
Activity of Cloves, Cinnamon and Thyme Essential Oils Against Some Oral Bacteria

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ABSTRACT

Background:

Gastronomic natural flavors and spices have been used as both flavoring agents and preservatives in food preparation for many years. They have also been utilized in medical field for their biological properties shown in previous studies.

Objective:

The study aimed to evaluate the inhibitory effect of the oil extracts from Cloves, Thyme, and Cinnamon compared with ampicillin on some oral bacteria (*Staphylococcus aureus*, *Streptococcus mutans*, *Lactobacillus* spp., *Pseudomonas aeruginosa*, and *Proteus* spp.).

Method:

Evaluation of the antibacterial effect of herbal and spices essential oil was performed using Agar well diffusion test. Its effect was assessed against oral bacteria, which diagnosed in laboratory by culturing on enriched and selective media and by biochemical test.

Results:

High inhibitory effect of cinnamon, clove against some Gram positive and Gram negative isolated oral bacteria (*Staphylococcus aureus*, *Streptococcus mutans*, *Lactobacillus* spp., *Pseudomonas aeruginosa*, and *Proteus* spp. comparing with ampicillin. While thyme essential oil shows inhibitory effect for the bacterial isolates except for *Pseudomonas aeruginosa* but less than the inhibitory effects of other essential oils and ampicillin.

For *Pseudomonas aeruginosa* result shows the bacteria have resistance to ampicillin and Thyme essential oil while both cloves and cinnamon have antibacterial effect.

Conclusions:

Cloves, thyme and cinnamon essential oil have a wide antimicrobial activity against some oral bacteria, both cinnamon and cloves essential oils recorded superior antibacterial activity than ampicillin for (*Proteus spp* and *Lactobacillus*), competence action with ampicillin for *Staphylococcus aureus* and *Streptococcus mutans* and novel antibacterial action against *Pseudomonas aeruginosa* which resist to ampicillin which may be a promising antibacterial management approach and resist development of antibiotic resistance strain if used instead than the antibiotic. Also, suggestion for using these oils orally may provide a degree of protection in oral cavity against some oral pathogens.

Key words: Clove, thyme, cinnamon oil, antibacterial.

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فعالية الزيوت الاساسية للقرنفل والدارسين والزعر ضد بعض البكتريا الفموية

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المستخلص

تم استخدام النكهات والتوابل الطبيعية للطعام كعوامل منكهة ومواد حافظة في تحضير الطعام لسنوات عديدة. كما تم استخدامها في المجال الطبي لخصائصها البيولوجية الموضحة في الدراسات السابقة. هدفت الدراسة إلى تقييم التأثير التثبيطي لمستخلصات زيت القرنفل والزعر والقرفة مقارنة بالأمبيسلين على بعض البكتريا الفموية ومنها

Staphylococcus aureus, *Streptococcus mutans*, *Lactobacillus spp.*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Proteus spp*

تم إجراء تقييم التأثير المضاد للبكتيريا للمستخلص العشبي (الزيت) باستخدام اختبار فحص الانتشار من الحفر، تم تقييم تأثيره ضد بكتيريا الفم، والتي تم تشخيصها في المختبر عن طريق الزراعة على الاوساط الاغنائية والانتقائية والاختبار البيوكيميائي.

النتائج: ارتفاع مستوى التأثير المثبط للقرفة والقرنفل وزيت الزعر على بعض بكتيريا الفم الموجبة والسالبة الجرام (*Staphylococcus aureus*، *Streptococcus mutans*، *Lactobacillus spp*، *Escherichia coli*، *Pseudomonas aeruginosa* و *Proteus spp*) مقارنة بالأمبيسلين.

أظهرت بكتريا *Pseudomonas aeruginosa* مقاومة للأمبيسيلين وزيت الزعتر الأساسي بينما كل من القرنفل والقرفة لهما تأثير مضاد لهذه البكتيريا.

استنتجت الدراسة الحالية بان الزيوت العطرية للقرنفل والقرفة والزعتر تملك نشاط مضاد للميكروبات واسع النطاق ضد بعض بكتيريا الفم، كلا من الزيوت العطرية للقرفة والقرنفل سجلت تأثير مضاد اعلى من التأثير المثبط للامبسلين على كل من بكتريا *Proteus spp* و *Lactobacillus* وتأثيرا منافس للامبسلين بالنسبة لبكتريا *Staphylococcus aureus* و *Streptococcus mutans* كما لها تأثير مضاد لبكتريا *Pseudomonas aeruginosa* التي تقاوم الامبسلين. هذه الدراسة تعتبر طريقة واعده في التضاد البكتيري ومقاومة ظهور سلالات مقاومة للمضادات الحياتية عند استخدامها بدلا عن المضاد الحياتي. اقترح استخدام هذه الزيوت فمويا ربما يوفر درجة من الحماية في التجويف الفمي من بعض البكتريا المرضية الفموية.

