Preparation and Comparative Evaluation of Olea europaea Leaf Toothpaste with Artificial Toothpastes

Ruwaydah Mohammed Alsararie[1], Samira Omar Hribesh[*1], and Rabia Omar Eshkourfu[1], Rokaya Omar Amara[2], Atiga Said Aljenkawi[1]

[1] Department of Chemistry, Faculty of Science, Al-Mergib University Al-Khoums, Libya
[2]Libyan Biotechnology Research Center, Tripoli, Libya

***Abstract*:** This study evaluated the safety and effectiveness of a formulated herbal toothpaste compared to artificial ones. It focused on developing and testing a toothpaste made from Olea europaea extract and comparing it to commercial herbal and synthetic toothpastes. The Olea europaea leaf extract was analyzed for phytochemicals using GC/MS, revealing beneficial compounds like phenols, fatty acids, terpenes, flavonoids, carotenoids, and alkaloids. Phenolic compounds were also examined with HPLC. The extract's antioxidant activity was tested and showed high effectiveness with an IC50 value of 5.05 µg/ml, as measured by the DPPH radical scavenging method. The study also involved formulating the toothpaste with Olea europaea leaf extract and evaluating it on parameters such as color, taste, appearance, moisture content, pH, spreadability, foaming power, abrasive particles, homogeneity, and stability. The results were comparable to those of commercial herbal and synthetic toothpastes. Additionally, the formulated toothpaste was tested for antibacterial activity against E. coli and Staphylococcus aureus and showed significant effectiveness against both bacteria, outperforming commercial herbal and synthetic toothpastes.

***Index Terms*-** natural Toothpaste; flow ability; direct compression; Olea europaea Leaf

I. INTRODUCTION

Dental caries is a prevalent chronic oral infection that affects individuals worldwide [1]. The presence of oral pathogenic microorganisms contributes to the formation of dental plaques, dental caries, as well as gingival diseases [2]. Toothpaste formulations are frequently used to promote oral health and aesthetics [3]. Toothpaste, which can be paste or gel, is typically utilized with a toothbrush to maintain oral hygiene and enhance aesthetics. [5]. Various active pharmaceutical ingredients in toothpaste include abrasives, humectants, detergents, binders, sweeteners, preservatives, antioxidants, and flavors [3-4]. While these ingredients are widely used, some formulations may potentially cause harm to teeth and gums. Therefore, there is an increasing demand for safe, effective, and well-formulated dentifrices [6]. The rationale behind this combination is to combat bacteria associated with oral issues such as gum disease, dental cavities, and gingivitis. Numerous medicinal plants have been traditionally used for oral cleansing, treating oral diseases, maintaining oral health, and eliminating unpleasant oral odors often associated with gingivitis. Toothpaste plays a crucial role in preventing gingivitis, tooth decay, and more severe dental problems. It is available in various flavors and leaves the mouth and breath feeling fresh after brushing. Additionally, toothpaste can mask odors from strongly flavored foods like garlic or onions. It possesses properties that address other dental concerns, including tooth sensitivity, teeth whitening, tooth decay, and gum disease [7]. Medicinal plants are defined as plants containing substances within one or more of their organs that can be used for therapeutic purposes or as precursors for chemopharmaceutical semi-synthesis. Plants produce a diverse range of bioactive molecules, making them a valuable source of various types of medicines [8]. Throughout history, higher plants have played a significant role in maintaining human health, as they serve as sources of medicinal compounds [9-10]. Each plant or herb can be considered a complete pharmacy, containing active substances with therapeutic potential [11]. In recent times, there has been a growing interest in studying medicinal plants and their utilization due to their richness in secondary metabolites such as polyphenols, flavonoids, and saponins. These compounds are known for their antioxidant properties and ability to treat infections, making them a natural source for the treatment of various diseases. In fact, over 50% of all modern clinical drugs are derived from natural products, emphasizing the crucial role of natural products in pharmaceutical drug development programs [12-13]. Herbal medicines with antimicrobial properties can be incorporated into toothpaste formulations as they assist in preventing oral infections and diseases by combating oral bacterial flora. Many studies have incorporated some plant extracts into the formulation and preparation of herbal toothpaste including the study conducted in India, which produced a herbal toothpaste containing extracts of medicinal plants Bark of Acacia nilotica, Acacia catechu, and flower buds of syzygium aromaticum as herbal ingredients, the results of which showed that this prepared herbal toothpaste eliminates many types of bacteria that infect the mouth [14]. Another study conducted in India by Pallavi et al produced an herbal toothpaste containing Aloe vera along with a combination of sodium chloride. The results were that toothpaste containing a mixture of aloe and sodium chloride possesses antimicrobial activity [15]. Moreover, Oluwasina and his group studied the produced toothpaste containing extracts of Syzygium aromaticum, Dennettia tripetala, and Jatropha curcas latex, which showed that it has a better and significant antimicrobial effect compared to commercial toothpastes. The manufactured toothpastes have shown effective antimicrobial properties against the tested

pathogens due to the bioactive compounds in them [16]. The use of Olea europaea leaf extract in toothpaste formulation has not been reported previously, to the best of our knowledge. This study aims to address this gap by developing a toothpaste containing Olea europaea leaf extract and evaluating its antibacterial properties.

