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Research Article

Comparative Study of Color Change of Enamel Surface Using Natural Whitening Tooth Pastes Versus Commercial Whitening Tooth Paste

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Abstract

The oil of Cocos nucifera, along with the alcoholic extracts of Salvia officinalis herb, Curcuma longa rhizomes, Psidium guajava leaves, Citrus limon fruits peels and Fragaria ananassa fruits were used to prepare a toothpaste for each extract and applied on isolated natural teeth aiming to evaluate their effectiveness as teeth whitening agents. Coconut oil (*C. nucifera*) was subjected to GC/MS analysis while all other extracts were subjected to HPLC-MS/MS and spectrophotometric analyses. The formulated toothpastes were evaluated for its organoleptic and physical properties. The toothpastes were of different colors, smooth in nature, foamability around 10, pH-8.2, and extrudability 95%. Most of them were considered good and stable formulations. The best formulations were of *S. officinalis, C. longa* and *C. nucifera*, therefore, their corresponding tooth pastes were compared for whitening effects to the commercial Signal white after tooth brushing for one month and six months. Twenty extracted premolars were selected which were extracted for orthodontic reasons. It is evaluated using CIELAB measurement system at base line before brushing T0 and after brushing for equivalent one monthT1 and six months T2. Coconut oil showed no significant difference in color changes ΔL, Δb and Δa between T0 and T1 and between T0 and T2, while ΔE, showed no changes that can be seen by the observers clinically where $\Delta E < 1$. On the other hand, Turmeric showed no significant difference in ΔL, Δb and Δa when compared the color changes at T0, T1 and T2, while ΔE showed changes that can be easily observed where $\Delta E > 3.3$. Sage showed changes in which $\Delta E > 3.3$ which can be easily observed clinically. When comparing the three natural products with Signal white, there was change in color where $\Delta E > 3.3$ with lighter effect after one month, while after six months brushing it became slight darker but still lighter than the base line (T0).

Keywords: CIE Lab System; HPLC-MS-MS; GC-MS; Tooth Pastes Formulation; Coconut; Sage

Introduction

Tooth pastes have been continually developed to improve the treatment for a range of therapeutics and cosmetic oral conditions, such as caries, bad breath, gingivitis, tartar and dental hypersensitivity. In recent years, tooth whitening is one of the most rapidly growing oral care sectors fueled by consumers demand for both healthy and cosmetically attractive smiles. The majority of people the appearance of teeth is very important, and any discoloration or stain that may be formed on them will affect their esthetic qualities. Access to in-office treatments is restricted to part of the population. Therefore, there has been an interest in developing methods so that the patients can remove stains and apply tooth whitening at home. Therefore, the dentifrices, due to their ease of use and low cost, have been used as vehicles for whitening agents [1,2]. Tooth pastes are based on optimized abrasive technology to remove and control extrinsic stains which form in the acquired pellicle. Extrinsic stains form naturally on the tooth surface when chromogens from dietary sources (e.g. tannins from tea and coffee) or habits (e.g. tar from smoking) are incorporated into the salivary pellicle. Other factors, such as poor techniques of oral hygiene maintenance and the ability of a dentifrice to control stain by removal or prevention, also influence the accumulation of stains. An important part of the evaluation process for whitening toothpaste is determining its clinical efficacy in terms of removing extrinsic tooth stains [3,4]. Tooth brushing also has aesthetic functions such as refreshing the oral cavity, cleaning the teeth, and improving the appearance of the teeth; additionally, tooth brushing serves the primary purpose of preventing dental caries and periodontal disease. Recently, toothpastes that contain ingredients for a variety of specific purposes are commercially

available; among these, whitening toothpastes, which are most closely associated with favorable aesthetics. However, despite the fact that the availability of whitening toothpastes that are easier-to-use is increasing, unlike the existing whitening methods, their whitening effects and impact on teeth have not yet been analyzed properly.

Most studies have used the CIE lab system to observe enamel color changes, which is based on three elements; hue, chroma and value in which L represents lightness, while a represents the color opponent dimensions on a red/green and b represents yellow/blue axis. Along a positive value indicates a tendency towards red and negative values indicates a tendency towards green. Along the b axis, positive values indicate a tendency towards yellow and negative values a tendency towards blue [4]. It's the most widely used system in different studies and it is recommended by the ADA. Also, there are delta values associated with this scale (ΔL*, Δa*, Δb*) which indicates how the sample differs from the standard for L*, a*, b* and are used to calculate total color difference ΔE by the formula ΔE = [(ΔL*)2, (Δa*)2, (Δb*)2]1/2. It's very important not only to observe the ΔE, but also the changes from red to green and yellow to blue and luminosity when evaluating the change of colors or staining [5].

Whitening dentifrices includes different active ingredients in their composition, such as chemical agents, and high amount of abrasives. Even though the chemical action is not well elucidated, abrasive components associated with tooth brushing seem to be the main factor leading to tooth whitening effect as a result of stain removal. Also, it is important to point out that dentifrices with higher amount of abrasive may produce increased surface roughness in dental tissues [6,7]. Home remedies have been used for a long time in many cultures, and there are some teeth whitening home remedies that use natural plants, that were found to be effective and much safer than chemicals in typical teeth whitening products. These plants are sage, turmeric, coconut oil, strawberry, lemon, guava leaves. Therefore, the aim of our study to use natural tooth paste products that may affect tooth color without the abrasive components present in the commercially available whitening tooth pastes. Comparison of the whitening effect of the Signal white and natural tooth pastes, Sage, Turmeric and Coconut after tooth brushing for one month and six months.

High performance liquid chromatography-Mass spectrometry/Mass spectrometry (HPLC-MS/MS):

HPLC-Ms/Ms analysis was carried out on a XEVO TQD triple quadruple instrument, Waters® corporation, Milford, MA01757 USA, mass spectrometer. The sample (100µg/mL) solution was prepared using high performance liquid chromatography (HPLC) analytical grade solvent of MeOH, filtered using a membrane disc filter (0.2 µm) then subjected to LC-ESI-MS analysis.

