J Herbmed Pharmacol. 2017; 6(3): 130-135.

JHP

Journal of Herbmed Pharmacology

Journal homepage: http://www.herbmedpharmacol.com

Formulation and physicochemical evaluation of toothpaste formulated with *Thymus vulgaris* essential oil

Mohammad Kiani[1,2], Farzin Firoozian[3], Shirin Moradkhani[1,2*]

[1]Medicinal Plants and Natural Products Research Center, Hamadan University of Medical Sciences, Hamadan, Iran
[2]Department of Pharmacognosy and Pharmaceutical Biotechnology, School of Pharmacy, Hamadan University of Medical Sciences, Hamadan, Iran
[3]Department of Pharmaceutics, School of Pharmacy, Hamadan University of Medical Sciences, Hamadan, Iran

ARTICLE INFO

Article Type:
Original Article

Article History:
Received: 1 December 2016
Accepted: 7 March 2017

Keywords:
Thymus vulgaris
Essential oil
Herbal toothpaste
Drug formulation
Physicochemical properties

ABSTRACT

Introduction: *Streptococcus mutans* is the most common cause of tooth decay. Parabens, and other commonly used as anti-*Streptococcus* agents in toothpaste industry have numerous side effects such as discoloration of teeth. *Thymus vulgaris* essential oil has profound antimicrobial activity against a wide range of species. The aim of present study was the aim of the present study was to formulate and evaluate the physicochemical properties of a kind of toothpaste formulated with Thymus vulgaris essential oil. Thyme oil components were also analyzed using gas chromatography–mass spectrometry (GC-MS).

Methods: Toothpaste was formulated in forms of gel and opaque and Thyme essence was added to it. The formulation was evaluated in terms of stability in different temperatures, pH, consistency, uniformity, taste, smell, and compatibility with special packaging for toothpaste at three temperatures. Profilometry was used to determine abrasivity. The rate of contaminations with lead and arsenic was determined by atomic absorption. The amount of fluoride was measured by potentiometry.

Results: Forty-one different components, representing 99.64% of the total oil were identified in essential oil. Addition of thyme essence to formulation had no deleterious effect in stability, consistency, taste and smell. The pH of opaque and gel formulations was 7.02 and 7.45, respectively. The abrasiveness of opaque and gel formulations was in standard ranges. The fluoride content was 1000 ppm. Lead and arsenic were not detected at all.

Conclusion: Formulation of toothpaste with *T. vulgaris* essential oil was acceptable and might be considered as a desirable herbal toothpaste.

Implication for health policy/practice/research/medical education:
Addition of *T. vulgaris* essential oil to toothpaste and evaluation of the physicochemical properties of this new formulation was the aim of present study. Addition of herbal components such as *T. vulgaris* essential oil to toothpastes may make them interesting for introducing new candidates in oral hygiene products.

Please cite this paper as: Kiani M, Firoozian F, Moradkhani S. Formulation and physicochemical evaluation of toothpaste formulated with *Thymus vulgaris* essential oil. J Herbmed Pharmacol. 2017;6(3):130-135.

Introduction

The oral cavity infections are the most common types of infections. Dental caries, an infectious disease, results in damage and infection of enamel and dentine (1). If left untreated, the infection continues and will lead to tooth loss. The mouth normal flora consists of opportunistic bacteria which are normally non-pathogenic. The imbalance of this situation creates infection and tooth decay. *Streptococcus mutans* is considered as the main species involved in the development of dental caries (2). *S. mutans,* acid producing bacteria, causes fermentation of carbohydrates which results in tooth decay (3). Toothpaste, toothbrush, mouthwash, and toothpaste that contain antimicrobial agents are commonly used as products which improve oral hygiene. Their uses dates back to ancient times and continues up to now (4). Toothpaste, as an irreplaceable agent in effective home care system, is a gel or paste dentifrice used with a toothbrush as an accessory to clean and maintain health of teeth in order to enhance oral hygiene (5).