II. Materials and Methods

1. Plant materials

A fresh leaf sample of the Olea europaea plant was collected from the north of Leptis Magna in the city of Al Khums, Libya region (32°38'11" N and 14°15'36" E) in March 2022. The studied plants were identified by a specialist taxonomist from the Botany Department, Faculty of Science, El Mergib University. The fresh leaf plant samples were washed with tap water and then with distilled water. They were subsequently dried in the shade at room temperature. The fresh leaf plant samples were washed with tap water and then with distilled water. They were subsequently dried in the shade at room temperature. Finally, they were ground into a fine powder in an electrical blender and sieved to give a particle size of 50-150 mm, then they were stored properly for future use. The analytical grade of all solvents and chemicals was obtained from BDH Chemicals Ltd.

2. Extraction and preparation of crude extract

The Soxhlet apparatus was used to extract 5 grams of plant leaves using 100 ml of 80% ethanol solvent at a temperature of 50°C. It took 4-6 hours for the extraction process to be carried out. After completion, the extracts were allowed to cool to room temperature and then filtered. Using a rotary evaporator, the filtered extracts were evaporated at room temperature under vacuum. The concentrated extracts were stored in a refrigerator at a temperature of 2-8°C until further use [17].

3. Phytocomponent identification by GC-MS

The chemical composition of the sample was performed using a Trace GC1310-ISQ mass spectrometer (Thermo Scientific, Austin, TX, USA. direct capillary column TG–5MS (30 m x 0.25 mm x 0.25 µm film thickness). The column oven temperature was initially held at 35 °C and then increased by 3°C /min to 200°C held for 3 min. increased to the final temperature 280°C by 3°C /min and hold for 10 min. The injector and MS transfer line temperatures were kept at 250, and 260°C, respectively; Helium was used as a carrier gas at a constant flow rate of 1 ml/min. The solvent delay was 3 min, and diluted samples of 1 µl were injected automatically using Autosampler AS1300 coupled with GC in the split mode. EI mass spectra were collected at 70 eV ionization voltages over the range of m/z 40–1000 in full scan mode. The ion source temperature was set at 200 °C. The components were identified by comparison of their retention times and mass spectra with those of WILEY 09 and NIST 11 mass spectral database [18,19,20].

4. Evaluation of antioxidant activity by DPPH radical scavenging method:

Free radical scavenging activity of ethanolic extract from Olea europaea leaves was measured by 1, 1- diphenyl-2-picryl hydrazyl (DPPH). In brief, a 0.1 mM solution of DPPH in ethanol was prepared. This solution (1 ml) was added to 3 ml of different extracts in ethanol at different concentrations (3.9, 7.8, 15.62, 31.25, 62.5, 125, 250, 500, 1000 µg/ml). Here, only those extracts solubilized in ethanol and their various concentrations were prepared by the dilution method. The mixture was shaken vigorously and held at room temperature for 30 min. then, absorbance was measured at 517 nm by using a spectrophotometer (UV-VIS Milton Roy). The reference standard compound used was ascorbic acid, and the experiment was done in triplicate. The IC50 value of the sample, which is the concentration of the sample required to inhibit 50% of the DPPH free radical, was calculated using the Log dose inhibition curve. The lower absorbance of the reaction mixture indicated higher free radical activity. The percent DPPH scavenging effect was calculated by using the following equation:

DPPH scavenging effect (%) or Percent inhibition = A0 - A 1 / A0 × 100.

Where A0 was the absorbance of the control reaction, and A1 was the absorbance in the presence of a test or standard sample.

5. Formulation of toothpaste:

Toothpaste formulation ingredients are shown in Table 1. A herbal toothpaste using Olea europaea leaves plant extract was formulated following the method approved by Pavan et al [21]. with some modifications. The herbal toothpaste was prepared using the following formulation procedure, as seen in Table 1: sodium lauryl sulphate (5 g) and calcium carbonate (25 g) were dissolved in 70 ml of distilled water. Methylcellulose (0.5 g) and glycerin (5 g) were then added to the solution and stirred to obtain a smooth gel consistency. Sodium chloride (0.5 g) and sodium Benzoate (0.25 g) were triturated and added to the mixture, followed by the incorporation of honey (0.3 g). Finally, Mint oil (2-3 drops) and approximately 0.18 grams of plant leaf extract were added to the previous mixture according to the proportions

specified in **Table 1**. The resulting toothpastes were then stored in tubes.

Table 1: Toothpaste Formulation Ingredients

S.NO	INGREDIENTS	Function	T.O
1	***Olea europaea*** **leaf extract**	Anti-inflammatory and anti-oxidant	0.18 g
2	**Calcium carbonate**	Filler/abrasive	25 g
3	**Sodium lauryl sulfate**	Foaming agent/ Detergent	5 g
6	**Glycerin**	Humectant	5 g
7	**Methyl cellulose**	Thickening agent	0.5 g
8	**Sodium Benzoate**	Preservative	0.25 g
9	**Sodium chloride**	Anti caries	0.5 g
10	**Honey**	Sweetening agent	0.3 g
11	**Mint oil**	Flavoring agent	2-3 drops
12	**Distilled water**	Solvent	70 ml

6. Evaluation of Toothpastes:

Physical Examination:

Color: The formulated toothpastes underwent a visual assessment to evaluate their color. The color was observed and examined.

