Effect of Some Essential Oils on Some Pathogenic Human's Mouth Bacteria

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Abstract:

Herbal remedies are now associated with safety, in contrast to synthetics, which are seen to be harmful to persons and the environment. This investigation aimed to test in vivo the effectiveness of herbs essential oils on oral bacteria. The antibacterial activity of myrrh (Commiphora molmol), thyme (Thymus vulgaris L.), sage (Salvia officinalis L.), chamomile (Matricaria recutita L.) and green tea (Camellia sinensis) essential oils (5 mg oil/100 ml water) dilution against total bacterial counts in saliva was studied. The counts of Streptococcus mutans and Lactobacillus rhamnosus were investigated. A standard susceptibility agar dilution method was used to assess the minimal inhibitory zones (MIZ) of myrrh, thyme, sage, chamomile, and green tea essential oils on Streptococcus mutans and Lactobacillus rhamnosus in order to look into their potential as antibacterial agents. Data were statistically processed. The experimental group showed a statistically significant reduction in colony counts of Streptococcus mutans and Lactobacillus rhamnosus relative to the control group. These findings showed the efficacy of the tested herbs oils against halitosis and oral flora, achieving a balance between the harmful and the harmless oral bacteria and also providing a promising practical application for the development of customised, all-natural antibacterial remedies.

Keywords: Streptococcus mutans - Lactobacillus rhamnosus – Halitosis- Myrrh – Thyme – Sage - Chamomile - Green tea - Essential oil.

Introduction

Herbal mouthwashes are in increasing demand since they target oral infections and provide immediate pain relief while having fewer adverse

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effects. Hydrogen peroxide and chlorhexidine, which are found in chemical mouthwash, instantly whiten, sterilise, and reduce tooth discomfort. However, they can discolour teeth and have adverse effects, but they are inexpensive **(Banu and Gayathri, 2016).**

It is generally known that oral conditions can affect a person's quality of life and can cause systemic and antecedent conditions. Many oral problems are strongly influenced by microorganisms. There is a constant demand for alternative therapies that are inexpensive, efficient, and non-toxic, such as plant and herbal medicines, due to the rise in bacterial resistance to antibacterial agents, the toxic side effects of some common antibiotics, and the cost of therapy **(Palombo, 2011).**

Halitosis is defined as foul breath caused by a range of factors, including but not limited to periodontal disease, bacterial coating of the tongue, systemic illnesses, and various foods **(American Academy of Periodontology, 2001)**. The exhaled air contains odoriferous chemicals that are linked to the aetiology of halitosis, particularly volatile sulphur compounds (VSC) produced by bacteria (**Rösing and Loesche, 2011)**.

Calculus, plaque, and infrequent dental visits were all linked to severe halitosis in a research conducted in Sweden by **Söder *et al*., (2002)**. Periodontitis and tongue coating was linked to VSC scores in a Japanese study by **Miyazaki et al., (1995).** In addition, patients with severe periodontitis had greater halitosis scores than non-periodontal patients. Tongue coating was regarded as an influencing factor for halitosis in two Swiss investigations by **Bornstein *et al*., (2009a) and Bornstein *et al.*, (2009b)**. Higher halitosis scores were linked to smoking and periodontal disease (**Bornstein *et al*., 2009a**). While **Yokoyama *et al.* (2010)** reported that halitosis has been linked to plaque buildup and tongue covering.

Gingivitis/periodontitis can produce odoriferous substances due to the presence of bacteria and inflammatory products. The presence of gingivitis or periodontitis was linked to halitosis in cross-sectional studies **(Quirynen *et al*., 2009).** The capacity of probable periodontal pathogens and inflammatory products to create volatile odoriferous chemicals was proven in vitro and in vivo studies (**Yoneda *et al.,* 2010; Salako and Philip, 2011)**. More over 60% of the 2000 patients at the breath clinic who had tongue coating, whether it was present alone or in conjunction with periodontal inflammation, had halitosis, according to **Quirynen *et al.* (2009).** The coating on the back of the tongue has been implicated in most investigations, It is in line with the fact that there are billions of bacteria there, including anaerobes, which can make odoriferous substances **(Kazor *et al*., 2003).** In vitro, bacteria from saliva, plaque removed from gingivitis/periodontitis and the tongue **(Kazor *et al*., 2003)** create odoriferous compounds, according to **Takeshita *et al*., (2010)**. These bacteria are reduced in intervention studies that provide a clinically relevant benefit in decreasing halitosis (**Shinada *et al*., 2010 ; Fedorowicz *et al*., 2008)**. **Silveira *et al*. (2011)** demonstrated that in people with periodontitis, robust supragingival plaque management can lower VSC and organoleptic ratings. Several studies have shown that halitosis can be reduced by eliminating germs on the tongue's dorsum. One of the most essential approaches for halitosis, according to a study, is tongue cleansing **(Faveri *et al*., 2006).** Because halitosis is caused by the presence of microorganisms from oral biofilms, every therapeutic method that affects the oral microbiota has the potential to reduce halitosis. Mouthwashes, particularly chlorhexidine and cetilpyridinium chloride, have been shown to reduce halitosis **(Shinada *et al*., 2010**).

