



# Investigation of Antimicrobial Activities of Some Herbs Containing Essential Oils and Their Mouthwash Formulations

## Uçucu Yağ İçeren Bazı Bitkilerin ve Gargara Formülasyonlarının Antimikrobiyal Aktivitelerinin Araştırılması

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### ABSTRACT

**Objectives:** The aim of this study was to prepare pharmaceutical formulations of mouthwashes and to examine the antimicrobial activities of essential oils obtained from plants used traditionally in Turkey for oral infections.

**Materials and Methods:** Essential oils were obtained from herbal drugs using water distillation with Clevenger apparatus. The antimicrobial capacities of mouthwash formulations containing a mixture of essential oils with proportions of 4.5% and 9.0% were examined using disc diffusion and microbroth dilutions.

**Results:** The inhibition zone diameters were determined to vary between 7 and 59 mm. The static and cidal activity was generally 50% and greater than 50% when pure essential oil samples were applied on microorganism specimens. Formulation F2, which contained a mixture of essential oils with proportions of 4.5%, showed 6.25% minimum bactericidal effect on *Staphylococcus aureus* ATCC 25923, and 3.125% the minimum inhibitory concentration and minimum bactericidal concentration on all other microorganisms. The antimicrobial effect of pure essential oil samples applied on microorganisms was lower than of mouthwashes formulations; the antimicrobial effect of F2, which contained a mixture of essential oils with proportions of 4.5% was higher than formulation F1, which contained a mixture of essential oils with proportions of 9%.

**Conclusion:** The results obtained by these methods allow us to conclude that the essential oils and the prepared F1 and F2 mouthwash formulations exerted activity against microorganisms affecting the oral cavity. The F2 formulation also had significant antimicrobial activity on the tested microorganisms.

**Key words:** Antimicrobial activity, *Laurus nobilis* L., *Origanum vulgare* L. ssp. *hirtum*, *Rosmarinus officinalis* L., *Salvia fruticosa* Mill.

### ÖZ

**Amaç:** Türkiye’de halk arasında yaygın olarak kullanılan bitkilerden elde edilen uçucu yağların antimikrobiyal etkilerini araştırmak ve bu etkilerden yola çıkarak ağız enfeksiyonlarında kullanılmak üzere gargara formülasyonu hazırlamaktır.

**Gereç ve Yöntemler:** Uçucu yağlar, bitkilerden (*Origanum vulgare* L. ssp. *hirtum*, *Rosmarinus officinalis* L., *Laurus nobilis* L., *Salvia fruticosa* Mill.) su distilasyonu metodu ile Clevenger aparatı kullanılarak elde edilmiştir. Elde edilen uçucu yağlar ve %4.5 ve %9.0 oranlarında uçucu yağların karışımını içeren gargara formülasyonlarının antimikrobiyal aktiviteleri disk difüzyon metodu ve mikrobroth dilüsyon metodu ile araştırılmıştır.

**Bulgular:** Bu çalışmada antimikrobiyal aktivite disk difüzyon metodu ve mikrobroth dilüsyon metoduyla değerlendirilmiştir. İnhibisyon zon çaplarının 7-59 mm arasında değişkenlik gösterdiği belirlenmiştir. Uçucu yağlar, mikroorganizmalar üzerine saf olarak uygulandığında statik ve sidal etkinliğin genellikle %50 ve %50’nin üzerinde olduğu gözlenmiştir. %4.5 oranında uçucu yağ karışımı içeren F2 formülasyonunun *Staphylococcus aureus* ATCC 25923 üzerindeki minimum bakterisidal etkisinin %6.25 olduğu ve diğer tüm mikroorganizmalar üzerinde minimum inhibitör konsantrasyon ve minimum bakterisidal konsantrasyonunun %3.125 olduğu bulunmuştur. Uçucu yağ örneklerinin mikroorganizmalar üzerine saf olarak uygulandığında gösterdikleri antimikrobiyal etkinin, gargara formülasyonlarına göre daha düşük olduğu; ayrıca %4.5 oranında uçucu yağ karışımı

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içeren F2 formülasyonunun, %9.0 oranında uçucu yağ karışımı içeren F1 formülasyonuna göre test edilen tüm mikroorganizmalar üzerine daha yüksek antimikrobiyal etkiye sahip olduğu bulunmuştur.