Thymus vulgaris (*Lamiaceae),* or thyme as common name, contains a useful essential oil. It has antiseptic, expectorant, antispasmodic and carminative activities (6). Thyme oil consists of high amounts of phenolic monoterpenes. These compounds have strong antimicrobial activity against *S. mutans* and a wide range of

***Corresponding author:** Shirin Moradkhani,
Email: shirin.moradkhani@yahoo.com

viruses, bacteria and fungi (6,7). Considering the ongoing use of plants, their lower side effects in comparison to synthetic substances and the wide range of antimicrobial activities of thyme oil, the aim of the present study was to formulate and evaluate the physicochemical properties of a kind of toothpaste formulated with *T. vulgaris* essential oil. Thyme oil components were also analyzed using gas chromatography–mass spectrometry (GC-MS).

Materials and methods

All substances were dental grade and purchased from Goltash Company (Iran). The essence was prepared from Giahessence Company (Iran).

Thymus vulgaris essential oil components identification

One microliter of oil was injected to GC-MS. The volatile compounds were analyzed by GC/MS (TRACE MS-Thermo Finnegan Polaris Q). The capillary column type was DB-5 (30 m * 0.25 mm * 0.25 µm). The oven temperature was programmed (60˚C for 1 minute, then 5˚C/min to 250˚C, and then left at 250˚C for 5 minutes). The injection port temperature was 250˚C and that of the detector was 280˚C (split ratio: 1/100). The carrier gas was helium (99.995% purity) with a flow rate of 1.1 mL/min. The MS conditions were as follow: ionization voltage, 70 eV; ion source temperature, 200˚C; electron ionization mass spectra were acquired over the mass range 40 to 460 m/z.

Formulation of clear gel and opaque toothpaste

In this study, 4 gel and 4 opaque toothpastes were formulated. Three formulations of each type had thyme oil and one in each type contained methyl and propyl parabene. The amounts of oil used in the formulations were based on microbial studies and measurement of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). The formulated toothpastes were packaged in laminate tubes.

Determination of physicochemical properties

pH

The pH of formulations was measured by the methods described in ISO3996 (grade 3) (13).

Homogeneity

Determination of homogeneity was studied by applying normal force to containers at 27±2˚C (14).

Tube inertness

Tube inertness was examined by cutting the tubes and investigating internal surface for any signs of deterioration in different storage conditions including normal and heating temperature at 45±2˚C for 180 days (14).

Sharp and edge abrasive particles

This test was performed considering the extradition of the content of 10 tubes and investigating the presence of sharp and hard edged abrasive particles (14).

Stability

The stability study was performed by soring formulations in three different temperatures and humidity conditions, viz. 4°C, 25°C± 2°C/60% ± 5% RH, 40°C ± 2°C/75% ± 5% RH for a period of 6 months considering ICH guidelines (15).

Fluoride ion

Potentiometry with fluoride ion sensitive electrodes was used for determination of fluoride ions.
Calculation: a graph was plotted on a log scale, taking the concentration of fluoride (y-axis) versus potential in mV (x-axis). From the calibration curve, the fluoride ion concentration (in ppm) of test solution was measured. Fluoride ion concentration (ppm) was calculated from the graph (16).

Heavy metals quantification

Sample preparation was carried out according to USP 231. Determination of heavy metals was done by atomic absorption spectrophotometer (17,18).

Dentine and enamel abrasion

Sample preparation was performed by use of brush machine and according to ISO 11609:2010 (16). The nominal Ra values were tabulated and analyzed using descriptive statistical software (SPSS version 18.0, IBM Corporation). The data of surface roughness were compared between the 2 experimental groups in each experimental period (standard formulation was control test and compared with F1 & F2 formulation roughness) using paired *t* test. $P<0.05$ was considered significant. Variation in roughness was calculated and compared with the 2 experimental groups by the same tests mentioned above.

Results

Thyme oil components identification

Analyzing thyme oil by means of GC-MS resulted in identification of 41 different components, representing 99.64% of the total oil (Figure 1 and Table 1). Identification was done by calculating the Kovats index for each

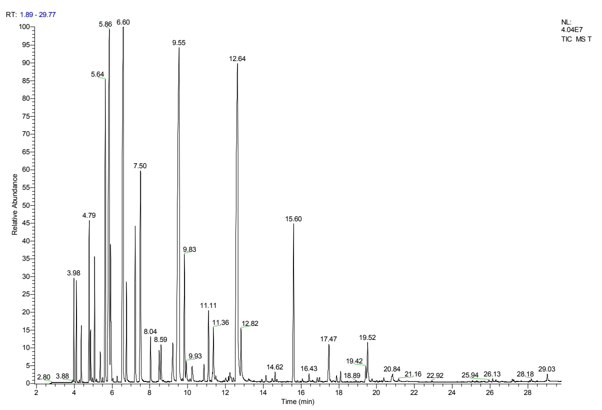

Figure 1. *Thymus vulgaris* oil components in GC-MS chromatogram.