Taste: The taste of the formulation was assessed by conducting a taste test.

Texture: To assess the texture of the toothpaste, it was rubbed between the fingers to determine its consistency and feel.

Determination of pH:

To determine the pH parameter in herbal toothpaste, a small amount of the toothpaste (1 g) was mixed with distilled water (9 ml), and the pH of the resulting mixture is measured using a pH meter. Combany (HANNA USA) [22].

Foaming power:

Weigh 5 g of toothpaste in a 100 ml glass beaker. 10 ml of water was added and covered the glass beaker with a watch glass and stand for 30 minutes. Heat the suspension gently to dissolve the detergent if present in it. Stir the suspension with glass rods and transfer it to a 100 ml measuring cylinder. Examine if no foam is produced (more than 2 ml). Transfer the residue retained in the beaker to the measuring cylinder by adding of 5-6 ml of water. Then make up the cylinder with 50 ml of water. Stir the contents with up-and-down movements to achieve a uniform suspension at 30 °C. Record V1 before shaking. After shaking, keep the cylinder standing for 5 minutes, and finally note the volume obtained and record V2. Calculate the Foaming power using the formula:

Foaming power (cm) = V2 – V1

V1 = Volume in ml of water without foam.

V2 = Volume in ml of water with foam.

Repeating the experiment and note the average value of three readings [23].

Moisture content:

5g of toothpaste was heated in an oven at 105°C for 24 hours. It was allowed to cool and reweighed. The heating and reweighing process continued until a constant weight was recorded in

consecutive checks. The weight loss was used to calculate the moisture content using the formula:

Moisture content %= (Original sample weight – dry sample weight) /Original sample weight ×100 [24].

Determination of spreadability:

Two grams of toothpaste were placed on a glass slide (20 x 20 cm), and covered with another glass slide. Then carefully place a 4 kg weight on the covered glass slide (sliding shall not take place). Measure the spreading (in cm) of the toothpaste after 30 minutes. Repeating the experiment and note the average value of three readings [25].

Determination of sharp and edge abrasive particles:

Extrude the contents 15-20 cm long on the butter paper, repeat the same process for at least ten collapsible tubes. Press with the contents of the entire length with a fingertip for the presence of sharp and hard-edged abrasive particles. Toothpaste shall not contain such particles [21].

Homogenecity:

The herbal toothpaste was placed in a collapsible tube and then squeezed on the tube to test the homogeneity of the herbal paste [19].

Stability:

The stability of the toothpaste shall be stable, but not deteriorate, ferment, or segregate during normal storage conditions and usage. The stability of toothpaste can be tested when it is exposed to 45±2 °C for one month. Also exposed to cool conditions such as 5 °C for 1 hour. After storage, no phase separation, fermentation, and

gassing can be observed, no obstruction of extrudable form from the container is observed, and studied for pH and appearance [20].

7. Antibacterial Activity:

The formulated paste was subjected to an in-vitro antibacterial study using the disc diffusion method. The study aimed to evaluate the paste's effectiveness against pathogenic bacterial strains, including E. coli and Staphylococcus aureus. Discs containing the formulated paste(TO) were placed on Mukker Hinton Agar medium plates to conduct the study. The extract of Olea europaea leaves (O) was used as a control. These plates were then incubated at 37ºC for 24 hours. This incubation period allowed for the assessment of the paste's antibacterial activity and ability to inhibit pathogenic bacteria growth [21].

III. Results and Discussions

1. The GC-MS analysis

In the present study, GC-MS analysis was utilized to identify the phytochemical compounds of the ethanolic extract of Olea europaea leaves. The GC-MS results of this plant revealed the presence of various phytochemicals, such as phenols, fatty acids, terpenes, terpenoids, flavonoids, carotenoids, esters, derivatives of amines, steroids, siloxane derivatives, alcohol derivatives and alkaloids as recorded in **Table 2.**

Table 2: GC-MS data of chemical compounds of Olea europaea leaves

Peak	Compound name	RT	Area %	Formula	MW
1	Phenol, 4-ethenyl-2,6-dimethoxy-	37.74	0.98	$C_{10}H_{12}O_3$	180
2	Cycloheptasiloxane, tetradecamethyl-	38.14	0.72	$C_{14}H_{42}O_7Si_7$	518
3	Duloxetine	41.23	10.35	$C_{18}H_{19}NOS$	297
4	(E)-4-(3)Hydroxyprop-1-en-1-yl)-2 methoxyphenol	43.79	1.25	$C_{10}H_{12}O_3$	180
5	6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a tetrahydrobenzofuran-2(4H)-one	44.02	0.69	$C_{11}H_{16}O_3$	196
6	2-Propenoic acid, 3-(3-hydroxy-2,6,6-trimethyl-1-cyclohexen-1-yl)-, methyl ester, (E)-	44.57	2.19	$C_{13}H_{20}O_3$	224
7	2(5H)-Furanone, 4-(2,3-dimethyl-2-buten-4-yl)-5-methoxy-	45.14	19.22	$C_{11}H_{16}O_3$	196
8	Spiro[4.4]non-3-en-2-one, 4-methyl-3-(1H-tetrazol-5-yl)-1 -oxa-	45.63	2.93	$C_{10}H_{12}N_4O_2$	220

