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Original article

Anti-Halitosis Tooth Paste: From Persian Manuscripts toward Clinic

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Abstract

Background and objectives: Halitosis as a common dentistry ailment which has a prevalence of around 50% of the adult population. There are many dental formulations in traditional Persian pharmacy called "Gharagher", "Mazmazeh", "Sonoun", etc for treatment of halitosis. In the present study, we have tried to describe the step by step modernization of traditional herbal advices for treatment of halitosis. **Methods:** Traditional Persian manuscripts were reviewed and dome herbs were selected for formulation of toothpaste. Qualitative and quantitative controls were performed on raw materials and toothpaste was formulated using the selected herbs. Pharmaceutical control tests including squeeze, centrifuge, conductivity, particle size, spreading, temperature related stability, and microbial limit tests were performed on the toothpastes. **Results:** A total of 31 medicinal plants from 24 plant families and 12 anti-halitosis "Sonoun"s possessing anti-halitosis related properties were found. *Syzygium aromaticum*, *Pistacia atlantica* var. *mutica*, and *Punica granatum* var. *pleniflora* were selected for the formulation. Results of quality contril assays were in the accepted range of pharmacopeias. GC-MS analysis showed 15 and 16 components in *P. atlantica* and *S. aromaticum* oils, respectively. GC-FID results showed 85.15% α-pinene in *P. atlantica* and 5.28% eugenol in *S. aromaticum*. ATR-IR spectrum was used for control of *P. granatum* flowers. The organoleptic properties, phase separation, particle size and microbial tests of formulations showed an accepted shelf life for performing clinical trials. The results proved a good texture property as well as desirable abrasive properties of the toothpaste. Post production quality control tests indicated the proper feature of final product after packaging. **Conclusion:** Such model would be a straight forward rout from traditional medical manuscripts toward clinic.

Keywords: anti-halitosis; Persian pharmacy; *Pistacia atlantica* var. *mutica*; *Punica granatum* var. *pleniflora*; *Syzygium aromaticum*

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Introduction

Today there is a trend for finding new opportunities for the treatment of incurable or not satisfactory treated diseases from eastern traditional medicines [1]. Such sources compiled in thousands of manuscripts from Persian and Arabic to Chinese languages presents us large amounts of formulations for such conditions. Halitosis as a common dentistry ailment has a prevalence of around 50% of the adult population while it is found around 27% in Iran, 27.5% in China and 25% in Jordanian adults [2-4]. It is classified into three categories: genuine halitosis, pseudo-halitosis, and halitophobia [5]. Genuine halitosis is a multi-factorial issue and involves both oral and non-oral regions, and it is reported that 80-90% of all cases are caused by oral conditions [6-8]. Oral malodor results from tongue coating, periodontal disease, peri-implant

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diseases, deep carious lesions, exposed necrotic tooth pulps, pericoronitis, mucosal ulcerations, healing wounds, imperfect dental restorations, unclean dentures and factors which cause decrease in salivary flow rate [9-15]. There are different dental dosage forms in Iranian traditional medicine including: "Gharagher": traditional gargles, "Mazmazeh": aqueous extract of medicinal plants, "Sonoun": powder of medicinal plants, oral "Barodat": powder of dried mixture of medicinal plants and medicinal plants' aqueous extract, and oral "Zarorat": fine powder of medicinal plants [16].

In this research work we have tried to describe the step by step modernization of traditional herbal advices for halitosis in the form of toothpaste. This process included the survey for anti-halitosis mono-ingredient or formulations from major traditional sources, identification of the components, providing a formulation from *Pistacia atlantica* var. *mutica*; *Syzygium aromaticum*; *Punica granatum* var. *pleniflora*, pharmacognostic controlling the ingredients, preparing and pharmaceutical controlling of the resulting toothpaste.

Material and Methods

Manuscript survey

Traditional Persian manuscripts of three main categories were reviewed: simple remedies (Materia Medica), multi-ingredient formulations ("Qarabadin"), and therapeutics. Information of simple remedies from animal, herbal and mineral sources were extracted Makhzan al-advia [17], Seydaneh fit teb [18], Al-aghraz al-tebbiehval-mabahes al-alayieh [19] and Tohfatolmomenin [20]. Information about "Sonoun" and their indications were gathered from "Qarabadin" texts such as "Qarabadin-e kabir" [21], "Amalesaleh (Qarabadin-e salehi)" [22] and "Qarabadin-e azam" [23]. The scientific equivalent names for raw materials were mainly chosen according to their descriptions and references [24,25].

