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Rim M. Harfouch *

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Research Article

Formulation and Preparation of a Novel Toothpaste Using the Essential Oil of *Salvia officinalis*

Rim M. Harfouch[1*], Manal Darwish[2], Mustafa Beesh[3], Nour Ibrahem[1], Hiba Dayoub[1], Rulla Zein[1], Soumya Ghosh[4], Shahin Ahamadi[5]

[1]Department of Biochemistry and Microbiology, Faculty of Pharmacy, Alsham private university, Syria.

[2]Department of Pharmacognosy, Faculty of Pharmacy, Al Andalus University, Tartous, Syria.

[3]Department of Pharamaceutical sciences, Faculty of Pharmacy, Al Andalus University, Tartous, Syria.

[4]Department of Genetics, Faculty of Natural and Agricultural Sciences, University of the Free State,

Bloemfontein, South Africa

[5]Faculty of Environmental Health, Zabol University of Medical Sciences, Zabol, Iran

***Corresponding Author:** Rim M. Harfouch, 1Department of Biochemistry and Microbiology, Faculty of Pharmacy, Alsham private university, Syria.

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Abstract

Dalmatian Sage (*Salvia officinalis* L., Lamiaceae) is known in the Middle East for its therapeutic properties, and is widely used in folk medicine, cosmetics, flavoring and preservative for food products. It's essential oil possess antimicrobial, antiviral, antifungal and antioxidant properties so that it is widely used to treat gingivitis and laryngitis. The present study has formulated a semi-solid pharmaceutical form (tooth paste) that contains essential oil extracted from the leaves of common sage and conducted the necessary observations and further control tests, in preface for preparation at the industrial level at the later stage. The Sage leaves were harvested from al-Qadmus area, in Tartous province, Syria, dried and extracted for essential oil by hydro-distillation. The extraction yield was estimated to formulate the toothpaste and observational tests for quality control (sensory tests, the absence of solid and sharp particles, spread ability, pH, moisture content, bacterial tests and stability test on shelf) were performed. Our formulated toothpaste characteristics were identical to the commercial toothpaste specifications from the following respect: Sensory tests, spread ability, pH, moisture content and stability on shelf, also the microbial parameters values were within permissible limits. Our study suggest using this formula to prepare a tooth paste with sage essential oil and perform further studies to evaluate its efficacy on dental cavities and gingivitis.

Key words: tooth paste; formulation; quality control; sage essential oil

Introduction

Common sage also known as *Salvia officinalis*- belongs to the Lamiaceae family, tribe Mentheae, subfamily Nepetoideae, and genus *Salvia*. Lamiaceae family is one of the largest and most famous families of the flowering plants, which is a small tree that does not exceed the height of 80-50 cm. It has oval long leaves with a whitish green color, thick, rough-textured and finely sharp edges [1, 2]. Salvia is derived from the Latin word 'salvaare' meaning 'to cure' [3] and is one of the most popular aromatic plants and also well known for its antimicrobial properties [4].

In the Middle East, S. officinalis L is known as therapeutic agent, and is widely used in folk medicine, cosmetics, and flavoring of food products [5]. Their essential oils possess antimicrobial properties [6] such as antiviral [7], antifungal [8], bactericidal [9]. Noticeably, the essential oils from this plant also possess antioxidant [6], hepatoprotective and anti-carcinogenic properties [10, 11].These properties of S. officinalis L. are attributed to its chemical composition that contains a wide range of phytochemicals such as phenolic compounds [coumarins, flavonoids, tannins], polyacetylenes, steroids, terpenes/terpenoids [monoterpenoids, diterpenoids, triterpenoids, sesquiterpenoids], waxes, alkaloids, carbohydrate, fatty acids, glyosidic derivatives [e.g., cardiac glycosides, flavonoid glycosides, saponins] and essential oils [12]. However, a wide range of phytochemicals, such as thujone, pinene ledene, camphor, elemene, humulene, cineole, caryophyllene and borneol, are derived mostly from the essential oils of S. officinalis L.[12]. The popular

use of this plantis gingivitis, in addition to laryngitis, abdominal cramps, and dyspepsia [13]. Moreover, as natural disinfectant S. officinalis essential oils could play a vital role in preventing the spread of pathogenic microorganisms and could effectively solve certain environmental pollutions connected with the use of synthetic chemicals [14].

