Original Article

Evaluation of Chemical, Biochemical and Anti-Microbial Effects of *Salvadora persica* and *Moringa oleifera* Extract to Produce Organic Disinfectant Products

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ABSTRACT

Oral hygiene is one of the most influential and important issues in people's health. People have been using herbal components to maintain their oral hygiene for centuries. Oral cancer develops in the oral cavity, and its origin always lies in the growth of malignant epithelial tissue cells. Due to the spread of this cancer in Iran, we intend to measure the antibacterial effects of the combination of *Salvadora persica* and *Moringa oleifera* extracts. Cariogenic bacteria are one leading cause of oral cancer. We used this extract in mouthwash, toothpaste, and chewing gum, and we expect that it would reduce cell proliferation and be used in prevention and treatment. The new organic mouthwash, chewing gum, and toothpaste were designed and prepared using *M. oleifera* oil, *S. persica*, *M. oleifera* extract, the powder of *S. persica* wood, and *M. oleifera* leaves. With the use of herbal compounds in the preparation of these products, the quantity of essential chemical ingredients in the prepared samples was decreased. We examined the quality and stability of mouthwash, toothpaste, and chewing gum that indicated the standard level of each substance. Furthermore, we evaluated the antibacterial effects of our products, which indicated that our products can significantly reduce the total bacterial count. For the first time, a combination of *S. persica* and *M. oleifera* extract replaced chemicals in mouthwash, toothpaste, and chewing gum. Natural herbal ingredients with antimicrobial activity are effective in maintaining low bacterial counts in the mouth, and as a result, improving oral hygiene and health.

Keywords: *Salvadora persica*, *Moringa oleifera*, Herbal products, Anti-microbial

1. Introduction

Oral health can have a huge impact on the quality of life and happiness, whereas poor oral health leads to several chronic and systemic diseases. With the growing prevalence of oral diseases, the global population's need for diverse prevention, safe, and effective treatment methods increased. Toothpaste and mouthwashes are used to keep the mouth healthy and clean; chewing gums are also used for the regular removal of dental plaque and food deposits (1).

Furthermore, toothpaste, mouthwash, and chewing gums that are produced by organic materials, are not only used for regular cleaning but also, because of their effective organic materials, they have a positive effect as an anti-plaque, anti-gingivitis, and anti-cariogenic properties, promote gingival wound healing, have whitening properties, can preserve orthodontic chain, and are biocompatible with oral cells (2).

Salvadora persica and *Moringa Oleifera* are two organic compounds we used for this purpose. The results of studies have shown various therapeutic properties for *S. Persica*, including antitumor, antibacterial, improving oral health (antiplaque and reducing gingivitis), enzyme inhibitor, antioxidant, anti-inflammatory, anticonvulsants, anti-osteoporosis, and hypoglemic and antiulcer activity (3). Aqueous extract of *S. persica* has positive impacts on oral cancer cells, and apoptosis has been observed in cancer cell lines (4). Ibrahim, El-Gengaihi (5) investigated the effect of *S. persica* extract on several human cancer cell lines. They reported that crude extract of *S. persica* had cytotoxic activity against different cancer cell lines, such as HCT116 (human colon adenocarcinoma), MCF7 (human breast adenocarcinoma), HEPG2 (human hepatocellular carcinoma), and A549 (lung carcinoma) (5). *Aggregatibacter actinomycetemcomitans* is a gram-negative bacterium that produces leukotoxin and attacks periodontal tissues. The secreted toxin can protect bacteria from dying through immune responses. Moreover, there is another bacterium called *Porphyromonas gingivalis* that causes tissue colonization and destruction as well as host defense. The findings of studies demonstrate that *S. persica* has antimicrobial activity against these bacteria (6). It has been reported that an extract of *S. persica* has beneficial or auxiliary antibacterial compounds and does not produce poisonous compounds; therefore, it improves oral health and cure infections (7). The antimicrobial activity of miswak is mainly against oral pathogens, such as *Streptococcus mutans*, *Tannerella forsythia*, and *Treponema denticola* (8). The employment of *S. persica* mouthwash resulted in a lower carriage amount of cariogenic bacteria (5). The presence of tannin and its derivatives in the tooth structure of the toothbrush inhibits the enzyme glucosyltransferase, thus preventing plaque formation and gingivitis (9). Gazi, Davies (10) reported that using *S. persica* led to the presence of calcium chloride and phosphate in saliva. However, since after 4 h, the amount of these compounds decreases, frequent use of Miswaks is recommended. The results showed that Miswak released substances that contributed to oral health (10). The findings of a study by Mohamed, Almulaiky (11) were indicative of the presence of alpha-amylase in Miswak extract. This enzyme digests glycogen and starch. It has been reported that the enzyme inhibitory effect of this plant, inactivates viruses. Besides, Miswak can inhibit the peptidase and protease of bacteria (12), the virulence of which are associated with periodontal disease (3). Free radicals in cells can destroy macromolecules, including lipids, nucleic acids, proteins, and carbohydrates. Therefore, free radicals are involved in causing numerous diseases, such as diabetes, cancer, and cardiovascular disease. There are two types of antioxidants; the first category is produced in the body, while the second one is received naturally from food (13). A balance between free radicals and antioxidants is essential for normal body function (14). Al-Dabbagh, Elhaty (15) reported that the flavonoid and phenolic compounds in the extract of *S. persica* had antioxidant activity. They also measured the anti-proliferative and anti-cancer effects of this extract on different cell lines (15). Research by Ibrahim, El-Gengaihi (16) has shown that