Samples injection volumes (10 µL) were injected into the UPLC instrument equipped with reverse phase C-18 column (ACQUITY® UPLC - BEH C18 1.7 µm particle size- 2.1 × 50 mm Column). Sample mobile phase was prepared by filtering using 0.2 µm filter membrane disc and degassed by sonication before injection. Mobile phase elution was made with the flow rate of 0.2 mL/min using gradient mobile phase comprising two eluents: eluent A is H_2O acidified with 0.1% formic acid and eluent B is MeOH acidified with 0.1% formic acid. Elution was performed using the above gradient. The parameters for analysis were carried out using negative ion mode as follows: source temperature 150 °C, cone voltage 30 eV, capillary voltage 3 kV, desolvation temperature 440 °C, cone gas flow 50 L/h, and desolvation gas flow 900 L/h. Mass spectra were detected in the ESI negative ion mode between m/z 100 -1000. The peaks and spectra were processed using the Masslynx 4.1® software and tentatively identified by comparing its retention time (Rt) and mass spectrum with reported data.

Determination of fatty acids and hydrocarbons (GC Analysis) For unsaponifiable matters

Unsaponifiable matters were separated using HP- Hewlett Packard GC-system, series 6890 equipped with Flame Ionization Detector (FID). A capillary column (HP-5 5% phenyl methyl siloxane, 30m x 320 µm, film thickness 0.25 µm) was used in the separation. The injector port temperature was set 240 °C (split mode) and the detector cell at 280 °C. The flow rate of the carrier gas, N2, was 20 ml/min, for H2 20ml/min and for Air 200ml/min. The column temperature was 80 °C for 1 min and then increased to 280°C by the rate of 8°C/min, with maximum column temperature 325°C then isothermally for a total run time of 20 minutes.

For fatty acid methyl esters

Fatty acid methyl esters were separated by the same GLC apparatus as unsaponifiable matters. A capillary column (HP- 5 5% phenyl methyl siloxane, 30m x 320 µm, film thickness 0.25 µm) was used in the separation. The injector port temperature was set 250 °C (spitless mode) and the detector cell at 280 °C. The flow rate of the carrier gas, Hydrogen flow 30 ml/min, Air flow 300 m; /min and N2 was 10 ml/min. The column temperature was 70 °C for 1 min and then increased to 220 °C by the rate of 4°C/min, then isothermally for a total run time of 20 minutes.

UV-visible spectrophotometer, Shimadzu UV (P/N 204 - 58000) was used to measure the absorbance in UV range.

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Methods

Preparation of plant extracts

The plant material is added to a container with 70% ethanol. The container is closed with foil and the mixture is kept at room

temperature for at least 2 days then filtered. This process is repeated until exhaustion. The alcohol is then evaporated from the collected extract using a rotary evaporator at a temperature not exceeding 50°C. The extract is then kept in a desiccator to dry.

Method of preparation of toothpaste

Six tooth pastes were formulated from sage extract, turmeric extract and coconut oil separately. Laboratory preparation of toothpastes was done by trituration method. A liquid base was prepared first with humectants, (glycerin) preservatives (EDTA) and water; to this base herbal extract was added, triturated well and kept aside for 15 min to allow the binding agent to swell. Next, after addition of all powders; surface active agent was added at the end and mixed slowly and thoroughly to prevent aeration or foaming. Mixing was continued till all constituents were evenly distributed. The finished product thus obtained was allowed to stand for 24 h. The paste was finally filled into collapsible tubes, stored and used for further studies [8].

Ingredient	Quantity (g)	Quantity (g)	Quantity (g)	Quantity (g)	Quantity (g)	Quantity (g)
Sage extract	1					
Turmeric extract		1				
Coconut oil			2ml			
Guava extract				1		
Strawberry extract					1	
Lemon extract						1
Sodium bicarbonate	0.5	0.5	0.5			
Sodium lauryl sulphate	0.4	0.4	0.4			
Hydroxy propyl methyl cellulose	0.7	0.7	0.7			
Glycerin	1ml	1ml	1ml			
Distilled water	q.s	q.s	q.s			

Table 1: Composition of chemicals encountered in each toothpaste formulation.

Evaluation of toothpastes

Composition

All ingredients should be complied with the global standards. Toothpaste is not composed of mono or disaccharides such as sucrose or fermentable carbohydrates.

pH

pH of formulated herbal toothpaste was determined by using pH meter. 10g of toothpaste placed in 150ml of beaker. Allow the 10ml of boiled and then cooled water. Stir vigorously to make a suspension [9].

Homogeneity

The toothpaste shall extrude a homogenous mass from the collapsible tube or any suitable container by applying of normal force at 27 ± 2°C in addition bulk of contents shall extrude from the crimp of container and then rolled it gradually [9].

Foamability

The foamability of formulated toothpaste evaluated by taking small amount of formulation with water in measuring cylinder initial volume was noted and then shaken for 10 times. Final volume of foam was noted [10].

Extrudability

In this method, the formulated paste was filled in standard capped collapsible aluminum tube and sealed by crimping to the end. The weights of tubes were recorded. The tubes were placed between two glass slides and were clamped. 500g was placed over the slides and then cap was removed. The amount of the extruded paste was collected and weighed. The percent of the extruded paste was calculated [11].

Teeth selection

Twenty extracted premolars were selected which were extracted for orthodontic reasons. Included teeth were free of caries and cracks or any damage from extraction, teeth were cleaned from any debris by scaling and stored in distilled water.

Specimens preparation and grouping

Selected teeth were sectioned at cementoenamel junction in a mesiodistal direction using a low speed micro-motor and Carborundrum disc to cut off the roots, and the crown were mounted on acrylic resin mold with labial surface facing upward. The specimens were then divided into four groups, where n = 5 according to the type of tooth paste which are listed in table 2, where group1with commercial tooth paste as control and groups 2 - 4 treated with natural tooth pastes.

Groups	Material
Group 1	Signal whitening tooth paste
Group 2	Sage tooth paste
Group 3	Coconut tooth paste
Group 4	Turmeric tooth paste

Table 2: Teeth grouping.

Base line color measurement

- Vita easy shade (spectrophotometer) was used to measure the color alteration. Tooth color was analyzed on the basis of color alteration (ΔE) Luminosity((ΔL) (alteration the green/red axis Δa, and alteration on the blue/yellow axis Δb. Co-ordinates from CIE lab color system [12]. The measurement was performed at T0 (Base line) before using any type of toothpaste.