Plant material

Samples of "Bane" gum were collected from Kavar (51 km southeast of Shiraz, Fars Province, Iran) in 2013; "Golnar" flowers from Janatshahr (15 km to Darab, Fars Province, Iran) in 2013 and "Mikhak" buds from herbal market in 2013. "Bane" was identified as *Pistacia atlantica* var. *mutica* Rech.f. (Anacardiaceae) (herbarium No. 785), "Golnar" as *Punica granatum* var. *pleniflora* Hayne. (Punicaceae) (herbarium No. 593) and "Mikhak" as *Syzygium aromatum* (L.) Merr. & L.M.Perry (Myrtaceae) (PM No. 699) by S. Khademian and herbarium or plant samples were deposited at the Herbarium of Faculty of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran.

Quality control of plant material

Quality control tests including total ash, moisture content, pH and microbial contaminates were applied according to United States pharmacopeia (USP) [26].

Essential oil analysis

Sample preparation

Pistacia atlantica var. *mutica* gum or *Syzygium aromatum* buds (50 g) were subjected to hydrodistilation (1 L water) for 4 h using a Clevenger-type apparatus according to the method recommended in British Pharmacopoeia [27] and the resulting essential oil was dried over anhydrous sodium bisulphate and stored at -20 ºC.

Gas chromatography-mass spectrometry analysis

The GC-MS analyses were carried out using GC instrument (Agilent 7890A), Mass specific detector (Agilent 5975C). The gas chromatograph was equipped with a fused silica capillary column (Agilent DB-1 MS; 30 m, 0.25 mm i.d.), He as the carrier gas (at 1.2 mL/min), mass spectrometer regulated in EI mode (70eV) and 30-600 m/z mass range with 280 ºC interface temperature. The injector temperature was 250 ºC. Temperature program was 3 °C/min at 70 ºC increased up to 280 ºC and kept 3 min at 280 ºC. Identification of components was based on a comparison of their RI and mass spectra with Willey (275) library spectra and literature [28].

GC-FID analysis

Quantification of α-pinene in *Pistacia atlantica* var. *mutica* gum and eugenol in *Syzygium aromatum* volatile oils were performed using a GC- 2014 (Shimadzu, Japan) equipped with a FID detector. Capillary column for separation was HP-5MS (30 m×0.32 mm×0.25 µm film thickness, Agilent USA). Helium with flow rate of 1.99 mL/min was used as the carrier gas. The ration was 1:10 and the injector and FID detector temperatures were 240 and 220 ºC, respectively. Temperature programming was executed as 1 min at 65 ºC up to 220 ºC at the rate of 3 ºC/min with holding time of 4 min. Quantitative analysis was done by using the calibration curve of α-

pinene (0.672-172 μg/mL) and eugenol (6.625-212 μg/mL).

ATR-IR spectroscopy

IR spectroscopy was used to obtain the fingerprint pattern and the operating groups in *Punica granatum* var. *pleniflora* as it described earlier [29]. Dried flower powder was subjected to a Bruker vertex-70 instrument using ATR apparatus. Before acquisition, the baseline for data was corrected in order to suppress the ingredients in the chamber. The transmittance values were obtained in the middle IR range 600-3400 cm^{-1}.

Preparation of toothpaste

For toothpaste preparation, freeze dried aqueous extract (1/10 w/v and 1 day extraction) of *Punica granatum* var. *pleniflora*, *Pistacia atlantica* var. *mutica* ethanolic solution and *Syzygium aromaticum* essential oil were diluted 5 folds with standard toothpaste vehicle consisting of sorbitol, glycerin, silica, methyl paraben, titanium dioxide, tetra sodium pyrophosphate, sodium fluoride, carboxy methyl cellulose and sodium lauryl sulfate. The resulted formulation was filled in polyethylene tubes under nitrogen flow and well-sealed by heat elements. The placebo formulation contained only the standard toothpaste vehicle, 5 % clove essential oil (compared to the amount of which was applied to the test formulation) for presenting the same odor, and safe food color was added to obtain more similarity.

