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Staining effect of herbal immune boosters used during Covid-19 pandemic on teeth shade: In-vitro study

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ABSTRACT

Objective: Assessment of staining effect of herbal immune boosters used during COVID-19 pandemic on teeth shade. *Material and method:* 70 extracted premolars (N=10) were assigned to different solutions which were formulated to mimic the impact of herbal immune boosters on teeth. A baseline reading was performed using in-vitro VITA Easy shade Advance 4.0 and one day later, seven days later, and fourteen days later. The data was entered into SPSS software version 26. *Results:* A statistically significant difference was noticed in orange, lemon, and curcuma. *Conclusion*: The consumption of some herbal products has an impact on teeth's shade. Moreover, some materials such as, orange, lemon, and curcuma had the ability to change the teeth shade faster and stronger effect on teeth shade.

Keywords: COVID-19, Dental Health, Dental Esthetics, Tooth Shade, Immunity, Herbal Immune Boosters

1. INTRODUCTION

Corona virus disease 2019 (abbreviated "COVID- 19") is a contagious disease with unfamiliar etiology identified by severe pneumonia that was first seen in Wuhan, China, in December 2019 (Naser et al., 2021). The World Health Organization (WHO), on 12th of March, 2020, announced that the COVID-19 disease is a pandemic (WHO, 2020). Worldwide, governments and health organizations try to contain the outbreak by applying restrictive rules to safeguard the populations from the spread of the disease. These regulations affected different sectors, including education, finance and tourism.

COVID-19 motivated many individuals to find ways to protect themselves by following preventive methods to avoid getting infected by the virus. These preventive measures were either physical such as using antiseptic solutions, wearing gloves and mask or by enhancing the immune system by being physically active, improving sleeping habits, and more importantly, introducing natural immune boosters to their diets (Alyami et al., 2020). Many

scientists recommend the sufficient intake of minerals, vitamins, and herbal products to help the immune system with supplies to lower the possibility and severity of infections, such as COVID-19 (Jayawardena et al., 2020; Nilashi et al., 2020; Panyod et al., 2020).

In study conducted by Iftikhar Ahmad et al., in 2020, there were 57.6% of participants used herbal foods to boost the immune system during the COVID-19 pandemic (Ahmed et al., 2020). Another study investigated herbal immune boosters during the COVID-19 pandemic. It included lemon, orange, myrrh, Curcuma, ginger and cinnamon as the most used herbal immune boosters (Aldalaan et al., 2022). Human teeth are a susceptible part of the body to changes; these changes could occur to shape, size, and shade (Lombardi, 1973). Dental shade could be changed throughout people's lives due to intrinsic, extrinsic, or both staining. Diet is one major factor that widely contributes to dental extrinsic staining (Veeraganta et al., 2015).

Thus, this study aims to assess and explore the staining effect of herbal immune boosters used during the COVID-19 pandemic on teeth shade by exposing teeth to materials used to boost immunity and observing their effects in-vitro settings.

2. MATERIALS AND METHODOLOGY

This experimental laboratory study was performed on tooth specimens immersed in different solutions, including orange, lemon, Curcuma, cinnamon, ginger, myrrh and saline. The research was reviewed and approved by the Institutional Review Board (IRB) of the King Abdullah International Medical Research Center (MRC) under reference NRC21R/157/03 before the commencement of the study. This experimental laboratory study was performed in Saudi Arabia from July 2021 to August 2021. Orange and lemon were natural with no additives and freshly juiced into one liter before the start of this experimental study. Both cinnamon and ginger were recently ground into one gram and poured into 333ml of water. Myrrh was added as a whole in 333ml. In total, there was one liter of solution for each material.