At the other end oral hygiene is the practice of keeping the mouth clean and is a means of preventing dental caries, gingivitis, periodontal disease, bad breath, and other dental disorders [15]. Regular cleanings, usually done by dentists and dental hygienists, remove tartar that may develop even with regular brushing and flossing. Professional cleaning may also include tooth scaling, which uses various instruments to remove deposits from teeth [16].

Paste is a semi-solid pharmaceutical form with a viscous sticky texture which has many therapeutic external and internal uses [17]. Toothpaste is a paste or gel dentifrice used, with a toothbrush, to clean and maintain the aesthetics and health of teeth [18]. Toothpastes are categorized into five classes such as Children's toothpaste, fluoride toothpaste, toothpaste for control of lime, toothpaste for sensitive teeth and bleaching toothpaste [16]. However, there are many additives that enter the toothpaste composition for example treatment materials, abrasive materials, foam materials, detergents, moisturizers, bonding agents, preservatives, sweeteners and flavouring agents [19].This study has focused to formulate a tooth paste from the laboratory generated sage oil and to compare and contrast the commercially available ones in terms of sensory tests, spread ability, pH, moisture content, stability on shelf and anti-microbial parameters. To the best of our knowledge, this formulation was designed for the first time of its kind at Al-Andalus University for Medical Sciences, Syria.

Materials and methods

Plant material

This study was conducted at the Department of Microbiology, Faculty of Pharmacy, Al Andalus University, Tartus, Syria. Common sage leaves were harvested from Al Qadamus area in Tartous cityand were dried by shade at room temperature 20-25 °C for one week.

Essential oil extraction

Thirty grams of dried sage leaves and fresh sage leaves samples were mixed with 200 mL of distilled water and were subjected to hydro-distillation in Clevenger-type apparatus for 4 hours at room temperature. The total oil yield was estimated and the pure oil was stored in sealed glass vials at 4-5 °C until formulation [10].

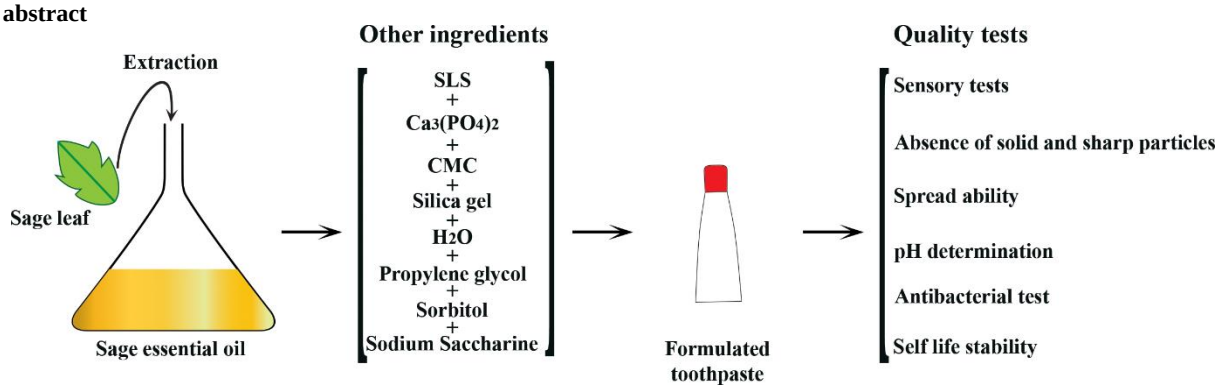
Toothpaste formulation

The following composition was used in order to prepare the toothpaste with sage essential oil (Table 1). Calcium di-phosphate (40 g), Sodium Lauryl sulfate S.L.S (1g) and carboxymethyl cellulose (2 g) were macerated in mortar while silica gel (1.5 g), propylene glycol (30 g), sorbitol (0.5 g) and sodium saccharine (0.5 g) were dissolved in water. The macerated powders were mixed with the prepared solution on a glass surface, followed by the addition of sage essential oil (0.1mL) to form paste (Table 1).

Ingredients	Quantities
Calcium di-phosphate	40 g
Sodium Lauryl sulfate S.L.S	1 g
Water	24g
Silica gel	1.5g
Carboxymethyl cellulose	2g
Propylene glycol	30g
Sorbitol	0.5g
Sodium Saccharine	0.5 g
Sage essential oil	0.1 mL

Table 1: *Composition of the toothpaste*

Graphical abstract



Quality tests for toothpaste formulation:

1. Sensory tests:

We evaluated the prepared tooth paste depending on a group of ten volunteers in terms of color, smell, taste and structure. All the participants gave their feedback as following: The color is white to grey white, the structure is soft, the smell and taste are aromatic and the cleaning ability is good.