Miswak extract has a high potential for anti-inflammatory activity. This extract can significantly reduce the secretion of inflammatory mediators, such as tumor necrosis factor-α (TNF-α), interleukin-6, interleukin-1β (IL-1β), and transforming growth factor-β1 (16). Toothbrush extract increases the activity of sodium pentobarbital. Increasing this substance enhances the improvement of sleep duration and reduces induction time. Besides, it has a protective effect against pentylenetetrazole. As a result, it increases the latency period (17). Research by Edge and Truscott (18) indicated that *S. persica* extract has an antiulcer effect. This property is because of its compounds, such as lycopene, α-Linolenic acid, oleic acid, lycoxanthin, and retinoic acid. The literature shows that the expression of proinflammatory cytokine genes, such as TNF-α and IL-1β, is reduced by *S. persica* extract. TNF-α expression promotes the release of free radicals and destroys cell membranes, resulting in tissue damage. Consequently, the extract of this plant reduces the products of these genes and heals wounds faster (18). Similar results have been reported by Yang, Yin (19).

Research has shown that the leaves of *Moringa oleifera* are rich in compounds, such as β-carotene, vitamin C, calcium, potassium, and protein. Various types of antioxidants have also been proven, including flavonoids, kaempferol, caffeoylquinic acid, ascorbic acid, quercetin, carotenoids, zeatin**,** and phenolics. *Moringa oleifera* contains simple sugar rhamnose (20). Numerous uses have been described for *M. oleifera*, among which one can mention the treatment of inflammation and dental caries; antimicrobial, anti-cancer, and anti-tumor activity; and anti-ulcer, anti-bacterial, anti-fungal, antibiotic, and antioxidant effects. The results of studies have also revealed that *M. oleifera* is highly effective in absorbing heavy metals, such as cadmium (21).

The current study aimed to assess the effect of adding the essence of *S. persica* and *M. oleifera* for oral health maintenance. So far, no study has used *S. persica* and *M. Oleifera* in combination with each other for improving oral health and the therapeutic properties of toothpaste, mouthwash, and chewing gum.

2. Materials and Methods

2.1. Preparation of Products

2.1.1. Preparation of *Salvadora persica* Wood Extract

Salvadora persica woods were collected from the Makoran area, Chabahar, Iran, washed with distilled water, dried in an artificial environment at low temperature (50-60 °C), finely powdered with an electric grinder, and used for extraction procedure utilizing various solvents. A percolation technique was used to obtain the extract of *S. persica* wood. In this method, a particular container (cylindric percolator) was used, and the flow rate of the extract could be well-adjusted using a suitable container valve. The sample was moistened with 30% of the desired solvent and kept for 2 h before entering the percolator. This prevented the plant contents inside the percolator from drying out and splitting. In addition, it created an excess volume in the mass containing the bioactive compounds. The moistened sample was then injected into the percolator through special sieves. The cell was subjected to an initial soaking process, which caused it to effectively absorb in the extraction solvent.. The plant material was inserted uniformly into the percolator and gentle pressure was applied to the plant mass. The surface of the wet sample was covered with filter paper and a few glass cylinders prevented the movement of plant powders on it. There was a cotton layer at the bottom of the percolator and the extract came out from the percolator after filtration on cotton, which was then collected. We added the rest of the solvent to the plant mass, while the percolator valve was open and the air inside the percolator was completely removed. As soon as the first drops of the extract started to trickle out, we closed the valve and continued the percolation process for 24 to 48 h while the solvent was completely covered on the plant mass.