Squeeze test

High internal pressure of toothpaste tubes may be resulted from air bubble produced during the filling or high temperature in the storage. The paste should be pulled out from the tube homogenously with no separation of phases. Existence of the air bubbles or non-homogenous features are unacceptable for toothpaste strips [26,27]. To evaluate the mentioned properties, the three tubes of the formulation and placebo were examined.

Odor and color tests

The odor of formulation should not change during the storage time. Any alteration in odor could be the result of probable oxidation, microbial growth, ingredient interactions, and formulation-container interactions [26,27]. Similar to the odor, color of formulation should be unchanged and the color of the various batches of toothpaste must be the same as possible. The tests were applied for placebo and formulation in triplicate.

Leakage test

This experiment was applied with 10 cleaned and well-sealed formulation tubes. According to the USP guideline [26], these tubes were lined on the filter paper in 60±3 °C for 8 h. None should leak during this time.

Centrifuge test

To evaluate the stability of the formulations, 10 g of toothpaste was centrifuged at 15000 g for 5, 10, 15, 30, 60 min intervals. No significant alteration and phase separation should be seen in toothpaste texture [26,27].

pH and conductivity determination

To determine the pH and conductivity of the formulation, the toothpaste samples were diluted 1:10 with deionized water and evaluated with glass probe of pH meter equipped with electrical conductivity meter (Crison Basic 20+). The fixity of pH and conductivity were followed to recognize any instability of formulations. Changes in conductivity indicate the creation or removal of existing ions that can relate to the stability of the product.

Weight variation

According to USP guideline [26,30], 10 tubes of formulation were weighted. Thereafter, they were emptied and re-weighted. The mass of tube content was calculated. For tubes between 60-150 g, all tubes must contain more that 95% of expressed values.

Particle size evaluation

The particle size and size distribution are important factors which affect the abrasive property of toothpaste. [26,27]. To analyze the particle size distribution, the toothpaste samples were diluted 1:10 with deionized water and evaluated by laser light scattering particle size analyzer (Shimadzu, Model Sald-10, Japan).

Spreading test

For this purpose, 0.5 g toothpaste was lined between 42 g glass-plates of extensiometer for 3 min. After 3 min intervals, 250 g was applied according to the standard procedure [26,30]and the radius of toothpaste extension was analyzed in each step. This experiment was applied in triplicate.

Temperature related stability test

Different samples of toothpaste formulations were kept at -10 °C and room temperature for 4 weeks. Then at room temperature and at 40 °C, by performing a shaking test for the samples shrinking, phase separation, crystal growth, bleeding and grittiness were checked for probable instability. [26,27].

Microbial limit test

To determine the possible microbial and fungal contaminations the initial powders and final formulation samples were tested for the presence of microorganisms such as *Escherichia coli*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and fungi or yeast types [26,27].

Results and Discussion

A total of 31 mono ingredient medicinal plants from 24 plant families possessing anti-halitosis properties were found in the main traditional manuscripts (table 1). Anti-halitosis effect was found in recent literatures for 18 plant materials and related effects such as odorant for 11 herbals, astringent for 4 materials and there were two reports for anti-gingivitis or dental infections. The most common traditional temperament as was reported by Naseri [31] was hot and dry (25 of 31 cases). Such holistic approach to natural products has been the subject of recent studies [32].

Table 2 describes herbal, animal or minerals containing anti-halitosis "Sonoun"s that were found in "Qarabadin" books. A total of 12 anti-halitosis "Sonoun" traditional formulations contained 3-26 ingredients were collected from "Qarabadin" manuscripts. The main reported properties for multi ingredient formulations were 11 odorants, 11 dental and periodontal reinforcements, 5 anti-halitosis and 2 reports as anti-gingival bleeding or anti-sialorrhea. Therefore, the main properties for multi ingredient formulations were odorant or dental and periodontal reinforcement. Three herbals were selected for preparing formulation and modernization from different classes of effectiveness i.e. anti-halitosis, odorant and astringent, including *Syzygium aromaticum* as anti-halitosis, *Pistacia atlantica* var. *mutica* as odorant and astringent, and *Punica granatum* var. *pleniflora* as astringents besides anti-halitosis.