The α-haemolytic *streptococcal* species *Streptococcus mutans* and *Lactobacillus rhamnosus* are the primary bacterial aetiological agents in caries. (**Taylor *et al*., 2005**). Streptococcus mutans (SM) is the most common bacteria in dental plaque and is the cause of caries (**Matalon *et al*., 2009**)

Many efforts have been made to exploit medicinal plants by increasing the number of strains resistant to antibacterial chemicals. Herbs have been used in traditional medicine all throughout the world since prehistoric times (**Hose, 2002**).

The stem of *Commiphora molmo*l (Family: Burseraceae), a tree that thrives in the Arabian Peninsula and northeast Africa, yields myrrh, an oleo gum resin (**Massoud *et al*., 2001**). In folk medicine, it is used for a range of diseases and is referred to as "a pharmacy unto itself." Myrrh has therapeutic properties against a variety of ailments, including antibacterial and anti-inflammatory properties (**Kumari *et al.,* 2011 and Cheng *et al.,* 2011**). As an antibacterial and anti-inflammatory, myrrh is used to treat tonsillitis, throat infections, and mouth ulcers. It's also utilised at funerals and as a perfume scent (**Van Wyk and Wink, 2004**). Staphylococcus aureus and other gram-positive and gram-negative bacterial species displayed effective antibacterial action when myrrh was utilized. Additionally an astringent, myrrh calms inflamed mouth and throat tissues. Research on the anticancer and analgesic effects of myrrh resin is ongoing **(Al-Harbi *et al*., 1994 and Dolara *et al*., 1996)**. Topical myrrh is used to treat mild oral and pharyngeal mucosal inflammations (**Sheir *et al*., 2001**).

Thyme extract mouthwash was successful as an antimicrobial agent. When compared to a strong antiseptic like chlorhexidine, it greatly decreased the total number of bacteria in children's saliva (**Abdel Hameed *et al*., 2020**). A gargle of sage tea is widely prescribed to cure a sore throat, mouth inflammations, and gingivitis in modern European herbal medicine. Sage is used to treat mouth, throat, and tonsil inflammation (**ESCOP, 1996**). To treat oral herpes, apply a salve comprised of thyme, myrrh, and goldenseal. Thyme can also be used to treat halitosis and persistent candidiasis (**Weiss, 1988**).

German Chamomile (GC) is an anti-inflammatory, antibacterial, bacteriostatic, wound-healing promoter, and deodorant that has been used as mouthwash or dentifrice with other herbal ingredients to reduce plaque formation and improve gingival health (**Gultz *et al.,* 1998).** *Matricaria Chamomilla* is a well-known component in alternative medicine. Dermatological, gastrointestinal, neurological, and mental conditions have all been treated with it (**Blumenthal *et al*., 2003**). Oral hygiene has been demonstrated to improve by using Chamomile as a a toothpaste or an oral rinse (**Pistorius *et al*., 2003).** A specific ingredient in the mouthrinse may be responsible for the anti-inflammatory properties of chamomile extract, which have been the subject of numerous research. In addition to being used in some toothpastes to treat oral irritations and minor infections, chamomile is also used as a mouthwash to treat gingivitis and periodontal disease **(Nissen *et al.*, 1998)**

Green tea stops bacteria from multiplying and causing tooth decay. Chamomile oil has antibacterial, anti-fungal, and anti-inflammatory effects. Chamomile oil also aids in the formation of granulation and epithelization. Thyme oil is used as an antibacterial and antiseptic agent against a variety of pathogens. Thyme oil can also help to reduce inflammation **(D'Amelio and Mirhom, 2010).** Anecdotal observations in the Japanese literature, such as "those who drink a big amount of green tea on a regular basis have less tooth decay" **(Kubo *et al., 1992*)** and "drinking green tea keeps the mouth clean" **(Sakanaka *et al*., 1989**). **Kaneko *et al*. (1993)** discovered that a four-week mouth washing routine with a weak catechin solution reduced periodontal disease-related halitosis; it was later shown that tea catechins deodorised methyl mercaptan, the principal source of halitosis (**Yasuda and Arakawa, 1995***)*.

Aim of work: The present investigation was carried out to study the antibacterial activity of myrrh, thyme, sage, chamomile and green tea essential oils (5*mg*/100 *ml* water) dilution against oral bacterial counts, *Streptococcus mutans* and *Lactobacillus rhamnosus*.

Materials and Methods

Materials

Myrrh (*Commiphora molmol*), thyme (*Thymus vulgaris* L.), sage (*Salvia officinalis* L.), chamomile (*Matricaria recutita* L.*)*, green tea *(Camellia Sinensis)* were purchased from local market and their essential oils were obtained from the National Research Center, Giza, Egypt.

Media: Mitis Salivarius Bacitracin Agar (MSBA), Mann Rogosa Sharpe agar (MRSA) and Nutrient Agar (NA) were obtained from Beta Company for chemicals in Mansoura city, Egypt.

Strains of oral bacteria: The bacterial strains used in this study were *S. mutans* ATCC 25175 and *Lactobacillus. rhamnosus* ATCC 7469 were obtained from Cairo Microbiological Resources Center (MIRCENT), Faculty of Agriculture, Ain Shams University, Egypt and checked for purity in laboratory.