Followingly, as the solvent entered regularly from above, the extract drops came out from the percolator. The extraction rate of the extract was adjusted between 4 to 6 drops per minute for every 100 g of plant powder being extracted. It was observed that the initial extracts that dripped out of the percolator contained high concentrations of bioactive compounds of plant and were thicker, which became thinner gradually.

2.1.2. Preparation of *Moringa oleifera* Extract

The Moringa plant was collected from the research farm of the medicinal plants, Taftan, Khash, Iran, and air dried in the shade. An aqueous method was employed for the preparation of the extract of this plant. The aqueous extract was prepared from the dried plant and used immediately. For extraction, 1 g of dried plant was mixed with 10 ml of distilled water in a round bottom flask. The extract should be prepared in the ratio mentioned in the relevant drug references.

2.1.3. Preparation of Organic Mouthwash

Specific amounts of disodium phosphate, sodium benzoate, calcium disodium EDTA, and xylitol were dissolved in distilled water and then moringa and *S. persica* wood extracts were added to the solution. The solution was homogenized by using a high-speed homogenizer (IKA_T25 digital ULTRA-TURRAX homogenizer equipped with a dispersing element, model S25N-25G, IKA Co. Germany) for 2 h.

2.1.4. Preparation of Organic Chewing Gum

Each ingredient was weighed accurately. The gum base was melted in a porcelain dish at about 30 ℃ to 40 ℃ in a water bath and followed by adding distilled water, xylitol, powder of *S. persica* wood, moringa leaves powder, and moringa oil, one at a time, and then mixed thoroughly with continuous stirring. The mint flavor was added at the end of the mixing. After 30 min of stirring, the mixture was allowed to cool.

2.1.5. Preparation of Organic Toothpaste

The organic toothpaste was formulated by preparing a toothpaste containing no agglomerates. Trisodium phosphate, hydrated silica, sorbitol, sodium mono fluorophosphate, potassium citrate, sodium dodecyl sulfate, and xylitol were added into the beaker containing a certain amount of distilled water while the solution was being homogenized in a high-speed homogenizer (IKA_T25 digital ULTRA-TURRAX homogenizer equipped with a dispersing element, model S25N-25G, IKA Co. Germany). Afterward, *S. persica* wood extract, powder of *S. persica* wood, moringa oil, moringa powder, and a gelling agent sodium carboxymethyl cellulose were added, one by one. In the end, a desired flavor was added, and the sample was homogenized for 2 h; finally, the toothpaste was deaerated and tubed.

2.2. Qualitative Test for Chewing Gum

2.2.1. Moisture Measurement

First, the porcelain dish was heated at the temperature of 100-150 °C, then 5 g of crushed sample was transferred to the dish. It is vital to weigh both the empty dish (w) and the sample-containing dish (w_1). Subsequently, after being heated until reaching a constant weight, the sample was weighed once more (w_2). Using the following formula, the amount of moisture was calculated:

$$\frac{w_1 - w_2}{w_1 - w} \times 100 = Moisture\ Weight\ Precent$$

2.2.2. Ash Measurement

Two grams of sample were added to the porcelain dish and weighed together (w_1). The sample was then heated until it was totally burnt. The sample-containing dish was placed in a furnace at 500-550 °C to obtain white ash. After cooling, the dish and ash were weighted (w_2), and we used the following formula to calculate the percentage of ash weight (w=empty porcelain dish weight):

$$\frac{w_2 - w}{w_1 - w} \times 100 = Ash\ Weight\ Precent$$

2.2.3. Acid Insoluble Ash Level

After turning chewing gum into ashes (w), 25 ml of dilute hydrochloric acid was added and put on the heat for 10-15 min. The contents passed through filter paper. Finally, the insoluble content was dried and weighed (w_2) (w_1=empty dish).

$$\frac{w_2 - w_1}{w} \times 100 = Acid\ insoluble\ Ash\ Weight$$

2.2.4. Heavy Metal Assessment

Concentrated nitric acid and oxygenated water were added to 1 g of gum and were heated for 1 h until completely dissolved in the solvent. In the next stage, by adding distilled water, the volume was increased to 10 ml. The number of heavy metals, such as Pb, As, and Cd, were measured using a spectrophotometer (v_s: sample volume; c_m: metal concentration; w_s: sample weight).