**Sonuç:** Elde edilen sonuçlar uçucu yağların karışımı ile hazırlanan F1 ve F2 formülasyonlarının, test edilen mikroorganizmalara karşı antimikrobiyal etkiye sahip olduğunu ortaya koymuştur. Ayrıca çalışma sonucunda F2 formülasyonunun test edilen mikroorganizmalar üzerinde önemli oranda antimikrobiyal aktiviteye sahip olduğu rapor edilmiştir.

**Anahtar kelimeler:** Antimikrobiyal aktivite, *Laurus nobilis* L., *Origanum vulgare* L. ssp. *hirtum*, *Rosmarinus officinalis* L., *Salvia fruticosa* Mill.

## INTRODUCTION

Diseases that affect the buccal cavity and teeth are a current public health concern. Mouth bacteria have been linked to plaque, tooth decay, and toothache. Plaque, which is a layer that forms on the surface of a tooth, principally at its neck, is composed of bacteria in an organic matrix that has been linked to gingivitis, periodontal disease, or dental carries.<sup>1</sup> Patient compliance and acceptance is extremely important for oral topical products. Ointments, creams, and some emulsions are rarely used for oral topical treatment, the patients have lower acceptance for application of ointments in the mouth.<sup>2</sup> Nowadays, mouthwash is one of the oral formulations that are available in the market. A mouthwash is identified as a non-sterile liquid solution used mostly for its deodorant, refreshing or antiseptic action, and also these rinses aim to decrease oral bacteria, eliminate food particles, temporarily decrease bad breath and offer a pleasant taste.<sup>3</sup> Mouthwashes are very useful in the reduction of microbial plaques.<sup>4</sup> It is important to make sure that formulated aqueous mouthwashes provide a comfortable feeling in the mouth during use, and it must have a pleasant flavor to obtain consumer acceptance.

Plants have been used for centuries in herbal tea preparations, as spices, and for therapeutic purposes.<sup>5</sup> The number of plants used for therapeutic purposes and spices is reported to be around 20,000. It is estimated that the number of plant species used for medical purposes in the world is 350,000 and 5% of these are formed by aromatic plants.<sup>6,7</sup> Herbal products have recently experienced more thorough investigation for their potential in preventing oral illnesses, particularly plaque-related diseases, such as dental caries.<sup>8</sup> Natural substances obtained from medicinal plants and used in alternative medicine were reported to possess antibacterial activity. The development of antibiotic resistant strains in recent years has become a serious health problem. In this direction, the antimicrobial effects of plant extracts and essential oils obtained from various parts of the plant against bacteria and fungus became important.<sup>9</sup> Researchers are trying to pay more attention to these natural products aiming to find an effective antimicrobial mouthwash that has the advantage of decreasing the adverse effects of synthetic products. The use of natural antimicrobials may conduce to control the disordered growth of oral microbiota, thus overwhelming problems caused by species resistant to conventional antimicrobials.<sup>10,11</sup> Natural materials have proved antibacterial action, mainly because most plants used in alternative medicine are composed of flavonoids, which act on bacterial cells disrupting the cytoplasmic membrane and inhibiting the enzymatic activity.<sup>12</sup>

Turkey is a Mediterranean country that is rich in medicinal and aromatic plants. Most of these are used in local folk tradition for many purposes.<sup>13</sup> *Laurus nobilis* L. is a plant belonging to

the Lauraceae family, which comprises approximately 2500 species. The genus *Laurus* is found in Europe and consists of two species, *Laurus azorica* and *Laurus nobilis*. Leaves of the plant, which are not shed during winter, are 5-10 cm long, 2-5 cm wide, and green in color. The fruits are small and olive-like.<sup>14,15</sup> The antimicrobial, analgesic, anti-inflammatory, acetylcholine esterase inhibiting properties of the essential oil of *Laurus nobilis* L. have been reported.<sup>15,16</sup>

