqueous extracts, this standard curve was used for comparison in the present study.

In their study, Hidaka & Oishi (2007), examined the effect of nutritional components on dental calculus formation, and used both calcium and phosphate ion final concentrations of 3 mM,

![Table 1. The effects of plant aqueous extracts on the nucleation and aggregation phases of calcium phosphate precipitation.](image)

Values were given as mean ± standard deviation; *p<0.05, **p<0.01: Significantly different from standard; ***p<0.01: Significantly different from *Petroselinum crispum*, *Eruca vesicaria ssp. sativa*, *Beta vulgaris* L.var.cicla, *Rumex crispus*; **p<0.01: Significantly different from *Petroselinum crispum*, *Beta vulgaris* L.var.cicla, *Rumex crispus*; *Cotinus coggygria* Student t test, aMann Whitney U test
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unlike our study. However, the concentrations they used did not fully reflect saliva conditions.

Plant extracts have an important place in modern medicine due to their chemical and medicinal ingredients. As their metabolites have a wide range of biological activities, the use of plants has also become widespread in dental and oral health (Sener & Kilic, 2019; Chandra Shekar et al., 2015; Gulfraz et al., 2011). It has been suggested that some plant extracts can be effective in the formation of calculus in different rates because of their phenolic components such as flavonoids, tannins, and coumarins (Ohtani & Nishimura, 2020; Ben Lagha, Dudonné, Desjardins, & Grenier, 2015; Weber, Hannig, Pötschke, Höhne, & Hannig, 2015; Ferrazzano et al., 2011). In studies conducted with high polyphenol content, plants have an inhibitory effect on calcium phosphate precipitation. Hidaka, Okamoto, Ishiyama, & Hashimoto (2008) reported that propolis and honey varieties known to contain flavonoids have an inhibitory effect on calcium phosphate precipitation, and that their inhibitory effects increase with increasing flavonoid content. Similar results were obtained in studies with green tea, R. rhizoma plant, and various Kampo plants with high polyphenol content (Torki et al., 2018; Anushya & Freeda, 2017; Hidaka et al., 1996). In the present study, Petroselinum crispum, Eruca vesicaria ssp. sativa, Beta vulgaris L. var. cicla, and Rumex crispus were shown to inhibit calcium phosphate precipitation.

However, the Cotinus coggyria aqueous extract showed an activator effect on calcium phosphate precipitation. It has been reported that Cotinus coggyria with a high flavonoid content has cytotoxic effects on bacteria, and shows inhibitory properties against the common components of dental plaque such as S. mutans and S. sanguinis (Ferrazzano et al., 2013; Wang et al., 2016). The contrasting effect of Cotinus coggyria, rich in polyphenols and expected to prevent calcium phosphate precipitation, may have arisen from its antibacterial effects and dye components.

Moreover, polyphenols in plants have been reported to inhibit the glucosyl transferase activity of Streptococcus mutans (Velez et al., 2016; Guven & Erkan, 2015; Smullen, Finney, Storey, & Foster, 2012). For this reason, polyphenols are considered as
important substances for oral hygiene in terms of dental caries and dental calculus formations (Dash, Singh, Gupta, Panwar, & Ramisetti, 2014). Its mechanisms of action is based on their affinity for proteins. When approached from this perspective, they bind to amylase and glycosyl transferase enzymes and they inactivate those enzymes.

**CONCLUSION**

The results of our study showed that *Petroselinum crispum*, *Rumex crispatus* DC, *Beta vulgaris* L.var.cicla, and *Eruca sativa* aqueous extracts can be effective at the beginning on the calcium phosphate precipitation which may reflect on dental calculus formation. In the limitations of our study, any effect of Conitus coggygia Scop. was found on precipitation. Nevertheless, more study needs to be done. Our study is the first attempt to apply the plant aqueous extracts to the area of oral health, such as their potential effects on calcium phosphate precipitation at the beginning of dental calculus formation. Furthermore our study also highlights that more plants can be examined in this way.

In our study, we examined the effects of plant extracts separately. Our aim was to determine the impact of each separately. Further studies may be done to see what kind of result can be obtained by mixing two or more of the plant extracts having synergic effects. This may be especially important in the process of producing more effective products to prevent dental calculus precipitation.

**Ethics Committee Approval:** Not necessary for this study.

**Informed Consent:** Written consent was obtained from the partici-

**Peer-review:** Externally peer-reviewed.

**Author Contributions:** Conception/Design of Study: A.Y., S.A.; Data Acquisition: B.S.Ç., A.Y.; Data Analysis/Interpretation: A.Y., B.S.Ç., A.Y.; Critical Revision of Manuscript: B.S.Ç., A.Y.; Final Approval and Accountability: A.Y., B.S.Ç., S.A., Ö.S., R.Y.; Acquisition: B.S.Ç., A.Y.; Critical Revision of Manuscript: B.S.Ç., A.Y.; Critical Revision of Manuscript: B.S.Ç., A.Y.; Final Approval and Accountability: A.Y., B.S.Ç., S.A., Ö.S., R.Y.; Data Analysis/Interpretation: A.Y., B.S.Ç., S.A., Ö.S., R.Y.; Final Approval and Accountability: A.Y., B.S.Ç., S.A., Ö.S., R.Y.

**Conflict of Interest:** The authors have no conflict of interest to de-

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