$$\frac{v_{s(ml)} \times c_{m\,(g/lit)}}{w_{s(mg)} \times 100}$$

2.3. Microbial Count Assay for Mouth-Wash and Toothpaste

Health products, such as toothpaste and mouthwash, need to be evaluated for a variety of microbes, including aerobic bacteria, fungi, yeast, and other microbes. For this reason, we must identify microbes that are capable of fermenting lactose at 37 °C. The studied samples were diluted according to the national standard number 356 of Iran. One ml of the sample was then transferred to the culture medium Lauryl sulfate tryptose broth and placed at 37 °C for 24 to 48 h. Finally, the number of colonies was studied using a colony counter machine. The results of counting different microbes in the mentioned products are reported in table 1.

2.4. Anti-Bacterial Activity of Products

To measure the antibacterial activity of the products, it was necessary to measure their effect on four strains of *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus,* and *Enterococcus hira*.

A mixture of the suspension of the bacterial test solution was added to the samples of the diluted product. This mixture was kept at 20 °C for 15 min. At the end of the contact period, a portion of the mixture was removed and its bactericidal activity was immediately measured. In this way, the number of live bacteria in each sample was determined and the reduction was calculated. The condition of performing an anti-bacterial activity is reported in table 2, and the results of each product and its impact on bacteria growth are shown in table 3.

Tabe 1. Result of the microbial count assay

Products	Type of analysis	The result of the analysis	Standard allowable limit
Mouthwash Toothpaste	Enumeration of mesophilic aerobic bacteria (cfu/gr)	1.4×10 <10	100 5×10^2
Mouthwash Toothpaste	Fungal mold (cfu/gr)	<10 <10	--- <10
Mouthwash Toothpaste	Yeast (cfu/gr)	1.2×10 <10	--- <10
Mouthwash Toothpaste	*Escherichia coli* (gr)	Negative Negative	Negative
Mouthwash Toothpaste	*Pseudomonas aeruginosa* (gr)	Negative Negative	Negative
Mouthwash Toothpaste	*Staphylococcus aureus* coagulase-positive (gr)	Negative Negative	Negative
Mouthwash Toothpaste	*Candida albicans* (gr)	Negative Negative	Negative

Tabe 2. Antibacterial activity under normal conditions of use

Storage conditions	Consumable diluent	Test and validation method	Neutralizer	Test temperature	Interfering material	Encounter Time
Normal	Tryptone sodium chloride	Dilution - Neutralization	Freshly diluted egg yolk up to 5%	37 ±1 degree Celsius	Cattle albumin in a ratio of 3 grams per liter	1 minute

Table ۳. Results of anti-bacterial test

Products	Microorganism tested	Reduction rate after exposure to antibacterial agents	Reduction limit
Mouthwash	*Pseudomonas aerugi nosa*	Reduce at least 10^5 bacteria	Reduce at least 10^5 bacteria
Toothpaste		Further reduction of 10^5 bacteria	Further reduction of 10^5 bacteria
Chewing gum		Further reduction of 10^5 bacteria	Further reduction of 10^5 bacteria
Mouthwash	*Escherichia coli*	Reduce at least 10^5 bacteria	Reduce at least 10^5 bacteria
Toothpaste		Further reduction of 10^5 bacteria	Further reduction of 10^5 bacteria
Chewing gum		Further reduction of 10^5 bacteria	Further reduction of 10^5 bacteria
Mouthwash	*Staphylococcus aureus*	Reduce at least 10^5 bacteria	Reduce at least 10^5 bacteria
Toothpaste		Further reduction of 10^5 bacteria	Further reduction of 10^5 bacteria
Chewing gum		Further reduction of 10^5 bacteria	Further reduction of 10^5 bacteria
Mouthwash	*Enterococcus hira*	Reduce at least 10^5 bacteria	Reduce at least 10^5 bacteria
Toothpaste		Further reduction of 10^5 bacteria	Further reduction of 10^5 bacteria
Chewing gum		Further reduction of 10^5 bacteria	Further reduction of 10^5 bacteria

3. Results

To confirm the quality and health of the manufactured chewing gum, mouthwash, and toothpaste, different tests were performed to determine the physicochemical, bacterial count, and antibacterial effect.

3.1. Physicochemical (qualitative) Evaluation of Chewing Gum

We performed this test to measure the chemical compounds of chewing gum. This test indicated the standard level of each substance (Table 4). The standard level of each substance is reported in table 4 as well.