There are four reports on the essential oil content and composition of *Origanum vulgare* L. ssp. *hirtum* of Turkish origin. This plant is known in Turkey as "İstanbul kekiği" and is widely used as thyme in the Marmara and Thrace regions.<sup>17,18</sup>

Rosemary (*Rosmarinus officinalis* L.), belonging to the Lamiaceae family, is a pleasant smelling perennial shrub that grows in several regions around the world. It is known as "Biberiye, Kuşdili" in Turkey.<sup>13,19</sup> Sage is one of the most valued herbs known for its essential oil richness and its plethora of biologically active compounds extensively used in folk medicine. Sages are cultivated in many countries. The garden sage (*Salvia officinalis* L.) is grown in Canada, the United States of America, Spain, Italy, Greece, Albania, Germany, France, Turkey, and England.<sup>20</sup>

Essential oils are volatile and oily mixtures with a strong odor, consisting of a large number of chemical compounds that give off the characteristic odor and color of the plant, which can be obtained from aromatic plants or from various herbal sources by methods such as water or hydro distillation. Essential oils can be found in different parts such as the root, stem, leaf, fruit, seed, wood, crust, bud, rhizome, and flower. Also, they are found in particular secretory canals such as the secretory trichome, secreting vesicle, or parenchyma and epidermal cells in the plants, depending on the family.<sup>5,21,22</sup>

Essential oils so called because they are fragrant, and "volatile oil" and "etheric oil" because they are volatile and easily evaporate.<sup>6,21</sup> The term "essential oil" was derived from the effective ingredient of the drug "Quinta essential", named by Paracelsus von Hohenheim in the 16<sup>th</sup> century.<sup>23</sup> Aromatic materials have been used scientifically and commercially for many purposes such as cosmetics, medicine, the food industry, perfumery, aromatherapy, and phytotherapy for many years.<sup>21</sup> Essential oils contain terpenic hydrocarbons and their oxygenated derivatives, as well as a small amount of volatile aliphatic hydrocarbons and mixtures of aromatic substances derived from phenylpropene. They consist of a many compounds that have different structures containing different functional groups. These functional groups also determine the characteristic chemical properties of volatile oils. Essential oils contain phenylpropene as well as terpenes. Terpenes are present in much greater amounts than phenylpropenes, and phenylpropenes are responsible for the odor and taste of essential oil.<sup>6,7</sup>

Hence, the purpose of the present study was to prepare and evaluate antimicrobial activities of mouthwashes.

Ethics committee approval was not required for the study.

## EXPERIMENTAL

The plant materials were provided from the market and identified in Pharmaceutical Botanical Department, İstanbul University Faculty of Pharmacy. Plant materials were determined as *Origanum vulgare* L. ssp. *hirtum*, *Laurus nobilis* L., *Rosmarinus officinalis* L., *Salvia fruticosa* Mill. Voucher specimens were kept in Medipol University.

In this study, sodium chloride, sodium bicarbonate, sodium saccharin, and ethanol were purchased from Sigma Aldrich (Germany).

### Obtaining essential oil

Plant materials were hydro distilled for 4 hours using a Clevenger apparatus. The temperature of the heater was set at  $100 \pm 2^\circ\text{C}$ . The obtained essential oils were dried over anhydrous sodium sulfate and stored at  $4^\circ\text{C}$ .<sup>24</sup> Plant materials and plant registration numbers are shown in Table 1.