Application of toothpaste

- Tooth pastes were applied on the specimens by tooth brushing machine and brushing was done for four minutes and 40 seconds, which was assumed to be equivalent to 1-month brushing, followed by color measurement using spectrophotometer. Then continuing brushing for 24 minutes which is equivalent to 5 months brushing, so when totaled, the brushing time would be equivalent to brushing 6 months [13]. It was done with 4 minutes and 40 seconds brushing in the first day, followed by 4 minutes brushing for 5 days and specimens were stored in distilled water between brushing. Tooth brushing was carried out using tooth brushing machine which was standardized at a speed of 365rpm, while loading was standardized at 200gm. Tooth brush used has rounded end, uniform length, flexibility and medium bristles.

Color measurement after one month and 6 months

Color measurement was taken after brushing for the equivalent of 1 month (T1) and 6 months (T2). In each specimen, measurements were carried out three times in different points and the total measurement results are then averaged.

Statistical analysis

The data were analyzed and conducted using Kruskal Wallis Mann Whitney test to compare between two groups in non-related samples, and the second test was Wilcoxon test which was used to compare between two groups in related samples that used to analyze level change between natural and commercial whitening tooth pastes and teeth after treatment.

Results

Colorimetric determination of total phenolic and flavonoids contents

- 100 mgs of the extracts of: *S. officinalis, P. guajava, C. longa, C. limon* and *F,ananassa* were used for spectrophotometric analyses to estimate the total flavonoids and total phenolic compounds.

Determination of total phenolic content

- The phenolic content is calculated as gallic acid equivalent [14] by measuring the intensity of the color formed from the phenolic compound-Folin-Ciocalteus phenol reagent complex, with a pre-established standard calibration curve as a reference.

- The plants of *S. officinalis, C. limon, C. longa, P. guajava, F. ananassa* were extracted with 70% ethanol, separately, on cold, till exhaustion. The residues were transferred to 100 ml measuring flasks and the volume was adjusted with distilled water.

- Known volumes of 100 mg of each ethanolic extract residue were transferred to separate 25 ml volumetric flasks, containing 9 ml of distilled water. 1 ml of Folin Ciocalteu phenol reagent was added into the mixture and the process continued as under the calibration curve.

Extract	Total Phenolic content Concentration mg/g
P. guajava	102.73
F. ananassa	2.35
C. longa	102.36
C. limon	51.6
S. officinalis	196.49

Table 3: Total Phenolic content of the extracts with Folin-Ciocalteu at λmax 750 nm.

S. officinalis shows the highest phenolic acid content followed by *P. guajava* and *C. longa.* While *C. limon* and *F. ananassa* have the lowest phenolic content. This could indicate that the whitening action of these plants is related to the presence of phenolic acids.

Determination of total flavonoid content

The flavonoid percentage was calculated as quercetin [15-38]. By measuring the intensity of the color formed from flavonoid-aluminium chloride complex, with a pre-established standard calibration curve as a reference.

The plants of *S. officinalis, C. limon, C. longa, P. guajava, F. ananassa* were extracted with 70% ethanol, on cold, till exhaustion.

The concentrated ethanolic extracts were transferred to 100 ml measuring flasks.

To each ethanolic extract residue, 5 ml of 0.1 M aluminum chloride solution were added and the process continued as under the calibration curves.

Extract	Total Flavonoids content Concentration mg/g
P. guajava	26.14
F. ananassa	1.70
C. longa	93.39
C. limon	5.75
S. officinalis	16.87

Table 4: Total Flavonoids content of the extracts with 0.1 M $AlCl_3$ at λmax 420 nm.

The high content of antioxidant flavonoids in *C. longa,* along with curcuminoids, could explain why turmeric has strong anti-inflammatory action and protects against dental caries and plaque formation, this action can also be seen with *P. guajava* and *S. officinalis*, thus, they are used in dental care products and home remedies to improve oral health.

HPLC-MS/MS analyses

- 20 mg of each of the following extracts: *Salvia officinalis, Psidium guajava, Curcuma longa, Citrus limon, Fragaria ananassa* were used for HPLC-MS/MS analysis. The peaks and spectra were processed using the (Masslynx® 4.1 software) and identified by comparing its retention time (Rt) and mass spectrum with reported data.

Peak no.	Rt	Mwt	M-H	Elemental composition	Identification	Refences
1	1.43	170	169	C7H6O5	Gallic acid	16
2	1.70	198	197	C9H10O5	Syringic acid	16
3	2.36	154	153	C7H6O4	Protochatecuic acid	16
4	3.14	344	343	C9H10O4	Homovanillic acid-*O*-hexoside	16
5	3.58	354	353	C16H17O9	Chlorogenic acid	16
6	4.84	180	179	C9H8O4	Caffeic acid	16
7	5.78	356	355	C16H20O9	Ferulic acid-*O*-hexoside	16
8	5.98	154.14	153	C10H18O	Eucalyptol	17
9	6.81	136.13	135	C10H16	Terpinoline	17
10	7.03	168	167	C8H8O4	Vanillic acid	16
11	7.90	164	163	C9H8O3	Coumaric acid	16
12	8.22	194	193	C10H10O4	Ferulic acid	16
13	8.68	610	609	C27H30O16	Rutin	16
14	9.08	464	463	C21H19O12	Quercetin-3-*O*-glucoside	16
15	9.15	448	447	C21H20O11	Kaempferol-3-*O*-glucoside	16
16	10.91	610	609	C28H34O15	Hesperidin	16
17	12.05	360	359	C18H16O8	Rosmarinic acid	16
18	12.19	434	433	C27H32O14	Naringin-*O*-hexoside	16
19	14.78	204.18	203	C15H24	β-Caryophylline	17
20	15.33	302	301	C15H10O7	Quercetin	16
21	16.4	202.17	201	C15H22	α curcumene	17
22	16.41	204.18	203	C15H24	Isoledene	17
23	17.40	272	271	C15H12O5	Naringenin	16
24	17.55	270	269	C15H10O5	apigenin	16
25	17.91	302	301	C16H14O6	Hesperitin	16
26	21.4	216.15	215	C15H20O	ar-Turmerone	17
27	21.6	218.16	217	C15H20O	Turmerone	17
28	22.4	218.16	217	C15H22O	Curlone	17