Volunteers: Sixty healthy volunteers ranging in age from 16 to 20 years from Mansoura University students. Ethical standards were upheld, and authorization was requested from the relevant department, Faculty of Specific Education, Mansoura University.

Methods:

Total phenolic compounds were assessed using the method of **Waskmundzka *et al*. (2007)** published in Food Technology Research Institute Agric. Rec. Cent., Egypt.

Identification of herbs essential oils by GC: The volatile oils were separated using a Ds chrom 6200 Gas Chromatograph equipped with a flame ionisation detector, as described by **Singh *et al.*, (2007).**

Experimental design of Saliva:

Sixty healthy volunteers were recruited and randomly divided into six groups (*n*=10):

* Group (A) participants were asked to rinse their mouths with (40 *mL*) of a placebo mouthwash (control).

* Group (B) participants were asked to rinse their mouths with (40 *mL*) of diluted myrrh essential oil (5 *mg*/100 *ml* water), for 1 minute, three times a day for a week.

* Group (C) participants were asked to rinse their mouths with (40 *mL*) of diluted thyme essential oil (5 *mg*/100 *ml* water), for 1 minute, three times a day for a week.

* Group (D) participants were asked to rinse their mouths with (40 *mL*) of diluted sage essential oil (5 *mg*/100 *ml* water), for 1 minute, three times a day for a week.

* Group (E) participants were asked to rinse their mouths with (40 *mL*) of diluted chamomile essential oil (5 *mg*/100 *ml* water), for 1 minute, three times a day for a week.

* Group (F) participants were asked to rinse their mouths with (40 *mL*) of diluted green tea essential oil (5 *mg*/100 *ml* water), for 1 minute, three times a day for a week.

Saliva samples were collected after 7 days.

Enumeration of total bacterial count (TBC): The total microbiological count in saliva was determined using plate count agar medium **(A.P.H.A., 1971).** 1.00 ml saliva was put to agar medium-coated Petri-plates, which were then incubated at 37°C for 48 hours. The total bacterial count was calculated using a logarithmic transformation of the average CFU/ml.

Enumeration of *Streptococcus mutans* and *lactobacillus rhamnosus:*

They were determined using *Lactobacillus rhamnosus* selective MRS agar (Himedia) for *Lactobacillus rhamnosus* screening and *Mitis salivarius* bacitracin agar (MSB) for *Streptococcus mutans* under aerobic incubation at 37°C for 3 days, according to the manufacturer's instructions **(Badet and Quero., 2011**). The dilution of Herbs essential oils was 5 mg/100ml water. The results are presented as a logarithmic transformation of the average CFU/ml.

Determination of minimum inhibitation zones (MIZ)

According to **Sagdic, *et al*. (2002),** the paper disc technique was used to test the sensitivity of oral bacteria to the selected herbal essential oils. Sterile paper discs (What man No 1, 6.0 mm in diameter) were soaked in essential oils and serially diluted. (5ml oil/100ml water) was the dilution utilised. The discs were then placed on the surface of the inoculated plate count agar medium with *Streptococcus mutans* and *Lactobacillus rhamnosus*.

Statistical analysis:

According to **McCormick and Salcedo (2017),** all tests were done using the computer application of statistical analysis programme (SPSS, version 24).

Results and discussion

Table 1 shows the total phenols and flavonoid contents (*mg/100g*) of the tested herbs. Total phenols were recorded (114.8, 941.7, 1916.2, 58.9 and 79.4 *mg/100ml*) for myrrh, thyme, sage, chamomile and green tea, respectively. Flavonoid contents recorded (87.6, 154.6, 1360.1, 716.4 and 37.2 *mg/100ml*) for myrrh, thyme, sage, chamomile and green tea, respectively. Phenolic acids and flavonoids have been identified as typical phenolics with an antioxidant action (**Kahkönen *et al*., 1999**). The total phenolic content of myrrh oil was discovered to be (3.3 percent). Myrrh oil has a total flavonoid concentration of 0.2 per cent (**Mahboubi and Kazempour, 2016**). The resin of the species C. myrrha has 44.77 per cent total phenolics (**Danial and Majrashi, 2016**). Total phenolic contents of thyme were 783.81 mg/l, while total phenolic contents of sage were 122.98 mg/l, according to **Viuda-Martos *et al*. (2010**). Sage has a total phenolic content of 27.94 mg GAE/100g DW and a total flavonoid content of 27.54 mg/CE/100 mg DW (**Atanassova *et al*., 2011**). Green tea extract with approximately 50% polyphenols, chlorophyll KK, oregano oil, peppermint oil, clove bud oil, lavender oil, thyme oil, cinnamon bark oil, eucalyptus oil, and mixtures there of **D'Amelio and Mirhom (2010)** found that each of these ingredients can be combined in various proportions to get the necessary antibacterial and anti-inflammatory characteristics in the final mixture. Green tea's chemical makeup is a muddled mess. Polyphenols, specifically flavonoids like catechins, catechin gallates, and proanthocyanidins, are the compounds in green tea that are most predominant (**Graham, 1992)**.