### Preparation of mouthwashes

The mouthwash was prepared according to Table 2. Mouthwash solutions were formulated 4.5-9% of essential oil, 1-2% of *Origanum vulgare* L. ssp. *hirtum* and *Salvia fruticosa* Mill, 2-4% of *Rosmarinus officinalis* L., and 0.5-1% of *Laurus nobilis* L. Ethanol, sodium chloride, and sodium bicarbonate were also added to the formulations. Saccharine sodium was used as a sweetener. Essential oils were weighed and dissolved in a part of the ethanol and the other ingredients were added gradually with the aid of a mechanical stirrer at 500 rpm for 30 mins. The mixture was filtered and the filtrate volume was made up to 10 mL with distilled water. No preservative was necessary to be added due to the high content of ethanol (>15%) in the formulations.<sup>25</sup>

### Determination of pH

The pH of the mouthwashes was determined using a calibrated pH meter (Mettler Toledo, Switzerland). Determinations were performed three times and an average of these determinations was taken as the pH of the prepared mouthwashes.

### Determination of antimicrobial activity of essential oils and mouthwash formulations

#### Kirby-Bauer disc diffusion method

The Kirby-Bauer disc diffusion method was used to determine the antimicrobial susceptibilities of microorganisms to essential oils. Antimicrobial activities of sage, rosemary, bay, and thyme essential oils were determined against various microorganisms (*Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 12228, *Enterococcus faecalis* ATCC 29212, *Bacillus cereus* DSM 4312, *Escherichia coli* ATCC 25922, and *Candida albicans* ATCC 10231). For this purpose, the bacteria were incubated in Brain Heart Infusion Agar (BHI) medium at  $37^\circ\text{C}$ , for 24 hours. *Candida albicans* was incubated in Sabouraud Dextrose Agar (SDA) medium at  $30^\circ\text{C}$ , for 48 hours. After incubation,

the microorganisms were adjusted to 0.5 McFarland turbidity standard ( $10^8$  CFU/mL) in 0.85% physiologic saline. The prepared microbial suspension was seeded on the Mueller Hinton Agar (MHA) medium with a swab. Petri dishes were allowed to stand for 15 mins to dry. At the end of the period, aseptic conditions, taking discs prepared from Whatman 42 number filter paper using a pen, 10  $\mu\text{L}$  of essential oil was dropped onto the disks and the disks were placed on the Petri dishes. After placement of the disks, the Petri dishes were allowed to stand for 15 mins, the bacterial specimens were incubated for 24 hours, and the yeast specimens were incubated for 48 hours. After the incubation, the zone diameters around the discs were measured using a scale and recorded, and the results were evaluated. The experiment was performed in double parallel.<sup>11</sup>

#### Microbroth dilution method

The microbroth dilution method was applied to determine minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) of F1 and F2 mouthwashes and essential oil samples. For this purpose, 100  $\mu\text{L}$  double-strength Mueller Hinton Broth medium for antibacterial activity and SDA medium for antifungal activity were added (100  $\mu\text{L}$ ) to each well of a 96-well plate. One hundred microliters of the essential oil samples and formulation samples were added and 50% dilutions were made. Subsequently, bacterial specimens (*Staphylococcus aureus* ATCC 25923, *Salmonella typhi* ATCC 14028, *Escherichia coli* ATCC 25922) incubated in BHI medium at  $37^\circ\text{C}$  for 24 hours, and yeast specimen (*Candida albicans* ATCC 10231) incubated in Sabouraud Dextrose Broth medium at  $30^\circ\text{C}$  for 48 hours were adjusted to a 0.5 McFarland turbidity standard ( $10^8$  CFU/mL) in the 0.85% physiologic saline. Microorganism samples adjusted the McFarland turbidity and was added 100  $\mu\text{L}$  to wells. The wells only containing the medium were used as a negative control, and the wells containing the microorganisms and the medium were used as positive controls. After incubation, MICs and minimum cidal concentrations were determined and recorded. The experiment was conducted in double parallel.<sup>12</sup>