Our study was conducted in a laboratory setting using seventy extracted premolars. The samples used in this study were extracted human premolar teeth with intact buccal or lingual/palatal surfaces, excluding discoloured and carious teeth. All sample teeth were stored in a saline solution before the study began, and it was divided into seven different groups. Each group included ten teeth (n=10), orange, lemon, cinnamon, ginger, Curcuma, and saline, as a control group. Thus, there were seven groups in total in this experimental study. Each tooth in every group was stored separately in a different container to ensure the validity of our results, and shade was recorded for each tooth four other times. Non-probability sampling was used to calculate the sample size of this study. In addition, the apical foramen of the samples was smeared with coloured varnish before treatment to prevent the penetration of the solutions into the specimen dentin tubules.

Shade readings were taken using VITA Easy shade Advance 4.0. Each tooth sample was immersed in 100ml (n = 10) of each group and stored in a glass container. The samples were preserved in dark glass bottles at room temperature between readings, with the solution information labelled on each container. The initial reading was taken before immersion into the medium. Then, the second shade reading was conducted after one day. Then, the samples were immersed in the same assigned solution and put back in the glass container for six more days to obtain seven days in total immersion time. At this time, the third shade reading was done again. Then, the samples were immersed one more time again in the exact assigned solutions and put back in the plastic container for seven days more to obtain fourteen days in total, after which the fourth and last shade reading was measured.

The difference between the four readings was interpreted in VITA classical shade guide. The difference in the shade was calculated in four different calculations as follows [1: Before immersion to one day as (1st period), 2: After one day to seven days as (2nd period), 3: After seven days to fourteen days as (3rd period) and 4: Before immersion to fourteen days as (periods' total)]. The collected data were arranged, organized and statistically analyzed using SPSS software (Statistical Package for the Social Sciences, version 26, SPSS Inc. Chicago, IL, USA). For quantitative data, the mean, range and standard deviation were calculated. In addition, the F-value of the ANOVA test was calculated for comparison between more than two means of parametric data, t. To detect the Significant used Post Hoc (Tukey HSD test). Significance was adopted at $P < 0.05$ for interpretation of results of tests of significance.

3. RESULTS

This experimental laboratory study was performed on tooth specimens immersed in different solutions, which included orange, lemon, Curcuma, cinnamon, ginger, myrrh and saline. Our study was conducted in a laboratory setting using seventy extracted premolars divided into seven groups (n=10).

Table (1) illustrates mean scores of shades in-vitro VITA Easy shade Advance 4.0. It was niticed that there was a statistically significant difference with orange by one-way ANOVA test (F-value = 4.112 and P value = 0.013). Furthermore, a highly statistically significant difference between lemon and Curcuma was made using the one-way ANOVA test (F-value = 10.635, 14.358 and P value = 0.0001), respectively. On the other hand, there was a highly statistically significant difference between materials in durations (1st

period), (2nd period) and (periods' total) by one-way ANOVA test (F-value = 14.751, 8,518, 9.590 and P value = 0.0001 each) respectively.

Table 1 Mean scores of shades in-vitro VITA (n=10)

Materials	Duration (n=10)				F-value	P
	1st Period (Day 0 to day 1)	2nd Period (Day 1 to day 7)	3rd Period (Day 7 to day 14)	Periods' Total (Day 0 to day 14)		
	Range Mean ± SD					
Orange	0 - 0 0.00 ± 0.000	0 - 2 0.70 ± 0.674	0 - 3 0.70 ± 1.159	0 - 4 1.40 ± 1.173	4.112	0.013*
Lemon	0 - 8 1.60 ± 2.796	0 - 14 5.10 ± 3.900	0 - 2 0.30 ± 0.674	4 - 14 7.00 ± 3.527	10.635	0.0001**
Curcuma	2 - 14 6.90 ± 4.653	0 - 3 0.30 ± 0.948	0 - 2 0.20 ± 0.632	2 - 14 7.40 ± 4.623	14.358	0.0001**
Myrrh	0 - 1 0.10 ± 0.316	0 - 0 0.00 ± 0.000	0 - 8 1.10 ± 2.469	0 - 9 1.20 ± 2.780	1.167	0.336
Ginger	0 - 2 0.30 ± 0.674	0 - 3 0.30 ± 0.948	0 - 9 1.40 ± 2.796	0 - 9 2.00 ± 2.788	1.683	0.188
Cinnamon	0 - 2 0.20 ± 0.632	0 - 10 1.30 ± 3.128	0 - 1 0.20 ± 0.421	0 - 12 1.70 ± 3.683	0.986	0.410
Control	0 - 0 0.00 ± 0.00	0 - 0 0.00 ± 0.00	0 - 0 0.00 ± 0.00	0 - 0 0.00 ± 0.00	---	---
F-value	14.751	8.518	1.186	9.590		
P	0.0001**	0.0001**	0.325	0.0001**		