Table 1. *Thymus vulgaris* chemical composition result

Peak Number	Compound	Retention time (RT)	Retention index (RI)	Percent
1	α-Thujene	3.98	926	1.2
2	α-Pinene	4.11	934	1.25
3	Camphene	4.37	949	0.7
4	Sabinene	4.79	974	2.34
5	β-Pinene	4.87	978	0.69
6	β-Myrcene	5.08	991	1.77
7	α-Phellandrene	5.38	1006	0.46
8	α-Terpinene	5.64	1018	6.19
9	p-Cymene	5.86	1028	12.09
10	Limonene	5.92	1031	2.48
11	1,8-Cineole	5.95	1032	0.49
12	γ-Terpinene	6.59	1061	11.61
13	cis-Sabinene hydrate	6.76	1069	1.56
14	α-Terpinolene	7.22	1090	2.63
15	Linalool	7.50	1102	5.23
16	cis-p-Menth-2-en-1-ol	8.04	1124	0.76
17	1-Terpineol	8.49	1141	0.62
18	Camphor	8.59	1145	0.63
19	Borneol	9.21	1170	1
20	4-Terpineol	9.55	1184	16.55
21	α-Terpineol	9.83	1195	2.6
22	cis-Piperitol	9.93	1199	0.37
23	trans-Piperitol	10.23	1211	0.39
24	Thymol, methyl ether	10.86	1235	0.29
25	Carvacrol, methyl ether	11.11	1245	1.3
26	trans-Sabinene hydrate acetate	11.31	1253	0.13
27	linalyl acetate	11.36	1255	1.02
28	Anethole<E->	12.24	1289	0.21
29	Thymol	12.64	1305	15.95
30	Carvacrol	12.82	1312	1.05
31	Neryl acetate	14.14	1364	0.1
32	Geranyl acetate	14.62	1383	0.17
33	E-Caryophyllene	15.60	1423	3.32
34	α-Humulene	16.43	1457	0.14
35	Bicyclogermacrene	17.47	1499	0.9
36	γ-Cadinene	17.88	1517	0.1
37	δ-Cadinene	18.09	1526	0.18
38	Spathulenol	19.42	1582	0.32
39	Caryophyllene oxide	19.52	1587	0.94
40	Iso-Spathulenol	20.80	1643	0.09
41	α-epi-Cadinol	20.84	1645	0.2

compound comparing the results with the library of mass apparatus and with those reported in the literature.

Physicochemical properties result

The results of physicochemical tests indicate the stability of both formula of Opaque and Jelly at room temperature (the temperature of 4°C of refrigerated and temperature of 40°C with germinator and 75% humidity). Also the formulations were satisfactory in terms of the status of consistency, uniformity, taste and smell. Also, there was no fermentation in two formulations (Figures 2 and 3). Also both formula had not any sharp and hard edged abrasive particles. The toothpaste container did not produce any corrosion or deterioration and Thymus vulgaris essential oil had perfect compatibility with container. Also, formulation F7 with parabens and without essence was stable and had proper consistency that was expected.

pH

At first the pH of Opaque and Jelly formulations before stability test was respectively 6.82 and 6.78. After stability test for a period of 6 months, the pH was 7.02 and 7.54 which were in the standard range.

Heavy metal content

The heavy metals of lead and arsenic test results indicated the absence of the two metals in the formulation and since the standard level of lead is less than 20 µg/g and arsenic is less than 2 µg/g, hence, both are in the standard range (Table 2).