## RESULTS AND DISCUSSION

In current study, the *Origanum vulgare* L. ssp. *hirtum*, *Laurus nobilis* L., *Rosmarinus officinalis* L. and *Salvia fruticosa* Mill. essential oils were collected (Table 1) and mouthwash formulations were prepared according to Table 2. There are studies showing antimicrobial activities of volatile oils obtained from various plants and spices.<sup>26</sup> Essential oils contain different components and therefore the antibacterial effect ratings vary depending on the variety and amount of the compounds. Essential oils have antimicrobial effects on various Gram (+) and Gram (-) bacteria and many other microorganisms. Carvacrol and thymol break down the bacterial membrane thus releasing membrane-related substances from the cell, terpenoids and phenylpropanoids have been reported to reach more internal parts of the cell by penetrating the bacterial wall due to their lipophilic nature. It is known that plant extracts and essential oils have antimicrobial effects on Gram (+) and Gram (-) bacteria, as well as against various fungi.<sup>27</sup>

In a study, Al-Howiriny extracted the essential oil of *Salvia lanigera* and reported that it had a good inhibitory effect against *Mycobacterium smegmatis*, *Candida albicans*, and *Candida vaginalis*.<sup>28</sup> Holley and Patel<sup>29</sup> showed that essential oils obtained from *Coriandrum sativum*, *Cinnamomum zeylanicum*, *Cymbopogon citratus*, *Satureja montana* (Coriander, cinnamon, lemon grass, geysey) were effective against *Aspergillus niger*, *Candida albicans*, *Rhizopus oligosporus*, and showed that essential oils obtained from *Thymus vulgaris*, *Pimpinella anisum*, *Cinnamomum zeylanicum* plants (thyme, anise and cinnamon) had fungicidal activity against *Aspergillus flavus*, *Aspergillus parasiticus*, *Aspergillus ochraceus*, and *Fusarium moniliforme*. Also, they showed that thyme essential oil was fungi toxic. This effect was thought to be due to the hydrogen bonds formed between the hydroxyl groups of phenolic compounds in the volatile oil composition and the active part of the target enzymes.<sup>30</sup> Another study showed that the essential oil obtained from *Rosmarinus officinalis* were effective against *Staphylococcus aureus*, *Salmonella typhi*, *Escherichia coli*, and *Pseudomonas aeruginosa*.<sup>31</sup>

For many centuries, plants have been used to provide food flavor and aroma, to extend the shelf-life of foods, and to treat diseases. The antimicrobial effects of plants that have been used for many years as traditional medicine have been investigated from the beginning of the 20<sup>th</sup> century. Due to the

**Table 1. Plant materials and plant registration numbers**

Turkish name	Latin name	Plant registration numbers
Kekik	<i>Origanum vulgare</i> subsp. <i>hirtum</i>	BK 1001
Defne	<i>Laurus nobilis</i> L.	BK 1002
Biberiye	<i>Rosmarinus officinalis</i> L.	BK 1003
Adaçayı	<i>Salvia fruticosa</i> Mill.	BK 1004

**Table 2. Amount of Ingredients used to prepare 10 mL of mouthwash formulations (F1 and F2)**

Ingredient/mouthwash formulations	F1	F2	Blank formulation	
Essential oil	<i>Origanum vulgare</i> subsp. <i>hirtum</i>	2%	1%	-
	<i>Salvia fruticosa</i>	2%	1%	-
	<i>Rosmarinus officinalis</i>	4%	2%	-
	<i>Laurus nobilis</i>	1%	0.5%	-
NaCl	0.1%	0.1%	0.1%	
NaHCO <sub>3</sub>	0.05%	0.05%	0.05%	
Sodium saccharine	0.01%	0.001%	0.001%	
Ethyl alcohol	60%	60%	60%	
Distilled water	q.s.10 mL	q.s.10 mL	q.s.10 mL	

increase in antibiotic resistant infections in recent years, there is a growing interest in natural compounds and essential oils obtained from plants in particular.<sup>7,32</sup> In this study, the *Origanum vulgare* L. ssp. *hirtum*, *Laurus nobilis* L., *Rosmarinus officinalis* L. and *Salvia fruticosa* Mill. essential oils were collected and used to prepare mouthwash formulations. Flavors are added to the formulas to improve consumer acceptability of the mouthwash ingredients. In this study, saccharine sodium was used as a sweetener. Sodium bicarbonate was used the F1 and F2 formulations. Several studies have shown that bicarbonate is one of the salivary components that potentially modify the formation of caries. It increases the pH in saliva, and in this way, creates a hostile environment for the growth of aciduric bacteria. Sodium bicarbonate can also change the virulence of the bacteria that cause tooth decay. Animal studies have shown that dentifrices containing sodium bicarbonate reduce the amounts of both *Streptococcus sobrinus* and *Streptococcus mutans*, and this may reduce caries. Studies on humans showed a statistical reduction in the number of mutant streptococci. Sodium bicarbonate can also prevent caries by reducing enamel solubility and increase remineralization of enamel.<sup>33</sup>