*Statistically Significant difference at (P<0.05)
** Highly Statistically Significant difference at (P<0.001)

It was noticed using Post Hoc (Tukey HSD test) that there was a statistically significant difference between the durations (1st period) and (periods' total) for orange consumption when the P value = 0.006. Also, it was noticed for lemon usage that there was a statistically significant difference between (1st period) and (periods' total) when P value = 0.002 and between (2nd period) and (3rd period) when P value = 0.005. Also, there was a highly statistically significant difference between (3rd period) and (periods' total) when the P value = 0.0001. In addition, for Curcuma, it was noticed that there was a highly statistically significant difference between (1st period) and (2nd period), and (3rd period) when P value = 0.0001 for both. Also, between (2nd period) and (periods' total) when P value = 0.0001. Also, between (3rd period) and (periods' total) when P value = 0.0001.

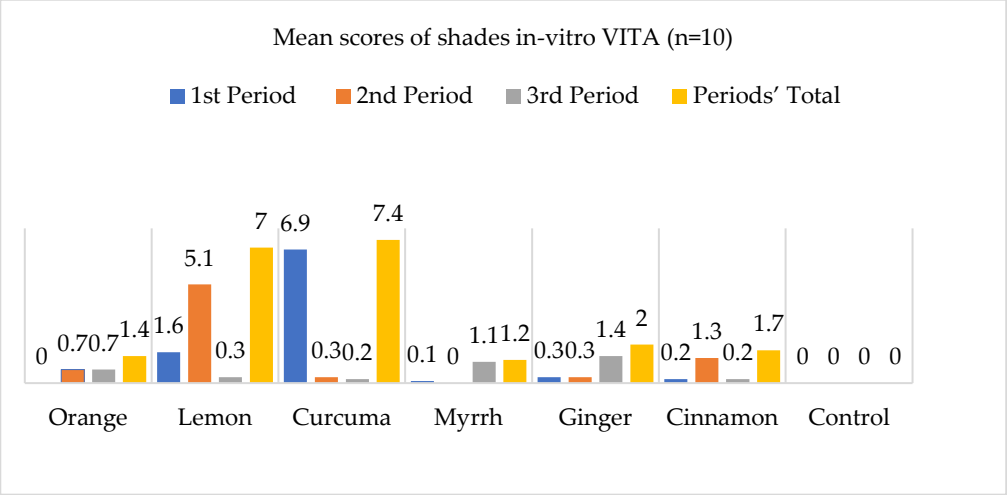

Figure 1 Mean scores of shades in-vitro VITA (n=10)

Table (2) Presented Post Hoc (Tukey HSD test) for using the materials related to (1st period). It was noticed that there was a highly statistically significant difference between Curcuma with control when the P value = 0.0001. Also, it demonstrates Post Hoc (Tukey HSD test) for using the materials related to (the 2nd period). It was observed that there was a highly statistically significant difference between lemon with control when the P value = 0.0001. In addition, it illustrates Post Hoc (Tukey HSD test) for using the materials related to (periods' total). It was observed that there was a highly statistically significant difference between Lemon and Curcuma with control when the P value= was 0.0001 for both.