In Table 2, the GC results of myrrh *(Commiphora molmol*) essential oil show that curzerene is the major constituent (40.0%) followed by Furanoeudesma-1,3-diene, β-elemene then 2-O-acetyle1-8,12-epoxy-germacra-1(10),4,7,11-tertraene, isomer with percentage of 4.5, 8.0 and 6.0%, respectively. When evaluated separately, a number of these chemicals showed strong antibacterial activity, according to several studies **(Ben Arfa *et al*., 2006; Bassolé *et al*., 2010 and Bajpai *et al*., 2012**). **Chen *et al.* (2013)** found that the highest percentage component in myrrh essential oil was(12.01%) 2-cyclohexen-1-one, 4-ethynyl-4-hydroxy-3,5,5-trimethyl, followed by -elemene, copaene, and aromadendrene (6.18, 5.50 and 4.62 % , respectively). In contrast, new components such as Coaene and -amorphene were found in myrrh oil (5.50 and 1.96 % , respectively). Other components of Ethiopian species include furanodiene (19.7%), furanoeudesma-1,3-diene (34.0%), and lindestrene (12.0%) (**De Rapper *et al*., 2012)**

The essential oils of the thyme *(Thymus vulgaris L.)* were examined by GC and represented in Table (3). Thymol is the major constituent (46.3%) followed by p-Cymene, γ-Terpinene then Caravcol (23.2, 18.3 and 1.5%, respectively). These results agreed with those of **Asllani and Toska (2003)** as they reported that the primary components found were thymol (21.38–60.15 %), p-cymene (7.76–43.75 %), -terpinene (4.20–27.62 %), carvacrol (1.15–3.04 %), and -caryophyllene (1.30–3.07 %). The terpene phenols thymol and carvacrol, according to **Stahl-Biskup (1989)**, are the most significant chemicals in the genus, followed by linalool, p-cymene, -terpinene, borneol, terpinen-4-ol, and 1, 8-cineol. Terpinen-4-ol (13.1 %), -terpinene (9.2%), cis-sabinene hydrate (7.6%), linalool (7.1 percent), and p-cymene (5.7 percent) were the primary ingredients in thyme essential oil (**Viuda-Martos *et al*., 2010**). Thymol, carvacrol, p-cymene, -terpinene, linalool, -myrcene, and terpinen-4-ol are the primary constituents. Some chemicals are glycosides in part (**Committee on Herbal Medicinal Products, 2013).** The phenolic components of Thymus and Origanum species, such as thymol and carvacrol, have been linked to high antibacterial activity (**Sokovi *et al*., 2009 and Hazzit *et al*., 2009**). Essential oils' antibacterial action is dependent on their chemical ingredients, according to **Boruga *et al.* (2014).** The content of phenolic chemicals (thymol) and terpene hydrocarbons (γ-terpinene), respectively, appears to be connected to the antibacterial activity of the essential oil studied (**Boruga *et al*., 2014**).

However, p-cymene has been linked to synergistic effects with thymol and -terpinene, which could be another explanation for the antibacterial activity found. However, multiple studies have found that essential oils have more potent antibacterial properties than their primary constituents or mixtures, indicating the synergistic effects of minor constituents and the significance of each component in connection to EOs biological activity **(Gill *et al*., 2002; Rota *et al*., 2008 and Dorman and, Deans, 2000).**

In Table (4), the GC results of sage (*Salvia officinalis* L.) essential oil show that α-Thujone is the major constituent (35.6%) followed by camphor, α-pinene and β- Thujone (23.0, 9.7 and 8.6%, respectively). According to **Craft (2017),** The sage oils were primarily composed of the monoterpenoids thujone (17–27%), 1,8-cineole (12–27%), and camphor (13–21%), with low amounts of thujone (3.8–6.0%), camphene (3.5–5.3%), and the sesquiterpene humulene (3.1-4.4%). This chemical profile is similar to many sage oil descriptions previously reported by (**Jirovetz *et al*., 2006; Alizadeh and Shaabani, 2012; Seidler-Ło˙zykowska *et al*., 2015**). Camphor (25.0%), 1,8-cineole (24.7%), and camphene were the predominant components in sage essential oil (7.6 percent). The most abundant compounds in clove essential oil were eugenol (85.5%) and β-caryophyllene (10.5%) **(Viuda-Martos *et al*., 2010**). The findings of **MITIĆ-ĆULAFIĆ *et al*. (2005)** show that thujone, among the primary sage monoterpenes, is harmful to all microorganisms examined. The antibacterial activity of EO and fractions, on the other hand, is unrelated to their amount of thujone, cineole, or camphor, implying that their antibacterial impact is likely due to a synergistic interaction of numerous elements. According to **Selim *et al*. (2022),** the GC study of sage (*Salvia officinalis*) EO revealed 21 different components, accounting for 89.94% of the total oil component. 1,8-cineole (39.18 %), β -caryophyllene (12.8 %), and α -terpineol were the most common chemicals (10.3 %).