Table 5: Results of HPLC-MS/MS analysis for *C. longa.*

Peak no.	Rt	Mwt	M-H	Elemental composition	Identification	Ref.
1	4.2	740	739	C24H23O14	Apigenin 7-O-neohesperidoside-6-Cglucoside	18
2	9.0	772	771	C33H40O21	Quercetin 7-O-glucoside-3-O-rutinoside	18
3	10.1	640	639	C22H22O12	Isorhamnetin-3-O-di-glucoside	18
4	12	624	623	C27H30O16	Diosmetin 6,8-di-C-glucoside	18
5	12.2	608	607	C28H32O15	Diosmetin 7-O-neohesperidoside (Neodiosmin)	18
6	17.5	578	577	C27H30O14	Rhoifolin	18
7	19.1	598	597	C27H32O15	Eriocitrin	18
8	31.2	300	299	C16H12O6	Diosmetin	18
9	31.5	594	593	C27H30O15	Luteolin 7-O-rutinoside	18

Table 6: Results of HPLC-MS/MS analysis for *C. limon.*

Peak no.	Rt	Mwt	M-H	Elemental composition	Identification	Ref.
1	11.7	316	315	C27H30O15	Acacetin-6,8-di-C-hexoside	19
2	13	464	463	C21H19O12	Quercetin-3-O-glucoside	20
3	15.31	448	447	C21H20O11	Kaempferol-3-O-glucoside	20
4	15.31	448	447	C21H20O11	Luteolin-3-O-glucoside	21
5	16.65	316	315	C16H12O7	Isorhamnetin	22
6	17.7	610	609	C28H34O15	Hesperidin	21
7	19.73	288	287	C15H12O6	Eriodictyol	21
8	21.1	300	299	C17H14O5	4-Hydroxy-5,7- dimethoxyflavanone	23
9	24.3	270	269	C15H10O5	Apigenin	24

Table 7: Results of HPLC-MS/MS analysis for *S. officinalis.*

Peak no.	Rt	Mwt	M-H	Elemental composition	Identification	Ref
1	4.6	434	433	C20H18O11	Guajaverin	25
2	5.9	170	169	C7H6O5	Gallic acid	26
3	6.5	281	280	C15H14O6	catechin	27
4	7.74	306	305	C15H14O7	gallocatechin	28
5	8.53	464	463	C21H20O12	isoquercitrin	29
6	12.5	578	577	C30H26O12	Procyanidin B isomer	30
7	24.46	272	271.0	C15H12O5	naringenin	31
8	30.8	464	463	C21H20O12	Hyperin	32
9	33.7	434	433	C20H18O11	Avicularin	32
10	46.5	318	317	C15H10O8	Myricetin	33
11	127.8	616	615		Quercetin-galloylhexoside isomer	34

Table 8: Results of HPLC-MS/MS analysis for *P. guajava.*

Peak no.	Rt	Mwt	M-H	Elemental composition	Identification	References
1	2.5	286	285	C15H10O6	kaempferol	20
2	3.0	382	381.1	C7H12O6	Quinic acid derivative	35
3	9.8	866	865	C45H38O18	Proanthocyanidin trimer	21
4	18.5	595	595	C27H31CLO15	Pelargonidin-3-o-diglucoside	36
5	19.262	580	579.2	C27H32O14	naringin	35
6	20.1	290	289	C15H14O6	catechin	35
7	27.1	580	579	C27H31O14	Pelargonidin-3-o-rutinoside	36
8	29.09	448	447		Methyl-EA-pentose	28
9	31.80	302	301	C14H7O8	Ellagic acid	37
10	32.2	478	477	C21H18O13	Quercetin-3-o-glucuronide	36
11	36.0	534	533	C30H32O19	Kaempferol-3-o-malonylglucoside	21
12	42.80	594	593	C30H27O13	k-coumaroyl-glucoside	34

Table 9: Results of HPLC-MS/MS analysis for *F. ananassa.*

Compound	Retention time (min.)	Relative %
Saturated fatty acids		
Caprylic acid C8	6.0	7.52
Capric acid C10	7.4	5.93
Lauric acid C12	9.5	53.11
Myristic acid C14	12.0	20.42
Palmitic acid C16	14.9	7.13
Stearic a C18	18.1	1.48
Unsaturated fatty acids		
Oleic acid C18(1)	18.5	3.95
Linoleic a C18(2)	19.6	0.46

Table 10: Results of GLC analysis of the identified FAME of the coconut oil.

Physical examination of toothpastes

The toothpaste formulations were prepared from sage, turmeric, coconut oil, Guava, Strawberry and lemon natural extracts and small amount of synthetic ingredients. The formulated herbal toothpastes were greenish brown, red, yellow to creamy in colour and showed the good homogeneity with absence of lumps. The resulted toothpaste was of different natural color, smooth in nature, foamability around 10, pH 5 to 8.2, and extrudability 95%.

Compound	Retention time (min.)	Relative %
C14 nTetradecane	11.31	0.09112
C15 Pentadecane	12.71	0.27581
C16 n-Hexadecane	14.01	0.76561
C17 nHeptadecane	15.33	2.55714
C18 n-Octadecane	15.99	2.93645
C19 n-Nonadecane	17.81	3.78145
C21 n-Henicosane	20.01	7.30840
C22 n-Docosane	21.11	7.85788
C23 n-Tricosane	22.31	9.01657
C24 n Tetracosane	22.92	7.88004
C25 nPentacosane	24.00	3.28157
C26 n-Hexacosane	24.71	3.05097
C27 nHeptacosane	26.21	8.59345
C28 n-Octacosane	26.52	1.99516
C29 n-Nonacosane	27.23	3.40541
C30 n-Triacotane	28.64	17.1048
Cholesterol	ND	ND
Campasterol	31.51	2.44505
Stigmasterol	32.90	0.44883
Alpha –amyrine	33.79	0.49936

Table 11: Results of GLC analysis of the identified unsaponifiable matter of coconut oil.

	Turmeric toothpaste	Sage toothpaste	Coconut oil toothpaste	Guava toothpaste	Strawberry toothpaste	Lemon toothpaste
Color	Reddish brown	Brown	Creamy	Brown	Red	Yellow
Evaluation Parameters						
pH	7.5	8	8.5	7	4.5	5
Homogeneity	good	very good	good	soft	soft	soft
Foamability	11(good)	10(good)	12(good)	6 (good)	7(bad)	10 (good)
Extrudability	95%	90%	85%	63 %	50 %	60%

Table 12: Physical examination of toothpastes.