GC-MS analysis showed 15 volatile components and the main components of *Pistacia atlantica* var. *mutica* essential oil as reported earlier [33,34] to be α-pinene (90 %) (table 3). Further quantification of α-pinene in the oil revealed the real quantity to be 85.15 %.

GC-MS analysis for *Syzygium aromaticum* buds essential oil (table 4) showed 16 volatile components while, α-caryophyllene (48.7%) and eugenol (29.5%) were reported earlier as the main components [35,36]. The percentage of eugenol was found to be 5.28 %. There is no report available on phytochemical analysis of *Punica granatum* var. *pleniflora*; therefore, ATR-IR spectrum was determined (figure 1) as a reproducible method for the raw materials [29].

GC-FID analysis showed a sum of 0.0001 % eugenol as well as 0.09 % α-pinene in the resulting toothpaste. The quality control tests on the toothpaste and related placebo according to references [26,37,38] have been summarized in table 5.

Studies on tube of toothpaste showed that there was no bubble and the tube was removed homogeneously with no separated phases. After examining the color, smell and taste of products on the first day, second day, third day, first week, second week, third week, first month and second month, there was no significant change in the smell, color and taste of the products. The results of the organoleptic tests have been shown in table 6. After centrifugation of the samples of toothpaste or placebo in 15000 rpm, no phase separation occurred and the products remained homogeneous. Minimum particle sizes of placebo and drug have been listed in table 5. The final toothpaste obtained from this study, is an exact instance of alteration from a traditional to a modern dosage form. This toothpaste presented a well-defined and proper characteristics accorded to the US pharmacopeia [29]. The stability tests including odor, color, centrifuge and temperature related stability test, pH and conductivity profile and microbial assays showed an accepted shelf life; which is necessary for the medical dosage forms.

Also, the particle size analysis and spreading test presented the quality of the product during the use by the patients. The results proved a good texture property as well as desirable abrasive properties of the toothpaste. Post production quality control tests including the leakage test, weight variation and squeeze test along with the previous tests indicated proper feature of final product after packaging.

Table 1. Medicinal plants reported as anti-halitosis related agents in the traditional medical manuscripts [17-20].