In Table (5), the GC results of chamomile (*Matricaria recutita L.*) essential oil show that α-bisabolol is the major constituent (46.4%) followed by Terpinen-4-ol (22.1%), β–bisabolol (7.3%) then Viridiflorene (6.1%). These findings are consistent with those of **Afify *et al*. (2012),** who found that α-bisabolol oxide A (35.251 %) and trans-α-farersene are the most abundant essential oils in chamomile (7.758 %). Bisabolol oxides, bisabolone oxide, α-bisabolol, spathulenol, enyne-dicycloethers, and chamazulene were shown to be the most physiologically active chemicals in chamomile oil, according to **Orav *et al*. (2010).** Meanwhile, **Raal *et al*. (2012)** discovered that chlorogenic acids, ferulic acid glycosides, dicaffeoyl quinic acids, and apigenin glycosides were the predominant phenolic components in chamomile infusions.

In Table (6), the GC/MS results of green tea *(Camellia Sinensis)* essential oil show that terpinen-4-ol is the major constituent (30.3%) followed by β-Linalool, γ-terpinene and α- terpinene (13.3, 10.8 and 10.0%, respectively). On the other hand, hexadecanoic acid, heneicosane, trans-geraniol, and nerolidyl acetate are the primary components of GT essential oil, according to **Hu *et al*. (2010),** accounting for 30.2 %, 7.36 %, 7.02 %, and 4.7 %, respectively. The strongest bactericidal effects were observed in EOs containing terpene alcohols, followed by those of aldehydes or phenols, such as cinnamaldehyde, citral, carvacrol, eugenol, or thymol. Other EOs, including -myrcene, -thujone, or geranyl acetate, have significantly less effects when combined with ketones or esters. Terpene hydrocarbon-containing volatile oils were primarily inactive **(Barros *et al*., 2009 and Ait-Ouazzou *et al*., 2011)**.

Sensory evaluation of the tested herb's volatile oils on rinsing are recorded in Table (7). Results show that *mouthwash*es with diluted essential oils (5 *mg*/100 *ml* water) of myrrh, thyme, sage, chamomile and green tea were chosen to be the best ones according to the sensory evaluation as they recorded the highest scores for taste, odor, texture color and overall acceptability as compared to (10 and 15) *mg*/100 *ml*. It could be noticed that these sensory parameters decreased significantly ($p<0.5$) by increasing the herbs oil concentration in *mouthwash*. Myrrh is utilised in spices, skin ointments, and fragrances because of its essential oil, and its tincture is used to treat oropharyngeal inflammations (**Brieskorn *et al.,* 1982**). Thyme is a single species herbaceous plant that grows in hilly locations and is used as a beverage instead of or in addition to tea. It is also used to some foods to give them a pleasing flavour. The plant is commonly used in folk medicine, where it is given to heal oral infections (**Mohamed *et al.*, 2013**). Sage has been used in the food industry as a flavouring ingredient (**Gali-Muhtasib *et al.*, 2000**). German chamomile flower heads have been used to make extracts and herbal beverages. German chamomile flower heads and extracts are used in a variety of herbal medicines, herbal teas, cosmetics, food tastes, dyes, and pest repellents (**Singh *et al.*, 2011**). Tea is one of the most popular beverages in many cultures, second only to water (**Pastoriza *et al.*, 2017**). Its peculiar flavour, scent, and health-promoting properties, as well as its socio-cultural associations, are highly loved around the world (**Komes *et al.*, 2010**).

Data represented in Table 8 showed that rinsing with the tested herbs Eos (5% concentration) significantly ($p<0.5$) controlled the total microbial counts in saliva compared to the control group. Saliva of the myrrh EO mouthwash group recorded the lowest total microbial counts (2.69±0.06 log cfu/ml), followed by the saliva of the thyme EO mouthwash group (2.75±0.27 log cfu/ml), saliva of sage EO mouthwash group (3.11±0.04 log cfu/ml), saliva of chamomile EO mouthwash group (3.30±0.06 *log/cfu/ml*), and saliva of green tea EO mouthwash group (3.36±0.04 log cfu/ml). A significant decrease in saliva total microbial counts was observed in the tested herbs groups comparing with the control group which recorded the highest total microbial count (4.20±0.04 log cfu/ml). Myrrh tincture is listed in the British Herbal Pharmacopoeia (**BHMA 1996**) as a mouthwash for gingivitis and ulcers. Myrrh was approved by the European Commission (**Blumenthal *et al*. 2000**) for topical treatment of mild oral and pharyngeal mucosa inflammation. Myrrh has recently been discovered to have antibacterial properties against resistant forms of *S. aureus, S. enterica*, and *K. pneumoniae* (**Rahman *et al*., 2008**). The greater antifungal activity of the myrrh extract film corresponds to the ayurvedic practice of using myrrh extract for oral and vaginal hygiene, antiseptic action, and as a natural antibiotic (**Treadway, 1998**). Additionally, *S. officinalis* EO at 5% concentration shown outstanding in vitro inhibitory action toward the biofilm formation of various S. enterica isolates, according to **Selim *et al.***

(2022). Ferrazzano *et al*. (2011) found that a green tea extract was effective against cariogenic oral bacteria. The antibacterial impact of the herbal mouth rinse was most likely the reason for its success. Antimicrobial effects of goldenseal were found in oral pathogens such as *S. mutans and Fusobacterium. nucleatum* **(Pourabbas *et al*., 2005).**