Figure 1

Curcumin toothpaste coconut oil toothpaste age toothpaste

- The tests were applied only on the best toothpaste formulations with good stability and foamability. Comparison of the whitening effect of the Signal white and natural tooth pastes of Sage, Turmeric and Coconut after tooth brushing for one month and six months.
- The mean and standard deviation values were calculated for each group in each test. Data were explored for normality using Kolmogorov-Smirnov and Shapiro-Wilk tests, data showed non-parametric (not-normal) distribution. Kruskal Wallis test was used to compare between more than two groups in non-related samples. Mann Whitney was used to compare between two groups in non-related samples. Wilcoxon test was used to compare between two groups in related samples.
- The significance level was set at $P \leq 0.05$. Statistical analysis was performed with IBM® SPSS® Statistics Version 20 for Windows.

Variables	ΔE				
	Immediate (T_0) and One month (T_1)		Immediate (T_0) and Six months (T_2)		p-value
	Mean	SD	Mean	SD	
Signal	17.82	1.31	13.15	0.99	0.068ns
Sage	3.39	1.15	8.05	2.41	**0.109ns**
Turmeric	5.67	1.54	5.02	2.08	**0.465ns**
Coconut	1.25	0.77	6.18	5.32	**0.109ns**
p-value	0.009*		0.035*		

Table 13: The mean, standard deviation (SD) of ΔE in different groups.

*; significant (p<0.05) ns; non-significant (p>0.05).

I-ΔE results

Effect of time

- **Signal:** No statistically significant difference was found between (T_0 and T_1) and (T_0 and T_2) where (p = 0.068).
- **Sage:** No statistically significant difference was found between (T_0 and T_1) and (T_0 and T_2) where (p = 0.109).
- **Turmeric:** No statistically significant difference was found between (T_0 and T_1) and (T_0 and T_2) where (p = 0.465).
- **Coconut:** No statistically significant difference was found between (T_0 and T_1) and (T_0 and T_2) where (p = 0.109).

Relation between groups:

Immediate (T_0) and One month (T_1)

- A statistically significant difference was found between (Signal), (Sage), (Turmeric) and (Coconut) where (p = 0.009).
- A statistically significant difference was found between (Signal) and each of (Sage), (Turmeric) and (Coconut) where (p = 0.034), (p = 0.020) and (p = 0.034).
- No statistically significant difference was found between (Sage) and each of (Turmeric) and (Coconut) where (p = 0.154) and (p = 0.050) respectively.
- A statistically significant difference was found between (Turmeric) and (Coconut) where (p = 0.032).

Immediate (T_0) and Six months (T_2)

- A statistically significant difference was found between (Signal), (Sage), (Turmeric) and (Coconut) where (p = 0.035).
- A statistically significant difference was found between (Signal) and each of (Sage), (Turmeric) and (Coconut) where (p = 0.021), (p = 0.021) and (p = 0.043).
- No statistically significant difference was found between (Sage) and each of (Turmeric) and (Coconut) where (p = 0.149) and (p = 0.564) respectively.
- No statistically significant difference was found between (Turmeric) and (Coconut) where (p = 0.999).

Figure 2: Bar chart representing change in color (ΔE) for different groups.

II-ΔL results

Effect of time

- **Signal:** No statistically significant difference was found between (T_0 and T_1) and (T_0 and T_2) where (p = 0.066).
- **Sage:** No statistically significant difference was found between (T_0 and T_1) and (T_0 and T_2) where (p = 0.285).
- **Turmeric:** No statistically significant difference was found between (T_0 and T_1) (T_0 and T_2) where (p = 0.285).
- **Coconut:** No statistically significant difference was found between (T_0 and T_1) (T_0 and T_2) where (p = 0.109).

Relation between groups

Immediate (T_0) and One month (T_1)

- A statistically significant difference was found between (Signal), (Sage), (Turmeric) and (Coconut) where (p = 0.014).
- A statistically significant difference was found between (Signal) and each of (Sage), (Turmeric) and (Coconut) where (p = 0.034), (p = 0.020) and (p = 0.034).
- No statistically significant difference was found between (Sage) and each of (Turmeric) and (Coconut) where (p = 0.074) and (p = 0.050) respectively.

- Also, no statistically significant difference was found between (Turmeric) and (Coconut) where (p = 0.721).

Immediate (T_0) and Six months (T_2)

- A statistically significant difference was found between (Signal), (Sage), (Turmeric) and (Coconut) where (p = 0.040).
- A statistically significant difference was found between (Signal) and each of (Turmeric) and (Coconut) where (p = 0.034) and (p = 0.021). While no statistically significant difference was found between (Signal) and (Sage) where (p = 0.149).
- No statistically significant difference was found between (Sage) and each of (Turmeric) and (Coconut) where (p = 0.593) and (p = 0.083).
- Also, no statistically significant difference was found between (Turmeric) and (Coconut) where (p = 0.480).

Variables	ΔL				
	Immediate and One month		Immediate and Six months		p-value
	Mean	SD	Mean	SD	
Signal	13.00	1.22	7.60	0.74	**0.066ns**
Sage	-3.33	1.10	0.77	5.77	**0.285ns**
Turmeric	-0.08	1.81	-2.90	2.96	**0.285ns**
Coconut	0.53	0.40	-5.80	5.34	**0.109ns**
p-value	**0.014***		**0.040***		

Table 14: The mean, standard deviation (SD) of ΔL in different groups.

*; significant (p<0.05) ns; non-significant (p>0.05).

Δa result

Effect of time

- **Signal:** No statistically significant difference was found between (T0 and T1) and (T0 and T2) where (p = 0.144).
- **Sage:** No statistically significant difference was found between (T0 and T1) and (T0 and T2) where (p = 0.109).
- **Turmeric:** No statistically significant difference was found between (T0 and T1) and (T0 and T2) where (p = 0.593).
- **Coconut:** No statistically significant difference was found between (T0 and T1) and (T0 and T2) where (p = 0.999).