The GC-MS analysis of Olea europaea leaves revealed the identification of twenty-seven phytochemicals, as shown in Table 1. Among these compounds, (2(5H)-Furanone, 4-(2,3-dimethyl-2-buten-4-yl)-5-methoxy) exhibited the highest peak area, accounting for 19.22% of the total composition. The following were Squalene (12.12%), Duloxetine (10.35%), and 1,4-Benzenedicarboxylic acid, bis (2-ethylhexyl) ester (6.97%). Conversely, the compounds (6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one) (0.69%), ([1,1'-Bicyclopropyl]-2-octanoic acid, 2'-hexyl-, methyl ester) (0.71%), and (Ethanol, 2-(octadecyloxy)-) (0.76%) showed the lowest abundance. Additionally, other compounds are present with varying peak area values. These results are consistent with Kamoun et al., who studied GC-MS analysis of Olea europaea leaves extract and identified similar compounds such as 3,7,11,15-Tetramethyl-2-hexadecen-1-ol and n-Hexadecanoic acid [26]. In the current study, most phytochemicals have pharmacological activity; for instance, 2(5H)-Furanone, 4-(2,3-dimethyl-2-buten-4-yl)-5-methoxy has demonstrated antibiotic activity [27]. Additionally, squalene has shown antioxidant, antibacterial, and anti-inflammatory properties [28,29], while duloxetine is a serotonin-norepinephrine reuptake inhibitor used to treat generalized anxiety disorder, neuropathic pain, and osteoarthritis [30]. Phytol exhibits anti-inflammatory, antinociceptive, and antioxidant effects [31], and n-Hexadecanoic acid has shown antimicrobial, antioxidant, and anti-inflammatory activities [34]. The GC-MS analysis of Olea europaea leaves highlights the presence of essential compounds involved in secondary metabolism. These compounds are biologically active, many displaying antibacterial, anti-inflammatory, and anti-allergic properties. Consequently, due to these properties, this extract was incorporated into the formulation of herbal toothpaste.

2. Composition of Phenolic Fraction:

The Phenolic and flavonoid compounds of Olea europaea leaves considered in this study are shown in Table 3. The quantification of total phenolic compounds in the leaves was carried out using HPLC based on their retention times. HPLC has proven to be one of the most useful techniques available to the chemist for separating complex mixtures of organic substances.

Table 3: Composition of Phenolic Compounds of Olea europaea leaves

Compound	*Olea europaea*
Gallic acid	3029.69
Chlorogenic acid	2105.01
Catechin	1512.98
Methyl gallate	41.69
Caffeic acid	53.57
Syringic acid	87.20
Pyro catechol	0.00
Rutin	1077.76
Ellagic acid	184.24
Coumaric acid	334.40
Vanillin	2548.53
Ferulic acid	759.74
Naringenin	494.53
Daidzein	79.95
Querectin	48.25
Cinnamic acid	7.91
Apigenin	64.77
Kaempferol	0.00
Hesperetin	0.00

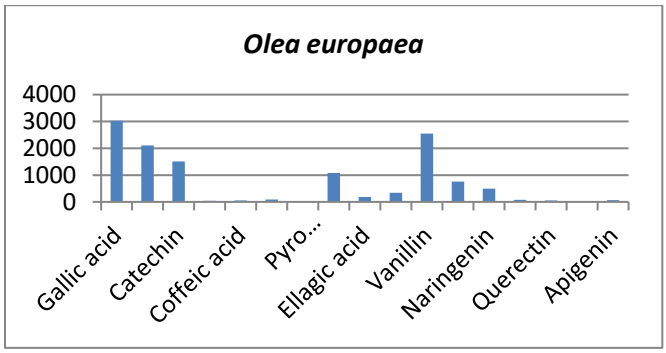

Figure 1: Summary of the composition of the phenolic compounds of Olea europaea.

3. Phenolic acids profile:

The present study aimed to analyze the levels of phenolic acids and flavonoids in the leaves of Olea europaea. Polyphenolic compounds have been identified to exhibit chemopreventive effects through various mechanisms, including antioxidant activity, antiproliferation, enzymatic detoxification, apoptosis induction, immune system modulation, estrogenic/antiestrogenic activity, and anti-inflammatory activity [35]. The results of this study showed the presence of eight different phenolic acids in varying proportions in the leaves of the studied plants: Gallic acid, Chlorogenic acid, Coffeic acid, Syringic acid, Ellagic acid, Coumaric acid, Ferulic acid, and Cinnamic acid. Specifically, among the examined phenolic acids, gallic acid was found to be the most abundant in Olea europaea leaves, quantified at 3029.69 mg/100g, while Cinnamic acid was the least abundant at 7.91 mg/100g. These findings align with prior studies conducted on Olea europaea leaves, which reported that gallic acid exhibits the highest concentration among the identified phenolic acids [36]. It is worth noting that these phenolic compounds possess various biological activities. Gallic acid, for example, has demonstrated significant antioxidant and anti-inflammatory properties in numerous studies [37]. Similarly, ellagic acid exhibits antioxidant, anti-inflammatory, and anti-allergic properties [38].