Inhibitory effect of tested herbs volatile oils on oral bacteria log cfu/ml) of saliva groups shown in Table 9. Significant ($p<0.5$) differences were observed between the control and herbs EO (5% concentration) mouthwash groups in *Streptococcus mutans* and *Lactobacillus rhamnosus* counts. Rising with herbs EOs controlled the oral bacteria in saliva groups. The lowest *Streptococcus mutans* count was for saliva of the myrrh EO mouthwash group (2.0±0.05 log cfu/ml), followed by saliva of the sage EO mouthwash group (2.30±0.17 log cfu/ml), saliva of chamomile EO mouthwash group (2.69±0.15 log cfu/ml), and saliva of green tea EO mouthwash group (2.77±0.22 log cfu/ml *l),* then saliva of thyme EO mouthwash group (2.84±0.10 log cfu/ml). Meanwhile, *Lactobacillus rhamnosus* counts were recorded (2.77±0.12, 2.69±0.09, 2.95±0.04, 2.84±0.04 and 2.90±0.05 log cfu/ml) for the same salivia groups, respectively. The control group recorded the highest *Streptococcus mutans* and *Lactobacillus rhamnosus* counts in saliva (3.30±0.11 and 3.11±0.03 log cfu/ml) respectively. In dentistry, herbal extract is utilized as a teeth cleanser, anti-inflammatory, antibacterial, and analgesic. Because of their safety and low cost, herbal remedies are used by 80 percent of the world's population for basic health care, according to the WHO. *Commiphora myrrha* is used in dentistry to treat bad breath and as an anti-inflammatory in periodontitis (**Kumar *et al*, 2013**). Antimicrobial activity of thyme essential oil against oral bacteria such as *Staphylococcus aureus, Streptococcus mutans, Lactobacillus spp., Pseudomonas aeroginosa,* and *Proteus spp.*was studied **(Al- Mahdy *et al*., 2021**). On oral bacteria strains, the effects of thymol, a plant-derived antibacterial agent, were investigated. The relationship between thymol leakage producing concentrations and minimal inhibitory and bactericidal concentrations suggests that membrane perforation is a major mechanism of action for this drug (**Shapiro and**

Guggenheim, 1995). The antibacterial properties of plant oils were evaluated against oral germs; the most potent essential oils were Australian lea tree oil, peppermint oil, and sage oil, with thymol and eugenol as potent essential oil components (**Shapiro *et al.*, 1994**). Catechins are inhibitory for *Streptococcus*. mutans and *Streptococcus*. sobrinus, according to several researchers, with MICs ranging from 50 to 1000 mg/ml, well within the quantities observed in brewed tea (**Sakanaka *et al.* 1989**; **Kawamura and Takeo 1989**; **Rasheed and Haider 1998**).

Zones of growth inhibition (*mm*) of tested herb's volatile oil components (5% concentration) on oral bacteria are registered in Table 10. *Streptococcus mutans* zone growth values were recorded (10, 8, 9, 7 and 8 *mm*), while *lactobacillus rhamnosus* zone growth values recorded (8, 10, 10, 6 and 8 *mm*) for myrrh EO, thyme EO, sage EO, chamomile EO and green tea EO, respectively. It is noticeable that myrrh EO was more effective in controlling *Streptococcus mutans* growth, followed by sage EO, thyme EO, green tea EO and chamomile EO. Meanwhile, thyme and sage essential oils were more effective in *Lactobacillus rhamnosus* growth inhibition, followed by myrrh and green tea EOs then chamomile EO. Oral ulcers, gingivitis, sinusitis, glomerulonephritis, brucellosis, and a range of skin ailments are treated with myrrh, which possesses antibacterial properties (**Shin *et al.*, 2019**). An interesting antimicrobial activity of myrrh extract was demonstrated by **Becheker *et al*. (2022)**, especially in relation to Gram-negative bacteria. The inhibition zones range from 16 to 30 mm, while the MIC values range from 15.62 to 250 *mg/ml*. The most effective against *Streptococcus mutans* was a 1 percent solution of thyme essential oil in ethanol, which might be deemed practical as a toothpaste ingredient, both in terms of cost and sensory profile. Furthermore, tests of the formulation's features revealed that the product is stable (**Gonçalves *et al.*, 2011**). The inhibitory impact of direct exposure was studied using the plate counting approach. Against the bacteria tested, all of the Thyme essential oils tested demonstrated considerable bacteriostatic activity. Against gram-positive bacteria, its action was more evident (**Marino and Bersani, 1999**). The number of *Streptococcus mutans* in tooth plaque was successfully reduced by using Sage mouthwash (**Beheshti-Rouy *et al*., 2015**). According to **Ahmed *et al*. (2017),** green tea and chamomile tea drinking reduces salivary pH and inhibits salivary *Streptococcus mutans* count. In impoverished nations like India, a green tea mouth rinse can be an effective preventive home treatment. In addition, **Neturi *et al*. (2014)** discovered that rinsing with green tea reduced the number of *S.mutans* in plaque when compared to the gold standard mouthwash as a control.