Scientific name	Family	Parts used	Persian name	Temperament	Properties
Allium porrum L.	Amaryllidaceae	Leaf	Koras	Hot and dry	Odorant
Aloexylum agallochum Lour	Thymelaeaceae	Wood	Ood	Hot and Dry	Anti-halitosis
Alpinia galanga (L.) Willd.	Zingiberaceae	Root	Kholenjan	Hot and Dry	Anti-halitosis
Artemisia dracunculus L.	Asteraceae	Leaf	Tarkhoon	Hot and dry	Odorant
Asperugo procumbens L.	Boraginaceae	Leaf	Badranjbooyeh	Hot and dry	Anti-halitosis
Calicotome spinosa (L.) Link	Leguminosae	Bark	Darsheysheyan	Hot and Dry	Anti-gingivitis
Camellia sinensis (L.) Kuntze	Theaceae	Leaf	Chaykhotaei	Hot and Dry	Anti-halitosis
Cinnamomum zeylanicum Blume	Lauraceae	Bark	Darcini	Hot and Dry	Anti-halitosis
Commiphora gileadensis L.	Burseraceae	Wood	Basham	Hot and Dry	Odorant
Commiphora myrrha (Nees) Engl.	Burseraceae	Gum	Mor	Hot and Dry	Anti-halitosis
*Cupressus sempervirens*L.	Cupressaceae	Fruit	Jozosarv	Cold and dry	Odorant
Cydonia oblonga Mill.	Rosaceae	Fruit	Safarjal	Hot and wet	Anti-halitosis; Astringent
Cyperus longus L.	Cyperaceae	Root	Soad	Hot and Dry	Anti-halitosis
Frankenia salina (Molina) I.M.Johnst.	Frankeniaceae	Root	Jarmilak	-	Anti-dental infection
Iris germanica L.	Iridaceae	Root	Irsa	Hot and dry	Anti-halitosis
Mangifera indica L.	Anacardiaceae	Wood	Anbaj	Hot and dry	Anti-halitosis
*Myristica fragrans*Houtt.	Myristicaceae	Fruit	Jozboa	Hot and Dry	Anti-halitosis
Nardostachys jatamansi (D.Don) DC	Caprifoliaceae	Root	Sonbol	Hot and Dry	Odorant
Ocimum africanum Lour.	Lamiaceae	Leaf	Franjmoshk	Hot and Dry	Odorant
Piper betle L.	Piperaceae	Leaf	Tanbol	Moderate in hot and dry	Odorant
Piper cubeba Bojer	Piperaceae	Fruit	Kababeh	Hot and Dry	Odorant
Pistacia atlantica var. *mutica*	Anacardiaceae	Gum	Samghe- Bane	Hot and dry	Odorant; Astringent
Pistacia lentiscus L.	Anacardiaceae	Gum	Mastaki	Hot and dry	Odorant; Astringent
Pistacia vera L.	Anacardiaceae	Fruit	Fostogh	Hot and dry	Odorant
Prunus armeniaca L.	Rosaceae	Fruit	Meshmesh	Cold and wet	Anti-halitosis
Punica granatum var. *pleniflora* Hayne.	Punicaceae	Flower	Golnar	Cold and dry	Anti-halitosis; Astringent
Rubus fruticosus G.N.Jones	Rosaceae	Leaf	Tameshk	Cold and dry	Anti-halitosis
Rumex hydrolapathum Huds.	Polygonaceae	Whole	Bartanighi	Hot and Dry	Anti-halitosis
Symplocos racemosa Roxb.	Symplocaceae	Bark	Armal	Hot and Dry	Anti-halitosis
Syzygium aromaticum (L.) Merr. &L.M.Perry	Myrtaceae	Bud	Gharanfol	Hot and Dry	Anti-halitosis
Vitis vinifera L.	Vitaceae	Vinegar	Khaal	Cold and dry	Anti-halitosis

There is no reliable anti-halitosis toothpaste in the market. At least toothpaste is suitable for traditional argues' modernization due to ease of use, acceptability, ease of handling, low cost and efficiency to improve individual health status. Furthermore, it is reported that most productive breath and anaerobic microbes gathering is in low oxygen areas and deep oral plaques or tongue papilla, therefore proper brushing and toothpastes should be used for removing food debris and plaque removal.

Here, three herbals were selected for preparing toothpaste from different classes of effectiveness on halitosis i.e. *Syzygium aromaticum* as anti-halitosis, *Pistacia atlantica* var. *mutica* as odorant and anti-halitosis, and *Punica granatum* var. *pleniflora* as astringent besides anti-halitosis. It is reported that eugenol has shown anti-biofilm effect [39]. Anti-gingivitis effects of *Punica granatum* var. *pleniflora* mouthwash have been indicated in a clinical trial [40]. Moreover, anti-dental plaque and subgingival effects of *Pistacia atlantica* var. *mutica* mouthwash have been reported in a randomized trial [41]. Therefore, well controlled toothpaste containing such ingredients would be safe and suitable for considering clinical trials.

Table 2. "Sonoun"s reported as anti-halitosis related agents in traditional compendium manuscripts.