4. Flavonoids profile

Flavonoids are found in plant leaves with notable health benefits as antioxidants and anti-inflammatories [39]. The results of this study showed the presence of eleven different flavonoids in varying proportions in the leaves of the studied plants: Catechin, methyl gallate, pyro catechol, rutin, vanillin, naringenin, quercetin, kaempferol, apigenin, hesperetin, and daidzein. The main flavonoids found in the plant leaves were rutin, 1077.76 mg/100g, followed by naringenin, 494.53mg/100g, and vanillin, 2548.53 mg/100g. While kaempferol was not found (ND). The findings of this study are in agreement with the results reported by other studies previously conducted on these plant leaves [36,40]. The present study provides valuable insights into the flavonoids of Olea europaea leaves. Specifically, it has been determined that vanillin is present in a significant concentration of 2548.53mg/100g. Conversely, the lowest concentration was observed for methyl gallate at 41.69mg/100g. Notably, Pyro catechol and Hesperetin were not detected in Olea europaea leaves. The major flavonoids have demonstrated various biological activities such Vanillin, has shown antioxidant properties, which help protect cells from oxidative stress and damage caused by free radicals. Also, it has shown potential anti-inflammatory effects by inhibiting pro-inflammatory molecules and pathways. Additionally, vanillin has been investigated for its antimicrobial properties, demonstrating activity against certain bacteria and fungi [41]. Whereas naringenin acts to protect neurons and reduce neuroinflammation, naringenin is considered a preventive agent in cancer treatment [42]. Rutin has shown anticancer and immunomodulatory properties, including enhancing the activity of certain immune cells and reducing inflammatory responses. Rutin has also demonstrated antimicrobial activity against various bacteria and fungi [43]. The findings of this study indicate that the leaves of Olea europaea possess significant levels of phenolic compounds with antioxidant activity. These plant varieties can serve as cost-effective sources of natural antioxidants, which can be utilized in a wide range of applications, including both food and non-food industries. Moreover, the incorporation of these phenolic compounds in the formulation of herbal toothpaste and the manufacturing of pharmaceutical compounds.

5. Antioxidant activity

Assessing the antioxidant properties of natural compounds holds significant importance due to their applications in medicine, food, and cosmetics [44,45]. Among the various colorimetric assays

available, the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay is widely recognized for estimating the radical scavenging capacity of plants and extracts. This method offers accuracy, simplicity, and cost-effectiveness, making it a valuable tool for screening the general antioxidant activity. The DPPH assay relies on the reaction between the stable synthetic radical, DPPH, and an antioxidant compound. As the antioxidant interacts with DPPH, its free radical property is neutralized, resulting in a visible color change from violet to yellow [46,47]. this color change serves as an indicator of the antioxidant activity of the tested compounds. In the context of this study, the antioxidant activity of ethanolic extracts from Olea europaea leaves was determined using the DPPH assay with standard ascorbic acid. Ascorbic acid is considered one of the essential compounds renowned for its high antioxidant activity. This is evident in **Table 3**. Also, the color change can be observed when using ascorbic acid. Ascorbic acid, also known as vitamin C, has long been recognized for its potent antioxidant properties. Its ability to neutralize free radicals and protect cells from oxidative stress has been extensively studied and documented. This makes it a crucial component in maintaining overall health and preventing various diseases associated with oxidative damage [48]. This study aimed to investigate the potential antioxidant properties of this plant extract and evaluate its efficacy in scavenging free radicals. The results are shown in **Table 4**.

Table 4. Comparison of Antioxidant Activity Results of Olea europaea leaves with Standard Ascorbic Acid:

Concentration	DPPH Scavenging% of Ascorbic Acid	DPPH scavenging% of olive
1000	97.0	**96.0**
500	94.5	**93.8**
250	92.7	**91.2**
125	86.4	**84.2**
62.5	78.0	**75.6**
31.25	71.2	**70.1**
15.625	64.2	**62.3**
7.8125	56.3	**54.6**
3.9	45.8	**44.1**
1.95	41.7	**39.2**
0	0.0	**0.0**