Antimicrobial activities of essential oil samples were determined by disc diffusion assays. Furthermore, the antimicrobial activities of the F1 and F2 mouthwashes and essential oil samples were determined using microbroth dilutions. The antimicrobial activities of the tested samples against various microorganisms (*Staphylococcus aureus* ATCC 25923, *Streptococcus epidermidis* ATCC 12228, *Enterococcus faecalis* ATCC 29212, *Bacillus cereus* DSM 4312, *Escherichia coli* ATCC 25922 and *Candida albicans* ATCC 10231) are given in Table 3. According to these results, the diameters of the zones ranged between 7 and 59 mm (Table 3).

*Bacillus* sp., *Enterococcus* sp., *Salmonella* sp., *Staphylococcus* sp., *Streptococcus* sp., and *Candida* sp. are found in the oral microbiome. Therefore, we worked with these bacteria in our study.<sup>34,35</sup>

*Origanum vulgare* L. ssp. *hirtum* essential oil had the largest inhibition zone (59 mm) on *Candida albicans* ATCC 10231 and *Salvia fruticosa* Mill. essential oil had the smallest inhibition zone (7 mm) on *Staphylococcus aureus* ATCC 25923 and *Bacillus cereus* DSM 4312; *Rosmarinus officinalis* essential oil had the

**Table 3. Determination of antimicrobial activity using disc diffusion. Values are given in millimeters**

Microorganism/plants	<i>S. aureus</i> ATCC 25923	<i>S. epidermidis</i> ATCC 12228	<i>E. faecalis</i> ATCC 29212	<i>B. cereus</i> DSM 4312	<i>E. coli</i> ATCC 25922	<i>C. albicans</i> ATCC 10231
<i>O. vulgare</i> subsp. <i>hirtum</i>	43	37	21	37	39	59
<i>S. fruticosa</i>	7	-	-	7	9	-
<i>R. officinalis</i>	7	9	-	9	9	9
<i>L. nobilis</i>	13	9	-	9	11	9

smallest inhibition zone (7 mm) on *Staphylococcus aureus* ATCC 25923. As a result of the study, the essential oil obtained from *Origanum vulgare* subsp. *hirtum* showed the largest zone diameter in the tested microorganisms (Table 3).

The antimicrobial activity results determined using microbroth dilutions against various microorganisms (*Staphylococcus aureus* ATCC 25923, *Salmonella typhi* ATCC 14028, *Escherichia coli* ATCC 25922 and *Candida albicans* ATCC 10231) are given in Table 4. According to these results, the static and cidal activity was generally 50% and more than 50% when pure essential oil samples were applied on microorganism specimens. the MIC of the essential oil *Salvia fruticosa* Mill. was registered as 6.25% on *Escherichia coli* ATCC 25922 and *Salmonella typhi* ATCC 14028.

Formulation F1 contained 9.0% of essential oil and it was observed that the MIC and bactericidal concentration were 25% on *Escherichia coli* ATCC 25922 and *Salmonella typhi* ATCC 14028, 50% on *Staphylococcus aureus* ATCC 25923, and was over 50% on *Candida albicans* ATCC 10231. The F2 formulation contained 4.5% of essential oil in its composition, has been found that was 6.25 % the minimum bactericidal activity on *Staphylococcus aureus* ATCC 25923. Also, the F2 formulation were 3.125% the MIC and MBC on all other microorganisms. The solvent formulation did not exhibit antimicrobial activity (Table 4).