Table (1): Total phenol and flavonoid *mg/100g* contents of dry herbs

Herbs essential oils	Total phenol *mg/100ml*	Flavonoid *mg/100ml*
Myrrh	114.8	87.6
Thyme	941.7	154.6
Sage	1916.2	1360.1
Chamomile	58.9	716.4
Green tea	79.4	37.2

Table (2): Volatile components of myrrh essential oil.

Compounds	%	Compounds	%
Curzerene	40.0	*cis*-β-elemenone	0.9
Furanoeudesma-1,3-diene	14.5	γ-cadinene	0.7
β-elemene	8.0	β-caryophellene	0.7
2-O-acetyle1-8,12-epoxy-germacra-1(10),4,7,11-tertraene, isomer	6.0	β-bourbonene	0.6
2-O-methyl-8,12-epoxy-germacra-1(10),4,7,11-tertraene, isomer	4.0	9-*epi*- caryophellene	0.5
γ-eudesmol	2.5	α-Humulene	0.4
γ-elemene	2.2	γ-Muurolene	0.3
7-*epi*-α- eudesmol	2.0	Elemol	0.2
Alloaromadendrance	1.9	2-hydroxyfuranodiene	0.2
Furanodiene	1.3	Dehydroaromadendrance	0.1

Table (3): Volatile components of thyme essential oil.

Compounds	%	Compounds	%
Tymol	46.3	Terpineol	0.9
p-Cymene	23.2	α-Pinene	0.9
γ-Terpinene	18.3	Linalool	0.8
Caravcol	1.5	Sabinene	0.8
α-Terpinolene	1.4	Camphene	0.7
β –Myrcene	1.3	1,8-Cineol	0.5
α-Terpinene	1.1	Geranic acid	0.3
α- Terpineol	1.1	Citral	0.2
4-Terpineol	1.0	Anisole	0.2
α-Thujone	1.0	Geraniol	0.1

Table (4): Volatile components of sage essential oil.

Compounds	%	Compounds	%
α-Thujone	35.6	β-Caryophyllene	0.9
Camphor	23.0	β –Pinene	0.8
α-Pinene	9.7	Tricyclene	0.7
β- Thujone	8.6	α-Terpinolene	0.5
Camphene	7.9	γ-Terpinene	0.4
1,8-Cineole	5.9	Terpinene-4-ol	0.4
Limonene	1.7	α- Terpinene	0.2
α-Humulene	1.6	Thymol	0.1
Borneol	1.4	α-Phellandrene	0.1
Myrcene	1.2	Myrtenol	0.1

Table (5): Volatile components of chamomile essential oil.

Compounds	%	Compounds	%
α-bisabolol	46.4	Trans-nerolidol	1.0
Terpinen-4-ol	22.1	Guaiazulene	0.6
β –bisabolol	7.3	Spathulenol	0.5
Trans-trans-farnesol	6.6	α-phellandrene	0.4
Viridiflorene	6.1	Cis-β-farnesene	0.3
β-bisabolene	2.1	Chamazulene	0.3
α-cubebene	1.9	Methyl acetate	0.3
α-pinene	1.4	Sabinene	0.3
α-bisabolol oxide A	1.4	T-terpinene	0.3
Caryophyllene oxide	1.2	β-pinene	0.1

Table (6): Volatile components of green tea essential oil.

Compounds	%	Compounds	%
terpinen-4-ol	30.3	α- pinene	2.0
β-Linalool	13.3	p-Cymene	2.0
γ-terpinene	10.8	Nerol	1.7
α- terpinene	10.0	1,2-Epoxylinalool	1.4
trans-Geraniol	6.8	1,8- Cineole	1.2
Heneicosane	6.5	Geranyl acetone	1.0
Nerolidyl acetate	4.9	1β-Cadin-4-en-10-ol	0.9
α-Terpineol	3.2	n-Docosane	0.8
α-terpinolene	2.3	n-Pentacosane	0.5
6-Pentadecen-1-ol	2.1	Solanone	0.1

Table (7): Sensory evaluation of mouthwashes with herbs essential oils.

Essential oils Samples	Taste (10)	Odor (10)	Texture (10)	Color (10)	Overall acceptability (10)
5% Myrrh	8.4±0.15a	8.7±0.35a	8.5±0.38a	8.9±0.15a	8.6±0.23a
10% Myrrh	8.0±0.60ab	8.4±0.43ab	8.1±0.60ab	8.5±0.29ab	8.2±0.33a
15% Myrrh	7.4±0.31b	8.0±0.41a	7.5±0.41a	7.9±0.69b	7.6±0.26b
5% Thyme	8.2±0.44a	8.0±0.76a	8.5±0.29a	8.8±0.57a	8.3±0.16a
10% Thyme	7.9±0.85a	7.5±0.55ab	8.0±0.39ab	7.7±0.51b	7.7±0.54b
15% Thyme	7.3±0.25a	7.0±0.36b	7.8±0.25b	7.2±0.38b	7.3±0.21b
5% Sage	8.6±0.47a	8.0±0.79a	8.2±0.31a	8.5±0.45a	8.3±0.33a
10% Sage	8.0±0.72ab	7.9±0.72a	7.8±0.60a	8.3±0.25a	8.0±0.36a
15% Sage	7.6±0.38b	7.2±0.58a	7.1±0.29b	8.0±0.79a	7.4±0.15b
5% Chamomile	8.1±0.60a	8.4±0.15a	8.0±0.79a	8.2±0.44a	8.1±0.16a
10% Chamomile	7.5±0.41a	7.4±0.29b	7.6±0.38a	7.2±0.67b	7.4±0.02b
15% Chamomile	6.4±0.29b	6.8±0.60b	6.1±0.69b	6.9±0.34b	6.5±0.09c
5% Green tea	7.4±0.29a	7.8±0.58a	7.5±0.76a	7.5±0.73a	7.5±0.07a
10% Green tea	7.2±0.60a	7.2±0.67ab	7.6±0.93a	7.2±0.75a	7.3±0.55a
15% Green tea	6.5±0.38b	6.6±0.44b	6.3±0.60a	7.1±0.29a	6.6±0.28b