The ethanolic extracts derived from Olea europaea leaves demonstrated notable inhibitory effects, as indicated by the findings of this study. When tested at a concentration of 1000 µg/ml, the extract displayed a significant inhibition percentage of 96% compared with ascorbic acid exhibited an inhibition of 97 %. Furthermore, even at a concentration of 500 µg/ml, the extract continued to exhibit significant inhibition values of 93.8% while ascorbic acid exhibited inhibition of 94.5 %. These findings highlight the sustained effectiveness of the extract at lower concentrations. At a medium concentration of 31.25 µg/ml, the mean inhibition remained substantial; leaf extracts showed mean inhibition values of 70.1% whereas ascorbic acid exhibited inhibition of 71.2 %. This further emphasizes the efficacy of the extract even at lower concentrations. Overall, the results of this study provide strong evidence for the inhibitory properties of the ethanolic extract derived from Olea europaea leaves. Additionally, Figure 2 illustrates the percent inhibition plotted against the concentration for the extract derived from Olea europaea leaves. This plot was utilized to calculate the IC50 values, which represent the concentration of the extract required to inhibit 50% of the oxidative activity. Based on the calculations, the IC50 value for Olea europaea extract was determined to be 5.05 µg/ml. These values indicate the concentration at which the extract exhibits significant antioxidant activity, inhibiting half of the oxidative activity. According to the parameters, the IC50 value category is very strong if the IC50 value is <10 µg/ml, strong if the IC50 value is between 10 and 50 µg/ml, mild if the IC50 value is between 50 and 100 µg/ml, weak if the IC50 value is between 100 and 250 µg/ml and not active if IC50 is above 250 µg/ml [49]. Based on the defined parameters, we noted that Olea europaea leaves exhibit very high antioxidant activity, as their IC50 values fall below the threshold of 10 µg/ml. The current study findings align with previous research conducted on the leaves of Olea europaea [40]

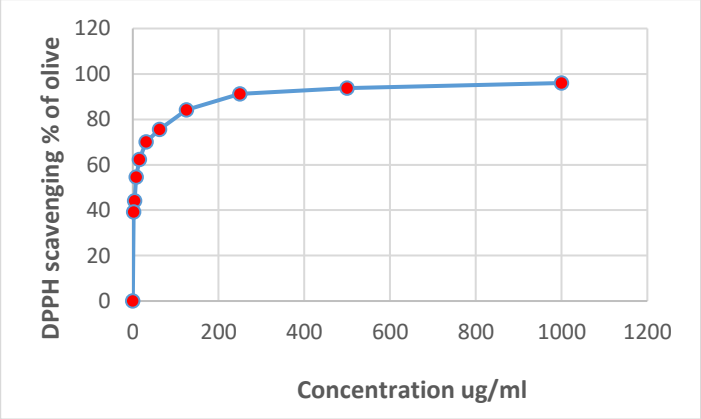

Figure 2. Presents a plot of percent inhibition versus concentration for the Olea europaea extract

6. Formulation of herbal toothpaste

In this study, one herbal toothpaste using Olea europaea leaves plant extract was formulated Figure 3, following the method approved by Pavan et al [21], with some modifications. This toothpaste was carefully examined, evaluated, and compared

against two commercially available toothpastes on the market. The results are as follows:

Figure 3. Herbal toothpastes using Olea europaea leaves plant extract.

Physical Examination:

Table 5 presents the results of the physical examination conducted on the toothpastes. The examination included assessments of the color, taste, and texture of the formulations.

Table 5: Results of physical examination of toothpastes
*T.O = Toothpaste with Olea europaea extract, T.C = Commercial toothpaste without extract, and T.C.E = Commercial toothpaste with miswak extract.

Properties	T.O*	T.C*	T.C.E*
Colour	Light Brown	Pink	White
Texture	Soft	Soft	Soft
Taste	Bitter and Stinging	Bitter and Stinging	Bitter and Stinging

Based on the findings presented in Table 5, it is evident that the studied toothpaste exhibits similar taste and texture characteristics. However, they vary in terms of color. This indicates that while the taste and texture of the toothpastes are comparable, there are distinct differences in their visual appearance.

Evaluation of toothpastes

Table 6 presents the results of the evaluation conducted on the toothpastes. The examination included assessments of PH, foaming power, moisture content, spreadability, sharpness and edge abrasive particles, homogenecity, and stability.

Table 6: Results of Evaluation of Toothpaste
*T.O = Toothpaste with Olea europaea extract, T.C = Commercial toothpaste without extract, and T.C.E = Commercial toothpaste with miswak extract.

Properties	T.O	T.C	T.C.E
PH	8.71	7.55	8.74
Foaming power (cm)	53 ± 0.05	40 ± 0.05	56.33 ± 0.03
Moisture content (%)	66.8%	34 %	31 %
Spreadability (cm)	9.43 ± 0.09	7.7±0.02	9.5±0.05
sharp and edge abrasive particles	Absent	Absent	Absent
Homogenecity	Pass	Pass	Pass
Stability	Good	Good	Good

PH: The pH measurement provides insights into the acidity or alkalinity of the toothpastes, which is crucial for maintaining oral health. Based on the information presented in the previous table, it is indeed evident that the pH value of the prepared herbal toothpaste was 8.71. Notably, this pH value closely resembles that of commercial toothpaste with miswak extract, which registered a pH of 8.74. It is worth noting that the pH value of the commercial paste without extract was 7.55, which indicates slightly lower alkalinity compared to the herbal toothpaste. The results of the current study are similar to the results of previous studies in which herbal toothpastes were prepared with other plant extracts [50,25]. The addition of herbal extract to the toothpastes may have contributed to their higher pH values, resulting in a more alkaline range. The herbal extract is known to possess alkaline properties, which can help create an environment in the mouth that is conducive to overall oral health [51].