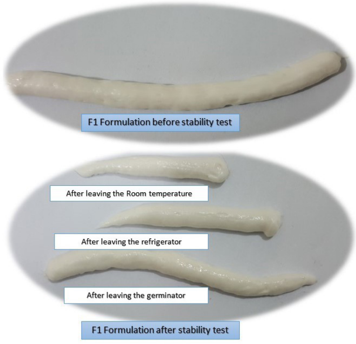

Figure 2. Physicochemical properties result of F1 formulation

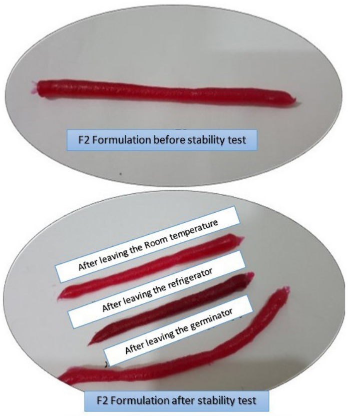

Figure 3. Physicochemical properties result of F2 formulation

Fluoride ion content

The graph was plotted for content of fluoride ion against potential difference on semi-logarithmic paper (Figure 4). The potential difference, in millivolts, was plotted on the X axis and the fluoride ion, in milligrams, on the Y axis. The amount of fluoride formulation was 1000 ppm according to fluoride ion that formulation was standard and acceptable (Table 3).

Toothpaste roughness on enamel and dentine

The amounts of abrasive of the formulations were determined. In opaque formula the amounts of dentin and enamel were 0.15, and 0.5 times of reference toothpaste, respectively, these amounts in Jelly formulas were 0.8 and 1.07 times of reference toothpaste, respectively. The abrasiveness of both formulae was acceptable and standard (Table 4). Also independent sample *t* tests were carried out to compare between F1 and F2 dentine and enamel roughness.

Independent sample *t* test was carried out to compare between F1 and F2 roughness in an effort to establish approximate differences in mean values between

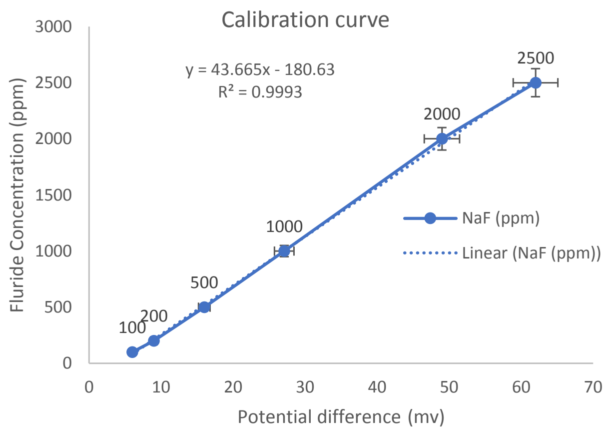

Figure 4. Fluoride ion calibration curve

Table 3. Content of NaF (ppm)

Sample	Measured potential difference (mv)	NaF (ppm)
Standard 1	6	100
Standard 2	9	200
Standard 3	16	500
Standard 4	27.1	1000
Standard 5	49	2000
Standard 6	62	2500
F1 & F2 Fluoride (ppm)	27.1	1000

dentifrices that were statistically significant (Table 5) ($P < 0.05$).

Discussion

The results of GC-MS analysis showed that *T. vulgaris* essential oil contained 15.95 % thymol and 1% carvacrol. In this way, Thyme oil effectiveness on *S. mutans* bacteria that causes oral infections is clear. The results of the initial investigation and after thermal & cooling physicochemical tests showed no changes in physical stability of formula. Also the pH of formula after tests did not change and remained in the range of 7 to 7.5 which indicates the stability of the *T. vulgaris* essential oil. Formulations containing *T. vulgaris* essential oil as preservative compared to formulations containing parabens had appropriate stability. A previous study on a cream made with *T. vulgaris* essential oil indicated appropriate and consistent formulation stability (19). *T. vulgaris* essential oil has been proven to possess antioxidant properties. This property is due to various components such as α pinen, β pinen, γ terpinen, 1,8 cineol, para-cimen, linalool, sabinen, eugenol and phellandrene. The antioxidant capacity of this components has previously proven (20,21).