2. The Absence of solid and sharp particles:

Three gram of toothpaste was spread on a butter paper, pressed in full width by fingers to feel any sharp or solid particles.

3. Spread ability:

Two gram of each of the formulated paste was placed on the center of a glass plate and another glass plate was superimposed from the top to sandwich the tooth paste between the 2 plates. The two glasses were left for 5 minutes to expel the air between the plates in order to form an uniform film of the paste between the glass plates. Following the drying, the diameter of the toothpaste was measured in centimeters[20].

4. Determination of pH:

I.One gram of toothpaste was taken in 100 ml beaker, shacked well until it formed a consistent suspension. The acidity in suspense was measured within five min [21].The pH of formulations was measured by the methods described in ISO3996 (grade 3) [22]**.**

II.Moisture content:

Five gram of toothpaste was dried at 105 ° C in the oven and cooled. Both pre- and post- weight of the toothpaste was estimated in order to measure the moisture content of the tooth paste.

$$\text{Humidity\%} = \frac{\text{original sample weight} - \text{dried sample weight}}{\text{original sample weight}} \text{x100}$$

5. Anti-bacterial tests:

The antibacterial test of the toothpaste was conducted on three different media namely Muller Hinton Agar (MH), Mannitol Salt Agar (MSA) and Eosin Methylene blue (EMB). The prepared tooth paste (0.1 g) was placed at the middle of a sterile petri plates followed by incorporation of sterile indicated media. The plate were incubated at 37°C for 72 hours initially and after that extended to 5 days.

6. Stability test on shelf:

The tooth paste was left on the shelf for a full month to observe any obvious changes in color, smell and texture.

Results

Essential oil yield:

The extracted essential oil yield was estimated as 0.8 mL per 100 grams of dried sageleaves, which is higher than the yield of essential oil of freshly harvested sage leaves (0.2 mL per 100 grams of leaves).

Quality tests for toothpaste formulation

I.Absence of solid and sharp particles:

. The results of absence of solid and sharp particles are indicated in table 2

Test results show that patch A has no scratches, while batch B showed presence of solid and sharp particles that need more smoothing.

Paste batch A	Paste batch B	The control commercial paste
No scratches	There are simple scratches that need more smoothing	No scratches

Table 2: *Existence of solid particles in prepared toothpaste in comparison to control toothpaste*

II.Spread ability

The results of determining the propagation limits **(Table 3).**

The paste	Diameter spread in centimeters
Paste meal A	55
The standard paste	55

III.Determination of pH:

The results showed both batches are slightly above neutral pH **(Table 4).**

The paste	pH
Paste batch A	8.57
Paste batch B	8.03

Table 4: The final pH for both batches was determined using pH meter:

IV.Moisture content:

Moisture content for paste A is11.37%, and moisture content for paste Bis 10.14%. While the moisture content of a commercial tooth paste is 15.04%. As we can see that batch A is more humid and less harsh than batch B, and both are less than commercial paste **(Table 5).**

Weight	Paste A (gr)	Paste B (gr)	Commercial paste (g)
Weight before drying	88.16	58.16	77.12
Weight after drying	78.13	52.26	65.52

Table 5: Humidity% = original sample weight - dry sample weight / original sample weight * 100

Table 5: Test results determine wet content

V.Bacterial tests

The results exhibited a limited number of colonies on general medium Muller Hinton, but showed no growth on two specific media (Mannitol salt agar for Staphylococci and Eosin methylene blue for *Enterobacteriaceae*) **(Table 6).** Noticeably, the microbial parameters values were observed within the permissible limits by World Health Organization and SyrianstandardsS.N.S:45/2007[23].

Medium	Growth after 72 hours	Growth after 5 days		
	Paste A	Paste B	Paste A	Paste B

Muller Hinton	2 colonies	3 colonies	6 Colonies	8 Colonies
Mannitol salt agar	No growth	No growth	No growth	No growth
Eosin Methylen blue	No growth	No growth	No growth	No growth

Table 6: Antibacterial activities depicted by Paste A and Paste B

VI.Stability test on shelf

We left the paste A and paste B on the shelf for a full month. After this period, the color and shape of the paste was not changed and the texture was not separated. We can say that the prepared toothpaste is stable in the conditions of conservation.