Table 2 Post Hoc (Tukey HSD test) for using the materials related to (1st period, 2nd period and periods' total)

Dependent Variable 1st Period						
Tukey HSD						
Materials	Materials	Mean Difference	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Control	Orange	0.00000	0.93248	1.000	-2.8400	2.8400
	Lemon	-1.60000	0.93248	0.608	-4.4400	1.2400
	Curcuma	-6.90000	0.93248	0.0001**	-9.7400	-4.0600
	Myrrh	-0.10000	0.93248	1.000	-2.9400	2.7400
	Ginger	-0.30000	0.93248	1.000	-3.1400	2.5400
	Cinnamon	-0.20000	0.93248	1.000	-3.0400	2.6400
Dependent Variable: 2nd Period						
Tukey HSD						
Materials	Materials	Mean Difference	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Control	Orange	Orange	-0.70000	0.88246	0.985	-3.3876
	Lemon	Lemon	-5.10000*	0.88246	0.0001**	-7.7876
	Curcuma	Curcuma	-0.30000	0.88246	1.000	-2.9876
	Myrrh	Myrrh	0.00000	0.88246	1.000	-2.6876
	Ginger	Ginger	-0.30000	0.88246	1.000	-2.9876
	Cinnamon	Cinnamon	-1.30000	0.88246	0.759	-3.9876
Dependent Variable: Periods' Total						
Tukey HSD						
Materials	Materials	Mean Difference	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Control	Orange	-1.40000	1.35518	0.944	-5.5273	2.7273
	Lemon	-7.00000	1.35518	0.0001**	-11.1273	-2.8727
	Curcuma	-7.40000	1.35518	0.0001**	-11.5273	-3.2727
	Myrrh	-1.20000	1.35518	0.973	-5.3273	2.9273
	Ginger	-2.00000	1.35518	0.758	-6.1273	2.1273
	Cinnamon	-1.70000	1.35518	0.870	-5.8273	2.4273

** Highly Statistically Significant difference at (P<0.001)

4. DISCUSSION

The presented study aims to assess and explore the staining effect of herbal immune boosters used during the COVID-19 pandemic on teeth shade by exposing teeth to materials used to boost immunity and seeing their effects in-vitro settings. Orange was one of the materials that had a noticeable effect on the teeth shade. The means of readings in the four differences show a statistically significant difference with the durations. The peak effect of orange in darkening teeth shade was observed in the 3rd period (day 7 to day 14); with the mean change on VITA classical shade guide 0.70 ± 1.159. According to these findings, orange has a staining

effect on teeth shade that can darken the shade when exposed for prolonged periods. That can be correlated to the erosion effect of orange, making enamel hardness decrease and roughness increase, which makes enamel more susceptible to stain (Ren et al., 2009).

Lemon was another material that showed changes in teeth shade when readings were taken with VITA Easy shade Advance 4.0. It was observed that the highest effect of lemon in changing the shade to a darker shade was during the 2nd period (day 1 to day 7), with the mean of change on VITA classical shade guide 5.10 ± 3.90. However, those teeth immersed in lemon showed whiteness to the naked eye, which could be attributed to the acidic nature of lemon on enamel which thinned the enamel to the degree that VITA Easy shade Advance 4.0 read dentin shade. Similarly, the findings of Grando et al., (1996) who found enamel colour changed to a lighter colour with lemon incubation, such changes were rationalized as results of acid attacks on dental enamel. Nevertheless, in our study, we depended on the readings of VITA Easy shade Advance 4.0, and these changes should be further investigated.

Curcuma showed the highest and fastest staining effect among all studied materials. It was observed that Curcuma had its peak effect on the 1st period (day 0 to day 1), with the mean of staining on VITA classical shade guide 6.90 ± 4.65. According to our findings, Curcuma has a profound ability to stain teeth compared to the control group and other materials. In conclusion, limited evidence is available regarding the staining effect of herbal immune booster products on teeth shade; further studies are needed to explore the effect of herbal immune booster products on teeth shade.