Foaming power: The results in Table 6 clearly show that the foaming power of the prepared herbal toothpaste was 53 cm. It is worth noting that the foaming power of the prepared herbal toothpaste falls between the foaming power of the commercial toothpaste with miswak extract (56 cm) and the commercial toothpaste without extract (40 cm). The fact that the prepared herbal toothpastes exhibit foaming power in a similar range to the commercial toothpastes is a positive outcome, indicating that they can provide an effective cleaning experience for users. The results of the current study are consistent with the results of previous studies in which herbal toothpastes were prepared with other plant extracts [52,23,53]. Foaming power is an important quality in toothpaste, as it enhances the perception of cleanliness and aids in spreading the toothpaste evenly during brushing [54].

***Moisture content*:** Based on the results in Table 3, it is evident that the moisture content of the prepared herbal toothpaste was 66.8 %. This finding was similar to the results of previous studies in which herbal toothpastes were prepared with other plant extracts [24,55]. This consistency in moisture content is important as it ensures that users will have a consistent experience when using the toothpaste, regardless of the specific herbal extract used. It also indicates that

the toothpaste has been formulated with precision to provide the desired moisture level for effective cleaning and oral care [56].

***Spreadability**:* The spreadability of the prepared herbal toothpaste is 9.43 cm Figure 4. There are many previous studies in which herbal toothpaste was prepared, confirming the results of the current study [23,57]. Spreadability is an important characteristic of toothpastes as it ensures that the product can be easily distributed and covers a sufficient area on the teeth and gums during brushing. The close range of spreadability values observed in the prepared herbal toothpaste suggests that the formulations have been optimized to achieve the desired consistency and texture for efficient cleaning and oral care [58].

Figure 4: Spreadability test result of herbal toothpastes using Olea europaea leaves plant extract.

Sharp and edge abrasive particles: the results indicate that the prepared toothpaste did not contain any sharp or abrasive particles Figure 5. This is important for the safety and comfort of users while brushing their teeth, and it is beneficial in preventing any potential damage to the tooth enamel or gum tissue. It also contributes to a gentle and smooth brushing experience, promoting overall oral health and hygiene [59,60]. The fact that the prepared toothpaste shows similar results to the commercial paste without extract and the commercial paste with miswak extract further confirms their quality and alignment with industry standards. This similarity in results indicates that the formulated toothpastes have been carefully developed to provide a safe and effective cleaning experience for users. These results are similar to the results of previous studies in which herbal toothpaste was prepared with other plant extracts [61,62].

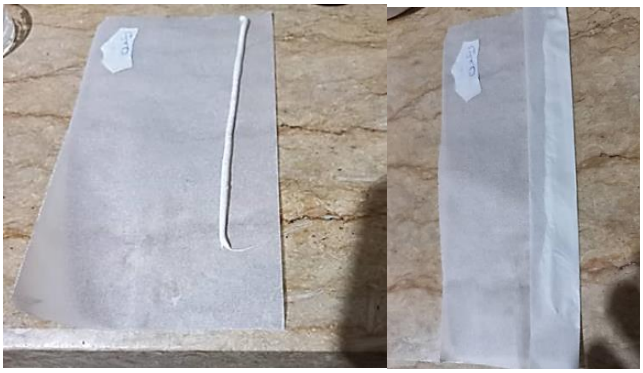

Figure 5: Results of the Sharp and edge abrasive particles of herbal toothpastes using Olea europaea leaves plant extract

Homogeneity: The prepared formulation that was tested for homogeneity has passed, indicating that there were no issues with obtaining the toothpaste from the tube through extrusion. This means that the toothpaste formulations have been successfully prepared to achieve a consistent and uniform texture throughout. This is an important factor as it ensures that users can easily and effectively dispense toothpaste from the tube without any difficulties. The successful homogeneity of the formulation further confirms their quality and suitability for practical use [63]. The results obtained for the homogeneity of the tested formulation are indeed identical to the results observed for the commercial paste without extract and the commercial paste with miswak extract. This indicates that the prepared herbal toothpaste exhibits the same level of homogeneity as the commercially available toothpastes. By achieving the same level of homogeneity as commercial toothpastes, the prepared herbal toothpaste demonstrates its effectiveness and compatibility with industry standards. This is an important aspect in building trust and credibility among consumers, who expect a consistent and reliable product experience [64]. There are many previous studies in which herbal toothpaste was prepared, confirming the results of the current study [65,61].

Stability: Based on the results, it is evident that the prepared toothpaste exhibited good stability, as indicated by the pH values and appearance shown in Table 7. These results are similar to the results of previous studies in which herbal toothpaste was prepared with other plant extracts [57,21,25].

Table 7: Results of PH and Appearance for the Stability Test

*T.O = Toothpaste with Olea europaea extract, T.C = Commercial toothpaste without extract, and T.C.E = Commercial toothpaste with miswak extract.