Discussion

The antimicrobial effects of medicinal plant essential oils and extracts corroborate it's use in many fields such as medicine, food industry, and pharmacy [24, 25]. Medicinal plants have constantly been used as sources for finding new drugs [24]. Numerous investigations have been conducted about the application of essential oils as antimicrobial agents and shown to increase the safety and shelf life of food products besides being used as a flavoring agent in foods [26].

Saliva officianalis is a plant that is native to the Mediterranean region, though it has been naturalized in many places throughout the world. It's essential oil contains cineole, borneol and thojone [24, 26]. Moreover, the sage leaves contain tannic acid, oleic acid, ursonic acid, carsonol, caffeic acid, flavones and estrogenic substances [23, 24, 26]. The current study, has used the Sage oil as one of the major constituents for the tooth paste formulation. Sage essential oil has proven as an antibacterial agent against oral bacteria that causes dental caries [24]. For example a previous study [9] from Southern Brazil on the antibacterial activities of the essential oils from Salvia officinalis L. and Salvia triloba L., exhibited bacteriostatic and bactericidal activities against Bacillus cereus, Bacillus megatherium, Bacillus subtilis, Aeromonas hydrophila, Aeromonas sobria, and Klebsiella oxytoca. Furthermore, the GC-MS analysis of these essential oils from S. officinalis and S. trilobarevealed α-thujone, 1,8-cineole, camphor, borneol, and β-pinene, and α-thujone, 1,8-cineole, camphor, and β-caryophyllene respectively that have also been observed to possess antimicrobial properties [25]. Intriguingly, the essential oil of S. triloba has further shown to inhibitthe growth of Staphylococcus aureusand Aeromonas hydrophilaeven at a concentration of 0.05 mg/mL [26]. Moreover, studies have also shown that there are preferences towards the antibacterial activities between the Gram positive and negative bacteria of these essential oils. For example, Rosmarinus officinalis essential oil has shown to exhibit higher antibacterial activity against Gram positive bacteria than against Gram-negative bacteria Hussain A. et al.[4, 27], and the chief compounds with antimicrobial effects are determined as 1,8-cineole, α-pinene, and camphor [28].

The current formulation also exhibited a higher pH [>8] which is in accordance to a previous study that showed herbal tooth pastes displace the pH of the saliva into the alkaline range which in turn reduces the probability of dental caries incidence [29]. Furthermore, the present toothpaste combination is devoid of chlorhexidine, which is advantageous since chlorhexidine causes tooth staining when used regularly[30]. Several studies have also proven the medicinal values of herbal tooth pastes, hence medicated herbal toothpaste can be safely used to control plaque and gingivitis [27, 31, 32]. For example, a previous study [33] conducted a study on the efficacy of an herbal based toothpaste containing sage in the control of plaque and gingivitis where it has been observed that there is statistically significant reductions in the gingival index and the plaque index scores within the test group. However, there were no statistically significant differences between the test and the control groups. The salivary pH changes were not statistically significant in the test group but were displaced more toward the acidic range in the control group [32].

In our study, formulated tooth paste properties were identical to the specifications of commercial tooth paste in following respects such as sensory tests, spread ability, pH, moisture content and stability on shelf, with better texture of paste A than paste B. In addition, the microbial parameters values of formulated toothpaste were within permissible limits. Although the formulations undoubtedly witnessed beneficial, further antimicrobial tests are required to conduct in order to examine the paste activity against dental cavities causing bacteria such as *Streptococcus mutans* and *Lactoacilli* [30-33].

Conclusion

Many studies have been conducted to evaluate natural treatments for bacterial infections. We demonstrate here the antibacterial efficacy of essential oils extracted from a medicinal plants. *Salvia officinalis* has aromatic, carminative, spasmolytic, antiseptic, astringent, antihydrotic actions. The thujone in the volatile oil has an antiseptic and antibiotic action and, when taken as a mouthwash, *Salvia* deals effectively with throat infections, dental abscesses, infected gums, and mouth ulcers. The phenolic acids in *Salvia* are particularly potent against *S. aureus*. *Salvia* also has an astringent action due to its relatively high tannin content. All these evidence the importance of *Salvia officinalis* in medical as well as dental treatments. Future study, should emphasize precisely in deciphering the active constituents from the essential oil of this *Salvia* from the Syrian origin.

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