Relation between groups

Immediate and One month

- A statistically significant difference was found between (Signal), (Sage), (Turmeric) and (Coconut) where (p = 0.042).
- A statistically significant difference was found between (Signal) and each of (Sage), (Turmeric) and (Coconut) where (p = 0.032), (p = 0.021) and (p = 0.034).
- No statistically significant difference was found between (Sage) and each of (Turmeric) and (Coconut) where (p = 0.714) and (p = 0.825) respectively.
- Also, no statistically significant difference was found between (Turmeric) and (Coconut) where (p = 0.724).

Immediate and Six months

- A statistically significant difference was found between (Signal), (Sage), (Turmeric) and (Coconut) where (p = 0.044).
- A statistically significant difference was found between (Signal) and each of (Turmeric) and (Coconut) where (p = 0.034) and (p = 0.021). While no statistically significant difference was found between (Colgate) and (Sage) where (p = 0.564).
- No statistically significant difference was found between (Sage) and each of (Turmeric) and (Coconut) where (p = 0.157) and (p = 0.083).
- Also, no statistically significant difference was found between (Turmeric) and (Coconut) where (p = 0.999).

Variables	Δa				
	Immediate and One month		Immediate and Six months		p-value
	Mean	SD	Mean	SD	
Signal	-2.88	1.03	-2.18	0.46	0.144ns
Sage	-0.20	0.35	-2.08	1.88	0.109ns
Turmeric	0.33	1.14	-0.23	0.40	0.593ns
Coconut	-0.23	0.42	0.25	1.55	0.999ns
p-value	0.042*		0.044*		

Table 15: The mean, standard deviation (SD) of Δa in different groups.

*; significant (p<0.05) ns; non-significant (p>0.05).

Δb result

Effect of time

- **Signal:** No statistically significant difference was found between (T0 and T1) and (T0 and T2) where (p = 0.068).
- **Sage:** No statistically significant difference was found between (T0 and T1) and (T0 and T2) where (p = 0.109).
- **Turmeric:** No statistically significant difference was found between (T0 and T1) and (T0 and T2) where (p = 0.285).
- **Coconut:** No statistically significant difference was found between (T0 and T1) and (T0 and T2) where (p = 0.785).

Relation between groups

Immediate and One month

- A statistically significant difference was found between (Signal), (Sage), (Turmeric) and (Coconut) where (p = 0.010).
- A statistically significant difference was found between (Signal) and each of (Sage), (Turmeric) and (Coconut) where (p = 0.034), (p = 0.021) and (p = 0.034).
- A statistically significant difference was found between (Sage) and (Turmeric) where (p = 0.034) while no statistically significant difference was found between (Sage) and (Coconut) where (p = 0.999).
- A statistically significant difference was found between (Turmeric) and (Coconut) where (p = 0.034).

Immediate and Six months:

- A statistically significant difference was found between (Signal), (Sage), (Turmeric) and (Coconut) where (p = 0.023).
- A statistically significant difference was found between (Signal) and each of (Sage), (Turmeric) and (Coconut) where (p = 0.021), (p = 0.034) and (p = 0.021).
- No statistically significant difference was found between (Sage) and each of (Turmeric) and (Coconut) where (p = 0.157) and (p = 0.248).
- No statistically significant difference was found between (Turmeric) and (Coconut) where (p = 0.724).

Discussion

This study is conducted with the aim of finding cheaper, safer, and more natural alternatives to conventional teeth whitening techniques and the evaluation and comparison of their effects. As the conventional methods use harsh chemicals for teeth bleaching such as; hydrogen peroxide, or its derivative; carbamide peroxide. These chemicals cause irritation of the gum tissue and have a damaging effect on the teeth, as well as being potentially carcinogenic due to free radicals produced by them. Natural plant alternatives are widely common in home remedies and on the internet with no scientific evidence of their effectiveness. Examples of these natural products are are sage (*Salvia officinalis,* F. Lamiaceae), turmeric (*Curcuma longa*, F. Zingeberaceae), coconut oil (*Cocos nucifera*, F. Arecaceae), strawberry (*Fragaria ananassa,* F. Rosaceae), lemon (*Citrus limon,* F. Rutaceae), and guava leaves (*Psidium guajava*, F. Myrtaceae.

At present, there would appear to be considerable demand for oral hygiene products which whiten teeth by eliminating or reducing extrinsic dental stain. The incorporation of abrasives such as the high cleaning, low abrasive silicas used in the commercially used pastes may help to physically remove stain. The concept of whitening formulations containing specific chemicals which reduce or inhibit stain independent of a physical effect would appear to be particularly attractive because reduced staining may be apparent in sites of the dentition where the abrasive effects of the toothpaste would be less obvious (38). Consumers prefer a dental whitening method that they can apply easily and conveniently for themselves. Thus, toothpastes with special purposes are easily accepted by many consumers. Among these numerous commercially available toothpastes, whitening toothpastes are popular in spite of their higher prices because they enhance tooth cleanliness, improve the aesthetic aspects of the teeth, and are easily accessible.

The goal of this study was to observe color changes in enamel surface after brushing with different tooth pastes. It is evaluated at base line before brushing T0 and after brushing for equivalent one-month T1 and six months T2. In this study brushing teeth was done for 120 seconds (2 minutes) for the entire surface of the teeth with each quadrant brushed for 30 seconds [39]. A study involving the use of an electric tooth brush on a subject who had an average of six teeth in each quadrant explained that the size of the head of the electric tooth brush was capable of reaching at least more than one tooth surface, so as to brush two surfaces simultaneously. This took an estimated five seconds, the same amount of time estimated for brushing one tooth surface [40]. Thus, if brushing one tooth surface is assumed to take five seconds, with the recommendations stating that the teeth should be brushed twice a day, brushing a tooth in one day was assumed to take 10 seconds, brushing for one month was assumed to take 280 seconds (4 minutes and 40 seconds), and brushing for 6 months 1,680 seconds (28 minutes) [41]. In this study, brushing was conducted for 280 second (equivalent to one month) followed by brushing for 1,400 seconds (equivalent to five months) for each tooth paste, so the total time would become 1,680 seconds which is equivalent to six months. Also brushing was carried out by means of a tooth brushing machine, in which the brushing was performed at a speed of 365 rpm, and the tooth brushing load was standardized at 200 gm. Tooth brushes were cut at the neck and fixed by screws at both sides and the top of the brush support. Correct adjustment of the screws allowed for proper levelling of the tooth brush [38], type of tooth brush used in this study has rounded end, uniform length, flexibility and medium bristles.