Table 2. Determination of heavy metal content

Formulation/Heavy metal	Lead	Arsenic	Standard range	LOD	LOQ	Result
F1	Not detect	Not detect	Pb: <20 µg/g & As_2O_3: <2 µg/g	0.3 ppm	1 ppm	**In rang**
F2	Not detect	Not detect				**In rang**

Table 4. Formulated toothpastes roughness on enamel and dentine in comparison to standard toothpaste

Group	Dentine roughness (µm) Mean ± SD	Roughness compared to standard (times)	Enamel roughness (µm) Mean ± SD	Roughness compared to standard (times)
Standard	0.324±0.011	-	0.315±0.007	-
F1 (Dicalcium phosphate)	0.463±0.011	0.15	0.360±0.008	0.5
F2 (Hydrated silica)	0.561±0.007	0.8	0.666±0.019	1.07

Table 5. Independent samples of statistical *t* test results

Formulation	Roughness Mean	Standard error	*P* value
F1 (Dicalcium phosphate)	0.36	0.009	P< 0.001*
F2 (Hydrated silica)	0.56	0.009	

*Significant at a significance level of 5%.

Periodontal disease is associated with an increase in reactive oxidative species (ROS) in the body and this increase in free radicals in the body causes chronic and worsens the symptoms. The essence of *T. vulgaris* can be used in oral products as a good antioxidant to improve inflammatory and infectious diseases of oral tissue (22). Preliminary results of this study showed that F1 formula had appropriate and suitable stability. In F3 formulation carbapol gelling agent was exchanged with CMC. In the gel formulation stability and consistency became lost so that it could not be packaged in its own tube. Carbapol has been shown to be not suitable for the opaque toothpaste formulation and affects the stability of toothpaste (23). Our findings in this study was consistent with that results. Also the study of Wilkinson and Moore in 1996 showed that CMC was a suitable gelling agent for the preparation of opaque toothpaste (24).

In F5 formulation abrasive silica was used instead of dicalcium phosphate. Due to changes form a paste formulation and heterogeneous particles in the formulation this change is not considered desirable. Kitchin about the history of the use of dicalcium phosphate abrasive showed that the abrasive since 1940 is used as an abrasive in opaque toothpaste (25).

The results of this study are consistent with the above findings. According to the dentine & enamel roughness result, the abrasivity of formulations F1 (dicalcium phosphate) and F2 (hydrated silica) both dentin and enamel roughness was in the range of standard, and both were good. After ensure the standardization of both samples F1 and F2 wear rate were compared with each other through statistical test (Tables 4 and 5). Thus it can be concluded that the average abrasivity of formulations with hydrated silica is more than the average abrasivity of formulations with dicalcium phosphate and repeating about the test result of the change cannot be achieved. Also, a study on 26 commercial toothpastes indicated that the roughness of toothpaste with hydrated silica abrasive (like Brill smile) was significantly different from that of toothpaste containing calcium phosphate (26).

Conclusion

An ideal plant dentifrice should provide optimum physicochemical, cleaning and polishing properties with maximum compatibility with dental tissue and minimum abrasion to the dental hard tissues. The formulated toothpaste with *T. vulgaris* essential oil was successfully evaluated using different standard parameters including testing pH, consistency, uniformity, change in colors, change in smell, standard roughness, temperature and tolerance. The formulated toothpaste may be safer compared to fully synthetic toothpastes and can be used as desirable toothpaste in terms of stability and physicochemical properties in the pharmaceutical market.

Acknowledgments

This article is a part of the thesis of Mohammad Kiani for the fulfillment of Pharm. D. degree in School of Pharmacy, Hamadan University of Medical Sciences, Hamadan, Iran. The authors are grateful to deputy of vice chancellor of research and technology for financial supports.

Authors' contributions

SM and FF were the supervisors of the thesis project, respectively. MK performed the experiments. All read and confirmed the manuscript.

Conflict of interests

The authors declare that there is no conflict of interest.

Funding/ Support

This study was financially supported by deputy of vice chancellor of research and technology, Hamadan University of Medical Sciences, Hamadan, Iran.

References

1. Bowen WH, Koo H. Biology of *Streptococcus mutans* derived glucosyltransferases: Role in extracellular matrix formation of cariogenic biofilms. Caries Res. 2011;45:69 86.
2. Wright JT, Hart TC. The genome projects: implications for dental practice and education. J Dent Educ. 2002;66:659-71.
3. Freedman ML, Tanzer JM. Dissociation of plaque formation from glucan-induced Agglutination in Mutants of *Streptococcus mutans*. Infect Immun. 1974;10(1):189-196.
4. Davies R, Scully C, Preston AJ. Dentifrices - an update. Med Oral Patol Oral Cir Bucal. 2010;15(6): 976-82.
5. Namba T, Tsunezuka M, Hattori M. Dental caries prevention by traditional Chinese medicines. Part II. Potent

antibacterial action of Magnoliae cortex extracts against *Streptococcus mutans*. Planta Med. 1982;44(2):100-6.