Properties		T.O	T.C	T.C.E
At room temperature	PH	8.73	7.55	**8.74**
	Appearance	Homogeneous	Homogenous	**Homogenous**
after storage at 45° C	PH	8.71	7.68	**8.94**
	Appearance	Homogeneous	Homogenous	**Homogenous**
after storage at 5 °C	PH	8.20	7.60	**8.99**
	Appearance	Homogeneous	Homogenous	**Homogenous**

The results demonstrate that the prepared toothpaste exhibited good stability, as evidenced by its similarity to the commercial toothpaste without extract and the commercial toothpaste with miswak extract. One key indicator of this stability is the minimal change in pH, with only a very small percentage variation observed. This suggests that the toothpaste maintained its intended alkalinity over time. Additionally, the fact that the toothpaste maintained a homogeneous appearance at room temperature further confirms its stability. This indicates that the ingredients remained well-mixed and did not separate or undergo any significant changes in texture [21,25],[16]. Furthermore, in previous studies, the stability of toothpaste has been tested by subjecting it to different storage temperatures. these studies aimed to know the ability of toothpaste formulation to withstand temperature variations and maintain its quality [21,25]. The toothpaste was subjected to storage conditions of 45 °C for a month and 5 °C for an hour. Even under these challenging conditions, the toothpaste maintained its stability and did not exhibit any noticeable changes in pH or appearance. Overall, the results confirm that the prepared toothpaste has good stability, which is an important characteristic for ensuring its quality and effectiveness in maintaining oral hygiene. Stability in toothpaste is an important characteristic, as it ensures that the toothpaste can withstand various storage conditions without compromising its effectiveness [66].

7. Antibacterial Activity of Olea europaea Extract Toothpaste

The findings of this study demonstrate that the toothpaste containing Olea europaea extract exhibits a good inhibition rate against both E. coli and Staphylococcus, as shown in Figure 6, with measurements of 11 mm and 12 mm, respectively, as presented in Table 8.

Table 8. Antibacterial Activity of Olea europaea Extract Toothpaste

*T.O = Toothpaste with Olea europaea extract, T.C = Commercial toothpaste without extract, and T.C.E = Commercial toothpaste with miswak extracts.

Bacterial strain	T.O	T.C	T.C.E
E. Coli	11	No R	**7**
Staphylococcus aureus	12	10	**No R**

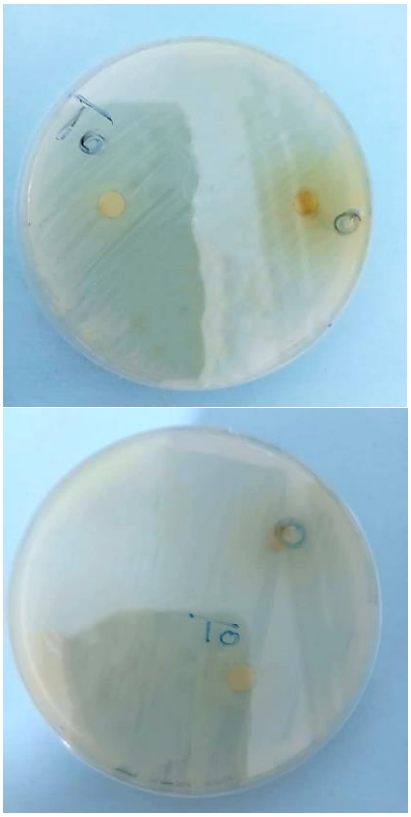

Figure 6: Antimicrobial screening test of Olea europaea Extract (O) and Olea europaea Extract Toothpastes (TO) on Staph. Aureus and E. Coli

Whereas the commercial toothpaste without extract exhibited an inhibition level of 10 mm on Staphylococcus aureus, with no effect on E. coli. Lastly, the commercial toothpaste with miswak extract showed an inhibition level of 7 mm on E. coli, with no effect on Staphylococcus aureus. These findings provide valuable insights into the effectiveness of Olea europaea toothpaste formulation in combating bacterial growth. They also underscore the potential advantages of incorporating specific plant extracts into oral care products. Several previous studies have prepared herbal toothpaste, confirming the results of the current study [52, 57, 53]. Overall, the study findings demonstrate that the formulated toothpastes outperform their commercial counterparts in terms of their ability to inhibit bacterial growth.

IV. CONCLUSION

Herbal toothpastes play a crucial role in promoting oral hygiene and preventing dental caries. In this study, Olea europaea toothpaste was formulated and evaluated using various standard parameters, including its antimicrobial properties. The results showed that the extract exhibited significant antimicrobial effects against both organisms tested. This suggests that the formulated toothpaste may be a safer alternative to fully synthetic toothpaste.

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AUTHORS

First Author – Ruwaydah Mohammed Alsararie, Department of Chemistry, Faculty of Science, Al-Mergib University, Al-Khoums, Libya,

Second Author – Samira Omar Hribesh, Department of Chemistry, Faculty of Science, Al-Mergib University, Al-Khoums, Libya.

Third Author – Rabia Omar Eshkourfu, Department of Chemistry, Faculty of Science, Al-Mergib University, Al-Khoums, Libya.

Fourth Author – Rokaya Omar Amara, Libyan Biotechnology Research Center, Tripoli, Libya.

Fifth Author: Atiga Said Aljenkawi, Department of Chemistry, Faculty of Science, Al-Mergib University, Al-Khoums, Libya,

Correspondence Author – Samira Omar Hribesh, Department of Chemistry, Faculty of Science, Al-Mergib University, Al-Khoums, Libya