This study used the CIELAB measurement system, which is appropriate for observing changes in brightness and analyzing saturation using the vita easy shade (spectrophotometer) in color space E*, L*, a*, b*, where L* indicates the lightness and a and b indicates the chromaticity coordinates, +a* indicates the red direction, -a* indicates the green direction, +b* indicates yellow direction and -b* indicates the blue direction. The increase in a and

b direction means that the point moves away from the center and increase in color saturation. By comparing the three natural tooth pastes at base line T0 and after 1-month T1 and after six months T2 there was no significant difference in the ΔL, Δb and Δa.

Coconut oil which is a natural antibacterial agent with the ability to kill micro-organism whose cell membranes contains lipids [42], stated that the 100% virgin coconut oil had a broad-spectrum antibacterial effect which has the same action as chlorohexidine [43]. Therefore, in our study we examined its effect on enamel color changes in the form of tooth paste. It showed no significant difference in color changesΔL, Δb and Δa between T0 and T1 and between T0 and T2, while ΔE, showed no changes that can be seen by the observers clinically where ΔE < 1. On the other hand, Turmeric has anti-inflammatory, anti-oxidant, anti-microbial, immune-stimulant and antiseptic effect, it was investigated by [43] its use in oral health as periodontal disease and they assumed that it can be used as pit and fissure sealant, mouth wash and subgingival irrigate [41]. In our study we investigated the color changes of enamel using the turmeric as tooth paste, it showed no significant difference in ΔL, Δb and Δa when compared the color changes at T0, T1 and T2, while ΔE showed changes that can be easily observed where ΔE >3.3. therefore, the turmeric can be used as mouth wash, but according to our study not recommended to be used in tooth pastes, as it induced color change which was observed toward the dark direction.

It has been reported that sage exerts a range of therapeutic activities including ant-bacterial, anti-viral, ant-fungal and anti-oxidant effects [44]. By evaluating the enamel color changes, it was found that there was no significant difference in ΔL, Δb and Δa, but ΔE showed changes in which ΔE > 3.3 which can be easily observed clinically. When comparing the three natural products with the commercially used tooth paste (Signal white), there was change in color where ΔE > 3.3 with lighter effect after one month, while after six months brushing it became slight darker but still lighter than the base line (T0).

Therefore, it is recommended that the natural products sage and coconut can be added in the tooth paste products for the anti-bacterial effect, but not as whitening agents, while turmeric is not recommended its addition in tooth pastes as it increase the +b (yellowness) so it is recommended to be used as mouth wash or gel application on the gingiva, not directly on tooth structure.

Conclusion

Commercial whitening tooth paste increased the lightness of the enamel surface after one month, but after six months it did not give the same effect. Meanwhile, using sage and coconut had no effect on tooth whitening after one and six months. Turmeric showed slight darker changes on the enamel surface. Further investigations to be done on these natural materials as they have multiple antibacterial and anti-inflammatory effects on tooth structure.

Conflicts of Interest

The authors declare no conflict of interest.

Bibliography

1. Karen P., *et al.* "Effect of whitening dentifrices: a double-blind randomized controlled trial". *Brazilian Oral Research* 30.1 (2016): 82.

2. Claydon N., *et al.* "Clinical study to compare effectiveness of a test whitening tooth paste with commercial whitening tooth paste at inhibiting dental stain". *Journal of Clinical Periodontology* 31 (2004): 1088-1109.

3. Joiner A., *et al.* "A review of tooth colour and whiteness". *Journal of Dentistry.* 36 (2008): S2-7.

4. Khan M K., *et al.* "Khan M U 2014 Extrinsic stain removal with a toothpowder: A randomized controlled trial". *International Journal of Health Sciences* 8 (2014): 269-74.

5. Hotwnak., *et al.* "Comparative invitro assessment of color stability of hybrid esthetic restorative material against various children beverages". *Journal of Conservative Dentistry* 1 (2014): 70-74.

6. Menzes M., *et al.* "Abrasion of eroded root dentin drushed with different tooth pastes". *Clinical Oral Investigations* 8.3 (2004): 151-155.

7. Peach K and Tracey MV. "Modern Methods of drug analysis". Springer- Verlag, Berlin, Cottingen, Heidelberg 3 (1955): 478.

8. Sharma PP. "Cosmetics- Formulation, manufacturing and quality control". 4th ed. Vandana publishing house, (2010): 539-554.

9. Dilip George., *et al.* "Comparative evaluation of the antimicrobial efficacy of aloe vera tooth gel and two popular commercial toothpastes: An in vitro study". *General Dentistry* 57.3 (2009): 238241.

10. Singh K., *et al.* "Comparative st dies between herbal toothpaste (dantkanti) and non-herbal tooth paste". *International Journal of Dental Research* 4.2 (2016): 53-56.

11. Solomon Charles Ugochukwu. "Arukwe UcheI and Onuoha Ifeanyi Asian". *Journal of Plant Science and Research* 3.3 (2013):10-13.

12. White DJ. "Development of an improved whitening dentifrice based upon 'Stain-Specific Soft Silica' technology". *The Journal of Clinical Dentistry* 12.2 (2001): 25-29.

13. Pisani Mx., *et al.* "Evaluation of the abrasiveness of dentifrices for complete dentures". *Journal of Prosthodontics* 19.5 (2010): 369-373.

14. Marinova. "Total phenolics and total flavonoids in bulgarian fruits and vegetables". *Journal of the University of Chemical Technology and Metallurgy* 40.3 (2005): 255-260.

15. Cook NC and Samman S. "Flavonoids-Chemistry, Metabolism, Cardioprotective Effects, and Dietary Sources". *The Journal of Nutritional Biochemistry* 7 (1996): 66-76.

16. Anna vallverdú-queralt., *et al.* "Characterization of the phenolic and antioxidant profiles of selected culinary herbs and spices: caraway, turmeric, dill, marjoram and nutmeg" *Food Science and Technology* 35.1 (2015): 189-195.