الكلمات المفتاحية: القرنفل، الزعتر، زيت الدارسين ومضاد بكتيري .

1- Introduction

Natural herbal elements and spices have been utilized as food additives (flavoring agent and preservatives) for hundreds of years. In addition, they have been used for medical purposes [1]

The increased exposure to antibiotics during eradication of bacterial infections has led to the development of resistant strains of bacteria. Not only for a certain antibiotic, but also there may be multidrug resistance. Recently, there has been a growing number of studies focusing on the use of natural botanicals as alternative antimicrobial agents (active or preservative) in topical preparation as well as in formulation for systemic use [2]. These cumulative works are mainly determined by safety and environmental concerning [3].

Essential oils have been prescribed to treat oral diseases as oral wash either conventionally or clinically in many periodontal disorders [4]. In addition to their ability to control resistant bacteria and other pathogenic microorganism [5].

Syzygium aromaticum L. belongs to family Myrtaceae. In Hindi this plant is famous as laung. It is an evergreen tree, native of Indonesia and Mallaca islands distributed in tropics of the old world. It is cultured in Tanzania, Indonesia, Penang, Malagasy, Mauritius as well as Srilanka. The extracted essential oil is used commonly as a food additive by Chinese. Also, it has been shown to improve digestion and inhibit growth of microorganism. Two of the main phenolic constituents of clove essential oil are eugenol and eugenyl acetate. Clove is a natural

analgaesic and antiseptic used mainly in dentistry for its main ingredient eugenol. It is used as home remedy for dental pain relief, mainly toothache [5] also used in approach to deal with antibiotic resistance problem and have synergistic outcome of combination of essential oils and antibiotics, and the possibilities of essential oil as the possible resistance modifying agent was highlighted [6].

The genus *Thymus* belongs to the Lamiaceae family. The leaves of thyme have been used in food as flavor, aroma and preservation. Its use as folk medicine is well known, particularly in Mediterranean countries since a long time ago [1].

The major components essential oil of thyme (*Thymus* Spp, *T. citriodorits*, *T. vulgaris-Labiatae/ Lamiaceae*) involve 20-40% thymol and carvacrol in addition to borneol, cineol, linalool, menthone, B-cymene, pinene and triterpenic acid. Thyme oil has a useful effect on human digestive system and can be used in the treatment of gastritis, enterocolitis and mouth ulcers. In addition, it showed activity against respiratory infections as well as effectiveness in cases of chronic obstructive airway disease. Furthermore, it has shown benefit in the management of gout, arthritis and other types of joint pain. Nevertheless, the antimicrobial effect of thyme is the most extensively investigated effect by studying the role of *Thymus vulgaris* L. (common thyme, German thyme) in inhibiting microbial growth. Several studies have shown that it is helpful in genitourinary tract infections [7]. In addition, it showed an effect against tooth infections [8] an effect that is thought to be a synergistic effect of thymol with other constituent in thyme or other essential oils. Its effect in decreasing dental caries may be due to its activity against *S. mutans* [7]. In patients with orthodontic brackets, a dental varnish containing thymol decreased the growth of *Streptococcus mutans* in supragingival plaque adjacent to bracket [8]. Thymol is among the essential oils that have antibacterial action in Listerine [9]. According to Gislene and colleagues [10], Hili and colleagues [11] and Nzeako and colleagues [12]. Cinnamon is a bark spice obtained from the inner bark of several tree species from the genus *Cinnamomum*. It is frequently used as spices. The main aromatic essential oil components of cinnamon giving its special flavor are cinnamaldehyde (up to 90%) and eugenol. In addition, at least 80 other constituents are recognized to be found in cinnamon oil, such as cinnamyl alcohol, cinnamyl acetate and various coumarone that add to its general taste and smell [1]. *Cinnamomum zeylanicum* such as Blume (cinnamon) is widely studied [11]. Cinnamon

essential oils have been incorporated widely in folk medicine for many years. Antibacterial activity of cinnamon oil has been recorded [11,13].

Antibacterial activity of cinnamon oil against oral pathogenic bacteria especially the pathogens in relation to periodontal disease, are still scanty [14]. Thus, this work concern for assessment the anti-bacterial activity of these herbal and spices essential oils against some oral bacteria in comparison with the broad spectrum anti-bacterial activity of ampicillin [15].