In this study, the antimicrobial effects of essential oils obtained from *Origanum vulgare* L. ssp. *hirtum*, *Salvia fruticosa*, *Rosmarinus officinalis*, and *Laurus nobilis* by water distillation were investigated on various microorganisms (*Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 12228, *Enterococcus faecalis* ATCC 29212, *Bacillus cereus* ATCC DSM 4312, *Escherichia coli* ATCC 25922 and *Candida albicans* ATCC 10231) using the disc diffusion method. According to the results, most of the tested plant materials were observed to have antimicrobial activity against microorganisms. The greatest antimicrobial activity was against *Candida albicans* ATCC 10231 strains by *Origanum vulgare* subsp. *hirtum* essential oil. In addition, the lowest antimicrobial activity was found with *Salvia fruticosa* essential oil against *Staphylococcus aureus* ATCC 25923 and *Bacillus cereus* DSM 4312 bacteria. The lowest antimicrobial activity of *Rosmarinus officinalis* essential oil was against *Staphylococcus aureus* ATCC 25923 bacteria.

Accordingly, *Origanum vulgare* subsp. *hirtum* essential oil was detected as having the strongest antimicrobial activity against the tested microorganisms.

According to the results of the microbroth dilution test, the essential oil samples showed antimicrobial activity at a certain rate against the tested microorganisms (*Staphylococcus aureus* ATCC 25923, *Salmonella typhi* ATCC 14028, *Escherichia coli* ATCC 25922, and *Candida albicans* ATCC 10231). The antimicrobial effect that essential oils showed separately on microorganisms was less effective than the mouthwash formulation. The antimicrobial effect of pure essential oil samples applied on microorganisms was lower than in mouthwash formulations; the antimicrobial effect of formulation F2, which contained mixture of essential oils with proportions of 4.5%, was higher than formulation F1, whose essential oils had a proportion of 9%.

## CONCLUSIONS

In this study, essential oils and essential oil-containing mouthwashes were successfully prepared. The results obtained by these methods allow us to conclude that the essential oils and prepared F1 and F2 mouthwash formulations exerted activity against the tested microorganisms, which affect the oral cavity. It was also concluded that static and cidal activity on the microorganisms of formulation F2 were markedly higher than in formulation F1. The static and cidal activity was generally 50% and more than 50% when pure essential oil samples were applied on microorganism specimens. Formulation F2, which contained 4.5% essential oils, had a 6.25% minimum bactericidal effect on *Staphylococcus aureus* ATCC 25923 and 3.125% MIC and minimum bactericidal concentration on all other microorganisms.

Formulation F2 contained less essential oil than formulation F1, yet the antibacterial and antifungal effect on the microorganisms of F2 was markedly higher than in F1.

The pH of a formulation is important for patient compliance. The pH of the prepared mouthwashes ranged between 7.37 and 7.63. The pH of the formulations was appropriate for mucosal delivery because they were iso-hydric. This indicated the non-irritancy of the formulation in oral mucosa.

Table 4. Determination of antimicrobial activity using microbroth dilutions

Microorganisms/plants	<i>E. coli</i> ATCC25922		<i>S. typhi</i> ATCC 14028		<i>S. aureus</i> ATCC 25923		<i>C. albicans</i> ATCC 10231	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MFC
<i>O. vulgare</i> subsp. <i>hirtum</i>	>50%	>50%	>50%	>50%	>50%	>50%	>50%	>50%
<i>S. fruticosa</i>	6.25%	>50%	6.25%	>50%	>50%	>50%	>50%	>50%
<i>R. officinalis</i>	50%	50%	50%	50%	>50%	>50%	50%	50%
<i>L. nobilis</i>	50%	50%	50%	50%	>50%	>50%	50%	50%
F1	25%	25%	25%	25%	50%	50%	>50%	>50%
F2	3.125%	3.125%	3.125%	3.125%	3.125%	6.25%	3.125%	3.125%

MIC: Minimum inhibition concentration, MBC: Minimum bactericidal concentration, MFC: Minimum fungicidal concentration

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