Table 4. Result of the physicochemical evaluation of the chewing gum

Description of test	Result of test	Unite	Standard allowable limit
Appearance	Solid–Dark green	---	---
Moisture	3.51	%	Maximum 4
Total ash	0.400	%	Maximum 1
Acid insoluble ash	0.0800	%	Maximum 0.5
Total gum	27.42	%	At least 25
Total sugar	13.248	%	4-20
Pb	0.09	ppm	< 0.1
As	0.014	ppm	< 0.1
Cd	0.001	ppm	< 0.1

3.2. Microbial Count Assay

The total number of microbes is one of the important indicators of hygiene management. This test is performed to determine the health of the products and showed how many microbes were present in them. In table 1, we reported the result of the assay. Just two items are included in these results: mouthwash and toothpaste.

3.3. Anti-Bacterial Assay

In this assay, the anti-bacterial properties of mouthwash were tested. This test was performed under the conditions listed in table 1. Four microorganisms were used to confirm this test, including *P. aeruginosa*, *E. coli*, *S. aureus*, and *E hira*. table 2 presents the test results. This test was carried out for all three items. How to conduct an antibiotic test is shown in table 2 and the results are presented in table 3. Output conditions for all three items were the same.

4. Discussion

Nowadays, natural herbal ingredients with antimicrobial activity need to be identified as a useful alternative way to replace chemical compounds. This study focused on the advantages of using *S. persica* and *M. oleifera* extracts instead of chemical compounds in mouthwash, toothpaste, and chewing gum. The main aim of designing these three products was to improve oral hygiene.

As we mentioned before, biological tests, including physicochemical, microbial count assay, and anti-bacterial, were examined for products. In fact, physicochemical and microbial count assays are both quality tests. Physicochemical evaluation which was done only for chewing gum (Table 4) indicated that almost all the items were in the standard range. The number of heavy metals (Pb, As, and Cd) has been acceptable too. Microbial counting included only mouthwash and toothpaste information. As we can see in table 1 these two products had appropriate ranges, and the data showed the healthy quality of mouthwash and toothpaste. All of the information from the results confirmed that the designed herbal products were safe for public use.

Table 3 presents the data on the anti-microbial activity of products to examine their pharmacological properties. A 2005 research by Aas, Paster (22) found that cavity was a significant sign of bacteria. Medicinal plant extracts and herbal phytocompounds demonstrated remarkable anti-microbial effects with low toxicity, and *S. persica* is not an exception. Indeed, *S. persica* extract has been used for commercial purposes, such as toothpaste, in different countries. The

pharmacological features of *S. persica* is an old practice, and this herb contains several biological constituents that would be effective against cavity (23).

In a study carried out by Dahot (24), it was revealed that different fractions of *M. oleifera* could have an anti-microbial effect. Various types of microbes, such as bacteria (e.g., *E. coli*, *Klebsiella aerogenes*, and *Bacillus subtillis*) and fungi (*Aspergillus fumigatus*, *Aspergillus niger*, and *Penicillium expansum*) lost their ability of growth when they were exposed to the aqueous extract of *M. oleifera* leaves (24). However, the results of research by Saadabi and Zaid (25) showed that the methanol extract of *M. oleifera* seeds lacked the quality to reduce the inhibition zone of *E. coli* significantly. Nevertheless, the aqueous extract was superior in the same case for suppressing the bacteria growth (25).

Numerous studies have analyzed different aspects of these two medicinal plants separately. These novel products might have other positive effects on oral cavity hygiene, such as anti-cancer, antiulcer, anticonvulsant, anti-inflammatory, antioxidant, and sedative. Several studies have shown that both plants have an anti-proliferative effect on cancer cell lines (26). It is suggested that more examinations be performed to investigate different aspects in the future. Due to the properties of the products, they can be effective in various pharmacological aspects. It is predicted that these products will have preventive and therapeutic properties against various diseases related to the oral cavity.

Authors' Contribution

Study concept and design: D. M. K. T.
Acquisition of data: F. B.
Analysis and interpretation of data: T. D.
Drafting of the manuscript: N. G. N.
Critical revision of the manuscript for important intellectual content: D. M. K. T.
Statistical analysis: M. S.
Administrative, technical, and material support: D. M. K. T.

Ethics

All procedures performed in this study, involving human participants, were in accordance with ethical standards.

Conflict of Interest

The authors declare that they have no conflict of interest.

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