Formulations	Anti-halitosis related properties	Ref.
Anacyclus pyrethrum (L.) Lag. (Asteraceae); *Acorus calamus* L. (Acoraceae); *Punica granatum* L.; *Punica granatum* var. *pleniflora* Hayne. (Punicaceae); *Quercus lusitanica* Lam. (Fagaceae); *Boswellia carteri* Birdw. (Burseraceae); *Rosa damascena* Herrm. (Rosaceae); *Iris germanica* L. (Iridaceae); *Terminalia chebula* Retz. (Combretaceae); *Bambusa arundinacea* Willd.(Poaceae); *Portulaca oleracea* L. (Portulacaceae); *Astragalus tragacantha* L. (Fabaceae); *Coriandrum sativum* L. (Apiaceae); *Lens esculenta* Moench (Fabaceae); *Piper nigrum* L. (Piperaceae); *Pistacia lentiscus* L. (Anacardiaceae); *Aloexylum agallochum* Lour. (Thymelaeaceae); *Laurus camphora* L. (Lauraceae); *Zygophyllum fabago* L. (Zygophyllaceae); *Sepia officinalis* L. (Sepiidae); *Corallium rubraum* (L.) (Coralliidae); *Helix pomatia* L. (Helicida); Cow horn; Starch; NaCl; Margarita; Chinese ceramic	Anti-halitosis; dental and periodontal reinforcement; anti gingival bleeding	[22,23]
Artemisia herba-alb Asso (Asteraceae); *Anacyclus pyrethrum* (L.) Lag. (Asteraceae); *Syzygium aromaticum (*L.) Merr. & L.M.Perry (Myrtaceae); *Rosa damascena* Herrm.(Rosaceae); *Cyperus longus* L. (Cyperaceae); Alum ($KAl(SO_4)_2.12H_2O$)	Odorant; strengthening gingival tissue	[22]
Santalum album L. (Santalaceae); *Rosa damascena* Herrm. (Rosaceae); *Cyperus longus* L. (Cyperaceae); *Tamarix aphyla* (L.) Karesten (Tamaricaceae); *Pistacia lentiscus* L. (Anacardiaceae); *Areca catechu* L. (Arecaceae); *Aloexylum agallochum* Lour. (Thymelaeaceae); *Phyllanthus emblica* L. (Phyllanthaceae); *Syzygium aromaticum* (L.) Merr. & L.M.Perry (Myrtaceae); *Terminalia chebula* Retz. (Combretaceae); *Laurus camphora* L. (Lauraceae); Ramak (a traditional formulation)	Odorant; strengthening periodontal tissue	[22]
Cyperus longus L. (Cyperaceae); *Saussurea costus* (Falc.) Lipsch. (Asteraceae); *Terminalia bellirica* (Gaertn.) Roxb. (Combretaceae); *Aristolochia rotunda* L. (Aristolochiaceae); *Nardostachys jatamansi* (D.Don) DC. (Caprifoliaceae); *Sepia officinalis* L. (Sepiidae); Potash (Ashkhar); Glass (SiO_2); Indian Salt	Odorant; strengthening roots and periodontal tissue	[22]
Hordeum vulgare L. (Poaceae); *Aloexylum agallochum* Lour. (Thymelaeaceae); *Nardostachys jatamansi* (D.Don) DC. (Caprifoliaceae); *Anacyclus pyrethrum* (L.) Lag. (Asteraceae); *Myrtus communis* L. (Myrtaceae); *Zygophyllum fabago* L. (Zygophyllaceae); *Syzygium aromaticum* (L.) Merr. & L.M.Perry (Myrtaceae); *Piper cubeba* Bojer (Piperaceae); *Sepia officinalis* L. (Sepiidae); Chinese ceramic; Alum ($KAl(SO_4)_2.12H_2O$); Potash (Ashkhar); Crystalline salt	Odorant; strengthening roots	[22]
Terminalia chebula Retz. (Combretaceae); *Anacyclus pyrethrum (*L.) Lag. (Asteraceae); *Brassica nigra* (L.) K.Koch (Brassicaceae)	Odorant; strengthening teeth	[22]