6. Zargari A. Herbal Medicine. Iran: Publishing and Printing Institute of Tehran University; 1996:353. [Persian].
7. Abdulsattar BA, Hassan AM, Hassan AS. In vitro antimicrobial activity of *Thymus vulgaris, Origanum vulgare* and *Rosmarinus officinalis* Against Dental Caries Pathogens. Haitham J Pure Appl Sci. 2011;25(2):1-7.
8. Richardson LM. International collaborative research of fluride. J Dent Res. 2000;79:893-904.
9. Kielbassa AM, Gillmann L, Zantner C, Meyner-Lueckel H. Profilometric and microradiographic studies on the effects of toothpastes and acidic gel roughness on sound and demineralized bovine dental enamel. Caries Res. 2005;39:380-6.
10. Amartey EO, Asumadu-Sakyi AB, Adjei CA, Quashie FK, Duodu GO, Bentil NO. Determination of heavy metals concentration in hair pomades on the Ghanaian market using atomic absorption spectrometry technique. Br J Pharmacol Toxicol. 2011;2(4):192-98.
11. Butler H, Poucher's Perfumes Cosmetics and Soaps. 9th ed. Netherlands: Kluwer Academic Publishers; 2000:981-6.
12. ISO E. 3696: Water for analytical laboratory use. Specification and test methods; 1997.
13. Benzian H, Holmgren C, Buijs M, van Loveren C, van der Weijden F, van Palenstein-Helderman W. Total and free available fluoride in toothpastes in Brunei, Cambodia, Laos, the Netherlands and Suriname. Int Dent J. 2012;62(4):213-21.
14. Bureau of Indian standard. 2001. https://law.resource.org/pub/in/bis/S11/is.6356.2001.pdf. Accessed September 2010.
15. Pope DG. Accelerated stability testing for prediction of drug product stability. 2. Drug & Cosmetic Industry; 1980; 127(6):48.
16. International Standard organization. https://www.iso.org/obp/ui/#iso:std:38010:en. Accessed September 2010.
17. US Pharmacopeia. Heavy metal analysis in toothpastes, 1990:1523-4.
18. British Pharmacopeia. Heavy metal analysis in toothpastes. 1990:87-8.
19. Manou I, Bouillard L, Devleescherouwer MJ, Barel AO. Evaluation of the preservative properties of *Thymus vulgaris* essential oil in topically applied formulations under a challenge test. J Appl Microbiol. 1998;84:368-78.
20. Przygodzka M, Zielińska D, Ciesarová Z, Kukurová K, Zieliński H. Comparison of methods for evaluation of the antioxidant capacity and phenolic compounds in common spices. LWT-Food Science and Technology. 2014; 58(2):321-6.
21. Nikolić M, Glamočlija J, Ferreira IC, et al. Chemical composition, antimicrobial, antioxidant and antitumor activity of *Thymus serpyllum* L., *Thymus algeriensis* Boiss. and Reut and *Thymus vulgaris* L. essential oils. Ind Crops Prod. 2014;52:183-90.
22. Tamaki N, Tomofuji T, Ekuni D, Yamanaka R, Yamamoto T, Morita M. Short-term effects of non-surgical periodontal treatment on plasma level of reactive oxygen metabolites in patients with chronic periodontitis. J Periodontol.2009;80(6):901-6.
23. Saeidi M, Azadbakht M. Formulation of herbal toothpaste from chamomile and myrah, apreliminary clinical evaluation on bleeding gum. Journal of Mazandaran University of Medical Science. 2003;13(40):61-9.
24. Wilkinson JB, Moore RJ: Harry's Cosmeticology. 7th ed. London: Longman Scientific & Technical; 1996:613.
25. Kitchin PC, Robinson HBG. How abrasive need a dentifrice be? J Dent Res. 1948;27:501-6.
26. Schemehorn BR, Moore MH, Putt MS. Abrasion, polishing, and stain removal characteristics of various commercial dentifrices in vitro. J Clin Dent. 2011;22(1):11.