17. Jueun Lee., *et al.* "Byeong Cheol Moon Do Hyun Ryu and Geum-Sook Hwang Secondary Metabolite Profiling of Curcuma Species Grown at Different Locations Using GC/TOF and UPLC/Q-TOF MS Molecules". 19 (2014): 9535-9551.

18. Anghel Brito., *et al.* "UV-MS Profiles of Phenolic Compounds and Antioxidant Activity of Fruits from Three Citrus Species Consumed in Northern Chile". *Molecules* 19.11 (2014): 17400-17421.

19. Anna Wojakowska., *et al.* "Maciej Stobiecki Structural analysis and profiling of phenolic secondary metabolites of Mexican lupine species using LC-MS techniques". *Phytochemistry* (2013).

20. Ga Hee Janga., *et al.* "Characterization and quantification of flavonoidglycosides in the Prunusgenus by UPLC-DAD-QTOF/MS". *Saudi Journal of Biological Sciences* 25.8 (2018): 1622-1631.

21. Ibrahim RM., *et al.* "HPLC-DAD-MS/MS profiling of phenolics from Securigera, securidaca flowers and its anti-hyperglycemic and anti-hyperlipidemic activities". *Revista Brasileira de Farmacognosia* 25.2 (2015):134-141.

22. Parejo I., *et al.* "Characterization of acylated flavonoid-O-glycosides and methoxylated flavonoids from Tagetes maxima by liquid chromatography coupled to electrospray ionization tandem mass spectrometry. Rapid Commun". *Mass Spectrometry* 18 (2004): 2801-2810.

23. Wang Y., *et al.* "Characterization of fifty-one flavonoids in a Chinese herbal prescription Longdan Xiegan Decoction by high-per-formance liquid chromatography coupled to electrospray ionization tandem mass spectrometry and photodiode array detection". *Rapid Communications in Mass Spectrometry* 22.12 (2008): 1767-1778.

24. Sánchez-Rabaneda F1, Jáuregui O, Casals I, Andrés-Lacueva C, Izquierdo-Pulido M, Lamuela-Raventós RM". *Journal of Mass Spectrometry* 38.1 (2003): 35-42.

25. Elixabet Díaz-de-Cerio., *et al.* "Exploratory Characterization of Phenolic Compounds with Demonstrated Anti-Diabetic Activity in Guava Leaves at Different Oxidation States". *International Journal of Molecular Sciences* 17.5 (2016): 699.

26. Eva Dorta., *et al.* "Screening of phenolic compounds in by-product extracts from mangoes (Mangifera indica L.) by HPLC-ESI-QTOF-MS and multivariate analysis for use as a food ingredient". *Food Research International* 57 (2014).

27. P Mena., *et al.* "Rapid and Comprehensive Evaluation of (Poly) phenolic Compounds in Pomegranate (Punica granatum L.) Juice by UHPLC-MSn". *Molecules* 17 (2012): 14821-14840.

28. Park AJ., *et al.* "Altered colonic function and microbiota profile in a mouse model of chronic depression". *Neurogastroenterology and Motility* 25.9 (2013): 733-e575.

29. Selmi S., *et al.* "Rosemary (Rosmarinus officinalis) essential oil components exhibit anti-hyperglycemic, anti-hyperlipidemic and antioxidant effects in experimental diabetes". *Pathophysiology* 24.4 (2017): 297-303.

30. Krishnamurthy PC., *et al.* "Identification of a mammalian mitochondrial porphyrin transporter". *Nature* 443.7111 (2006): 586-589.

31. David Olivier., *et al.* "Mussel and oyster culture in Saldanha Bay, South Africa: potential for sustainable growth, development and employment creation". *Food Security* 5.2 (2013): 251-267.

32. Simirgiotis MJ. "Antioxidant capacity and HPLC-DAD-MS profiling of Chilean peumo (Cryptocarya alba) fruits and comparison with German peumo (Crataegus monogyna) from southern Chile". *Molecules* 18.2 (2013): 2061-2080.

33. Kajdzanoska M., *et al.* "Comparison of different extraction solvent mixtures for characterization of phenolic compounds in strawberries". *Journal of Agricultural and Food Chemistry* 59.10 (2011): 5272-5278.

34. Seham S Elhawary., *et al.* "LC-MS/MS-based chemometric analysis of phytochemical diversity in 13 Ficus spp. (Moraceae): correlation to their in-vitro antimicrobial and in-silico quorum sensing inhibitory activities". *Industrial Crops and Products* 126 (2018): 15.

35. Zhang Y., *et al.* "Isolation and identification of strawberry phenolics with antioxidant and human cancer cell antiproliferative properties". *Journal of Agricultural and Food Chemistry* 56.3 (2008): 670-675.

36. Mullen W., *et al.* "Analysis of ellagitannins and conjugates of ellagic acid and quercetin in raspberry fruits by LC-MSn". *Phytochemistry* 64.2 (2003): 617-624.

37. Singh RK., *et al.* "Influence of diet on the gut microbiome and implications for human health". *Journal of Translational Medicine* 15.1 (2017): 73.

38. Joiner A., *et al.* "In vitro cleaning, abrasion and fluoride efficacy of a new silica based whitening toothpaste containing blue covarine". *Journal of Dentistry* 36 (2008): 32-37.

39. Tellefsen G. "Surface roughness- casual factors and its relationship to bacterial adhesion". *Dissertation Paper* (2013).

40. Wiegand A., *et al.* "Brushing force of manual and sonic tooth brushes affects dental hard tissue abrasion". *Clinical Oral Investigations* 17 (2013): 815-822.

41. Pisani Mx., *et al.* "Evaluation of the abrasiveness of dentifrices for complete dentures". *Journal of Prosthodontics* 19.5 (2010): 369-373.

42. Adita Gayatri., *et al.* "Anti-bacterial effect of virgin cocnut oil on the viability of chromogenic bacteria that causes dental black stain in children". *International Journal of Applied Pharmaceutics* 19 (2017).

43. Monika Nagpal and Sheeta Sood. "Role of curcumin in systemic and oral health: An overview". *Journal of Natural Science Biology and Medicine* 4.1 (2013): 3-7.

44. Maryam Beheshti-Rouy., *et al.* "The anti-bacterial effect of sage extract (saliva officinal) mouth wash against streptococcus mutans in dental plaque: a randomized clinical trial". *Iranian Journal of Microbiology* 7.3 (2015): 173-177.

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