Zygophyllum fabago L. (Zygophyllaceae); *Piper longum* L. (Piperaceae); *Elettaria cardamomum* (L.) Maton (Zingiberaceae); *Hordeum vulgare* L. (Poaceae); *Zingiber officinale* Roscoe (Zingiberaceae); *Sepia officinalis* L. (Sepiidae); Burnt Table Salt.	Odorant	[22]
Bambusa bambos (L.) Voss (Poaceae)*; Plantago major* L. (Plantaginaceae) - *Terminalia chebula* Retz. (Combretaceae); *Punica granatum* var. *pleniflora* Hayne. (Punicaceae); *Zygophyllum fabago* L. (Zygophyllaceae); *Olea europaea* L. (Oleaceae); *Knautia arvensis* (L.) Coult.(Caprifoliaceae); Alum ($KAl(SO_4)_2.12H_2O$)	Odorant; strengthening roots	[22]
Areca catechu L.(Arecaceae); *Quercus lusitanica* Lam. (Fagaceae); *Commiphora myrrha* (Nees) Engl.(Burseraceae); *Tamarix gallica* L. (Tamaricaceae); *Zygophyllum fabago* L. (Zygophyllaceae); *Pistacia lentiscus* L. (Anacardiaceae); *Punica granatum var. pleniflora Hayne.* (Punicaceae); *Aristolochia rotunda* L. (Aristolochiaceae); *Iris germanica* L. (Iridaceae); *Boswellia carteri* Birdw. (Burseraceae); *Rhus coriaria* L. (Anacardiaceae); *Cypraea moneta* L. (Vadae) (Cypraeidae); *Sepia officinalis* L. (Sepiidae); *Corallium rubraum* (L). (Coralliidae); Chinese ceramic; Alum ($KAl(SO_4)_2.12H_2O$); Salt	Odorant; strengthening roots and periodontal tissue	[22]
Quercus brantii Lindl. (Fagaceae); *Aloexylum agallochum* Lour. (Thymelaeaceae); *Piper longum* L. (Piperaceae); *Piper nigrum* L. (Piperaceae); *Elettaria cardamomum* (L.) Maton (Zingiberaceae); *Zingiber officinale* Roscoe (Zingiberaceae); *Sepia officinalis* L. (Sepiidae); Burnt Table Salt; Vinegar	Anti-halitosis; Odorant; strengthening roots and periodontal tissue; anti-sialorrhea	[22]
Pistacia lentiscus L. (Anacardiaceae); *Indigofera tinctoria* L.(Papilionaceae); *Zingiber officinale* Roscoe (Zingiberaceae); *Sulfuretum antimoniinativum* (Esmed); *Coriandrum sativum* L. (Apiaceae); *Bunium persicum* (Boiss.) B.Fedtsch. (Apiaceae); *Piper nigrum* L. (Piperaceae); Arabian stone	Odorant	[23]
Cyperus longus L. (Cyperaceae); *Santalum album* L. (Santalaceae); *Rhus coriaria* L. (Anacardiacea); *Punica granatum var. pleniflora Hayne.* (Punicaceae); *Elettaria cardamomum* (L.) Maton (Zingiberaceae); *Colchicum autumnale* L. (Colchicaceae); *Glycyrrhiza glabra* L. (Papilionaceae); *Zingiber zerumbet* (L.) Roscoe ex Sm. (Zingiberaceae); *Anacyclus pyrethrum* (L.) Lag. (Asteraceae); *Aloexylum agallochum* Lour. (Thymelaeaceae); *Pistacia lentiscus* L. (Anacardiaceae); *Rosa damascena* Herrm. (Rosaceae); *Embelica officinalis* Gaertn. (Phyllanthaceae); *Syzygium aromaticum* (L.) Merr. & L.M.Perry (Myrtaceae); *Piper betle* L. (Piperaceae); *Tamarix aphylla* (L.) Karesten (Tamariaceae)	Odorant; strengthening roots	[23]