5. CONCLUSION

All in all, COVID- 19 encouraged the community to improve their immunity by using different herbal immune booster products. The consumption of these products has an impact on teeth's shade. The result of these products was assessed and explored their staining effect on teeth shade in-vitro settings. The used materials varied in their impact on teeth shade in terms of rapidness and severity of staining. Orange, lemon and Curcuma were the materials that significantly affected the teeth shade. To conclude, limited literature is available, and further studies are needed to establish the consequences of prolonged consumption of these herbal immune booster products.

Author Contribution

All authors contributed equally to the manuscript

Ethical approval

This research was reviewed and approved under reference NRC21R/157/03 from the Institutional Review Board (IRB) of the King Abdullah International Medical Research Center (MRC) before the commencement of the study.

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Conflict of interest

The authors declare that there is no conflict of interests.

Data and materials availability

Data that support the findings of this study are embedded within the manuscript.

REFERENCES AND NOTES

1. Ahmed I, Hasan M, Akter R, Sarkar BK, Rahman M, Sarker MS, Samad MA. Behavioral preventive measures and the use of medicines and herbal products among the public in response to covid-19 in Bangladesh: A cross-sectional study. Plos One 2020; 15(12), e0243706.
2. Aldalaan K, Alhazmi K, Alrumi A, Alnashwan S, AlSwayyed T. Impact of covid-19 pandemic on the consumption of herbal products and its effect on oral and Dental Health: A cross-sectional study in Saudi Arabia. Med Sci 2022; 26(124):1–8.
3. Alyami HS, Orabi MA, Aldhabbah FM, Alturki HN, Aburas WI, Alfayez AI, Alharbi SA, Almasuood RA, Alsuhaibani NA. Knowledge about COVID-19 and patients' beliefs about and use of herbal products during the COVID-19 pandemic: A cross-sectional study in Saudi Arabia. Saudi Pharm J 2020; 1326–32.
4. Grando LJ, Tames DR, Cardoso AC, Gabilan NH. In vitro study of enamel erosion caused by soft drinks and lemon juice in deciduous teeth analysed by stereomicroscopy and scanning electron microscopy. Caries Res 1996; 30(5):373–8.

5. Jayawardena R, Sooriyaarachchi P, Chourdakis M, Jeewandara C, Ranasinghe P. Enhancing immunity in viral infections, with special emphasis on covid-19: A Review. Diabetes Metab Syndr: Clin Res Rev 2020; 14(4):367–82.
6. Lombardi RE. The principles of visual perception and their clinical application to Denture Esthetics. J Prosthet Dent 1973; 29(4):358–82.
7. Naser A, Dahmash E, Alsairafi Z, Alwafi H, Alyami H, Jalal Z, Al Rajeh A, Paudyal V, Alhartani Y, Turkistani F, Hassanin F. Knowledge and practices during the COVID-19 outbreak in the Middle East: A cross-sectional study. Int J Environ Res Public Health 2021; 18(9):4699.
8. Nilashi M, Samad S, Yusuf SY, Akbari E. Can complementary and alternative medicines be beneficial in the treatment of COVID-19 through improving immune system function? J Infect Public Health 2020; 13(6):893–6.
9. Panyod S, Ho C-T, Sheen L-Y. Dietary therapy and herbal medicine for COVID-19 prevention: A review and perspective. J Tradit Complement Med 2020; 10(4):420427.
10. Ren Y-F, Amin A, Malmstrom H. Effects of tooth whitening and orange juice on surface properties of dental enamel. J Dent 2009; 37(6):424–31.
11. Veeraganta SK, Savadi RC, Baroudi K, Nassani MZ. Differences in tooth shade value according to age, gender and skin color: A pilot study. J Indian Prosthodont Soc 2015; 15(2):138.
12. WHO announces COVID-19 outbreak a pandemic. Euro who int 2020. Available from: https://www.euro.who.int/en/health-topics/health-emergencies/coronavirus-covid-19/news/news/2020/3/who-announces-covid-19-outbreak-a-pandemic