Table (8): Total microbial evaluation (log cfu/ml) of saliva groups after rinsing with herbs EOs (5% concentration).

Groups / TBC	Group A (control)	Group B (Myrrh EO)	Group C (Thyme EO)	Group D (Sage EO)	Group E (Chamomile EO)	Group F (Green tea EO)
Saliva log cfu/ml	4.20±0.04a	2.69±0.06[d]	2.75±0.27[d]	3.11±0.04[c]	3.30±0.06[b]	3.36±0.04[b]

EO:essential oil TBC: Total bacterial count

Table (9): Inhibitory effect of herbs EOs (5% concentration) on oral bacteria (log cfu/ml) of saliva groups.

Groups / Oral bacteria	Control	Myrrh EO	Thyme EO	Sage EO	Chamomile EO	Green tea EO
Streptococcus mutans	3.30±0.11[a]	2.0±0.05 [c]	2.84±0.10[b]	2.30±0.17 [c]	2.69±0.15[b]	2.77±0.22[b]
lactobacillus rhamnosus	3.11±0.03 [a]	2.77±0.12 [bc]	2.90±0.05[b]	2.69±0.09 [c]	2.95±0.04[b]	2.84±0.04[bc]

EO:essential oil

Table (10): Zones of growth inhibition (mm) showing antibacterial activity for herbs EOs (5% concentration) on oral bacteria.

Groups / Oral bacteria	Control	Myrrh EO	Thyme EO	Sage EO	Chamomile EO	Green tea EO
Streptococcus mutans	0.0	10	8	9	7	8
lactobacillus rhamnosus	0.0	8	10	10	6	8

EO:essential oil

Conclusion

Daily use of an antimicrobial mouth rinse that has been shown to have strong antibacterial action against *Streptococcus mutans* and *Lactobacillus rhamnosus* would be a good complement to mechanical oral hygiene treatments and could be a valuable component of oral hygiene regimens. In the prevention and treatment of many human infections, phytopharmaceuticals based on an examined essential oil from myrrh, thyme, sage, chamomile, and green tea may be suitable.

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تأثير بعض الزيوت العطرية علي بعض بكتريا الفم الممرضة للإنسان

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الملخص العربي:

من المعروف أن صحة وسلامة المعدة مرتبطة بصحة وسلامة الفم، لذا تهدف هذه الدراسة الي اختبار فاعلية زيوت الأعشاب المختارة ، على بكتيريا الفم لمحتواها من الزيوت العطرية. حيث تم دراسة النشاط المضاد للبكتيريا من نبات المر (Commiphora molmol) والزعتر (Thymus vulgaris L) والمريمية (Salvia officinalis L) والبابونج (Matricaria recutita L) والشاي الأخضر (Camellia sinensis)، تمت دراسة تأثير الزيوت الأساسية بتركيز (٥ مجم زيت / ١٠٠ مل ماء) على العدد البكتيري الكلي في اللعاب، كما تم دراسة تأثير تلك الزيوت بذات التركيز علي العدد الكلي لكلاً من Streptococcus mutans و . Lactobacillus rhamnosus وكذلك الحد الأدنى من المناطق المثبطة (MIZ) من زيوت نبات المر والزعتر والمريمية والبابونج والشاي الأخضر على Streptococcus mutans و Lactobacillus rhamnosus باستخدام تقنية أجار الحساسية القياسية. حيث تمت معالجة البيانات إحصائيا، وأظهرت المجموعة التجريبية انخفاضاً ذا دلالة إحصائية في تعداد مستعمرات Lactobacillus rhamnosus . و Streptococcus mutans بالنسبة للمجموعة المقارنة. أظهرت هذه النتائج فعالية زيوت الأعشاب المختبرة ضد رائحة الفم الكريهة والفلورا الفموية ، وتحقيق توازن بين بكتيريا الفم الضارة وغير الضارة ، وكذلك فتح طريق واعد للتطبيقات السريرية في تحضير علاجات محددة وطبيعية مضادة للبكتيريا.

توصي الدراسة باستخدام زيوت الأعشاب موقع الدراسة بالتركيز المذكور في السيطرة علي بكتريا الفم الممرضة حفاظا علي صحة المعدة والجسم.

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** زميل تعليمي تخصص تغذية وعلوم الأطعمة بالهيئة العامة للمستشفيات والمعاهد التعليمية - مستشفى الجلاء التعليمي