Table 3. Chemical composition of *Pistacia atlantica* var. *mutica* essential oil

No	Components	%	KI
1	α-Pinene	90	939
2	ß-Pinene	0.307	946
3	Verbenene	0.180	948
4	Sabinene	0.433	967
5	Myrcene	1.147	973
6	ρ-Cymene	0.343	1013
7	Limonene	0.813	1022
8	Pinene oxide	0.216	1067
9	Linalool	0.180	1083
10	Camphenol	0.560	1104
11	*trans*-Pinocarveol	1.42	1123
12	*trans*-Verbenol	2.231	1129
12	ρ-Mentha-1,5-diene-8-ol	1.165	1146
14	ρ-Cymene-8-ol	0.316	1154
15	*trans*-Carveol	0.217	1197

Table 4. Chemical composition of *Syzygium aromaticum* essential oil

No	Component	%	KI
1	Benzyl ethanoate	0.097	1131
2	Ethyl benzoate	0.034	1143
3	Methyl salicylate	0.345	1167
4	Chavicol	0.204	1225
5	Eugenol	29.529	1334
6	Copaene	0.983	1372
7	α-Caryophyllene	48.664	1416
8	Humulene	6.467	1446
9	Naphthalene, 1,2,4a,5,6,8a –hexahydro-4,7-dimethyi-1-(methylethyl)	0.243	1466
10	ß-Selinene	0.082	1476
11	Eugenol acetate	10.516	1486
12	α-Farnesene	1.124	1496
13	γ-Cadinene	0.131	1502
14	δ-Cadinene	0.773	1511
15	2-Cyclopentene-1-one,3-methyl-2-(-2-pentenyl)	0.063	1532
16	Caryophyllene oxide	0.729	1562

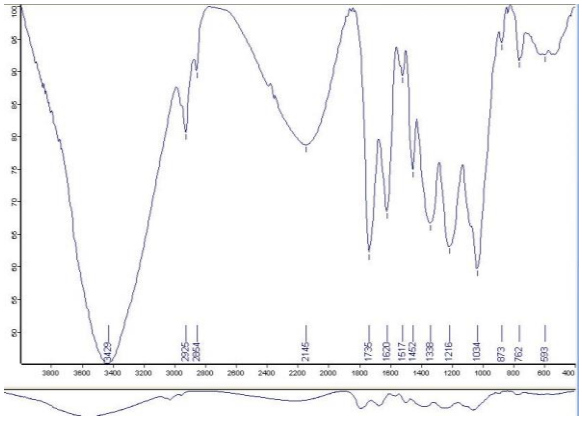

Figure 1. *Punica granatum* var. *pleniflora* IR spectrum

Rational approach to Persian medicine, as a source of thousands years experiences, would be an opportunity for current medical problems like halitosis. Today many clinical trials with traditional products are conducted without sufficient quality control assessments which would comprise of manuscripts' survey, identification and control of selected raw materials, formulation as well as pharmaceutical control of the resulting dosage form. It means cooperation between pharmaceutics, pharmacognosy and traditional pharmacy and traditional medicine should guaranty a product for clinical trial. Such model would be a straight forward rout from traditional medical manuscripts toward clinic.

Table 5. Pharmaceutical tests on anti-halitosis toothpaste and related placebo

QC tests		Test sample	Placebo sample
Squeeze test		Homogenous	Homogenous
Odor test (during 2 months)		No change	No change
Color test (during 2 months)		No change	No change
Leakage test		No leakage	No leakage
Centrifuge test (after 1h)		No phase separation	No phase separation
pH		6.65±0.03	6.60±0.02
during 2 months		No significant changes	No significant changes
Conductivity		236.00 mv±1.21	255.00 mv±0.89
during 2 months		No significant changes	No significant changes
Weight variation		72.72±0.39 g	72.81±0.32 g
Particle size evaluation		8.081±0.38 µm	6.626±0.44 µm
10%		1.90 µm	1.67 µm
50%		10.27 µm	7.68 µm
90%		33.59 µm	22.08 µm
Spreading test (Glass-plate)	50 g	961.62 mm^2	706.50 mm^2
	250 g	1589.62 mm^2	1319.58 mm^2
	500 g	1962.50 mm^2	1962.50 mm^2
	1000 g	2374.63 mm^2	2374.63 mm^2
	2000 g	2920.98 mm^2	2826.00 mm^2
Temperature related stability test		No significant changes	No significant changes
Microbial limit test		No growth	No growth

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Author contributions

Atefeh Arabzadeh prepared data and did research as part of Ph.D. thesis, Amir Azadi and Saeed Daneshamooz supervised pharmaceutical parts and also Amir Azadi supervised writing the pharmaceutical parts of the manuscript, Somayeh Karami did GC analysis, Mostafa Rezaei supervised toothpaste requirements for clinical trials and Abdolali Mohagheghzadeh supervised the whole research and writing the manuscript.

Declaration of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the content of the paper.

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Abbreviations

USP: United States pharmacopeia; GC-MS: Gas chromatography-mass spectrometry; ATR-IR: Attenuated total reflection-*infrared* spectroscopy; LOD: Limit of detection; RI: Retention index; QC: Quality control.