2- Materials and Methods

Herbal and spices essential oils

Herbal and spices essential oils (100% purity) were purchased from herbal shop in local markets these includes clove oil, thyme oil (HEMANI, Pakistan) and cinnamon essential oil.

Antibiotic

Broad spectrum ampicillin antibiotic [15] with concentration 10 mcg was used for study the antibacterial activity of herbal and spices essential oil in compare with activity of ampicillin.

Bacterial Isolates

Oral bacterial species (Gram positive & Gram-negative bacteria) were obtained from research unit-1 at Collage of Dentistry/ University of Babylon. These bacteria previously diagnosed in laboratory by culturing on enriched and selective media and by biochemical test.

In Vitro Assessment of Antibacterial Activity Using Agar Well Diffusion Assay

The agar well diffusion technique was applied for the detection of antibacterial action of herbs' essential oils. Swab from bacterial growth was inoculated into liquid media and incubated at 37 °C for 18 hours. Normal saline was used for diluting bacterial suspensions. The suspension turbidity was adjusted to be comparable with standard tube (McFarland 0.5) to get an average suspension containing 1.5×10^8 CFU/ml. Muller- Hinton agar was prepared and poured 25 ml for each Petri dish and streaked by swab of bacterial suspension according to Kirby- Bauer techniques, wells were created on the culture media using the head of blue, 1000 μ l 9.8 x 70 mm pipette tip (Then 50 μ l of herbal and spices essential oils were added, then incubated at 37 C for 24 hours. All readings were measured in triplicate and zones of inhibitions were measured as diameter of inhibition zones in millimeter [16].

Table 1: Bacteria isolates used in this work.

Gram positive bacteria	Gram negative bacteria
<i>Staphylococcus aureus</i>	<i>Pseudomonas aeruginosa</i>
<i>Streptococcus mutans</i>	<i>Proteus spp.</i>
<i>Lactobacillus spp.</i>	

The activity of essential oils was compared to the effect of standard antibiotics, ampicillin, a wide spectrum antibiotic with an activity against many Gram-positive and Gram-negative bacteria.

Statistical Analysis

Means of triplicate of zones of inhibition as well as standard division were calculated by excel computer program.

Graph were performed using GraphPad Prism version 8.0.0, GraphPad Software, San Diego, California USA, 2020).

3- Results

Our current study as explained in **table-2** and fig.1 shows that both cinnamon essential oil and ampicillin have antibacterial activity against the pathogenic bacteria *Staphylococcus aureus* followed by cloves and thyme essential oil. Cinnamon essential oil shows the highest antibacterial activity against cariogenic bacteria *Streptococcus mutans* followed by ampicillin, clove and thyme.

For *Lactobacillus spp.* cloves shows the highest activity followed by ampicillin, cinnamon and thyme.

For *Pseudomonas aeruginosa* result shows the bacteria have resistance to ampicillin and thyme essential oil while both cloves and cinnamon shows antibacterial effect.

Cinnamon essential oil shows the highest activity against *Proteus spp* followed by ampicillin and cloves essential oil. Table-2, **fig.1**.

Thyme essential oil shows inhibitory effect for the bacterial isolates except for *Pseudomonas aeruginosa* but less than the inhibitory effects of other essential oils and ampicillin.

Table-2: Zone of inhibition induced by essential oils under investigation compared to ampicillin in a variety of bacterial species (Numbers represent the triplicate mean \pm SD).

Treatment	Zone of inhibition for each bacterial isolate				
	<i>Staphylococcus aureus</i>	<i>Streptococcus mutans</i>	<i>Lactobacillus</i> spp.	<i>Pseudomonas aeruginosa</i>	<i>Proteus</i> spp.
Cloves	41.66 \pm 2.88mm	23.33 \pm 7.63mm	35 \pm 5mm	21.66 \pm 5.77mm	16 \pm 3.60mm
Cinnamon	43.33 \pm 2.88mm	31.66 \pm 2.88mm	30 \pm 5mm	16.66 \pm 10.4mm	36.66 \pm 5.77mm
Thyme	26.66 \pm 10.40mm	13.33 \pm 5.77mm	18.33 \pm 12.58mm	0 \pm 0	3.66 \pm 1.15mm
Ampicillin	43.33 \pm 2.88mm	26.66 \pm 7.63mm	33.33 \pm 5.77mm	0 \pm 0	21.6 \pm 5.7mm

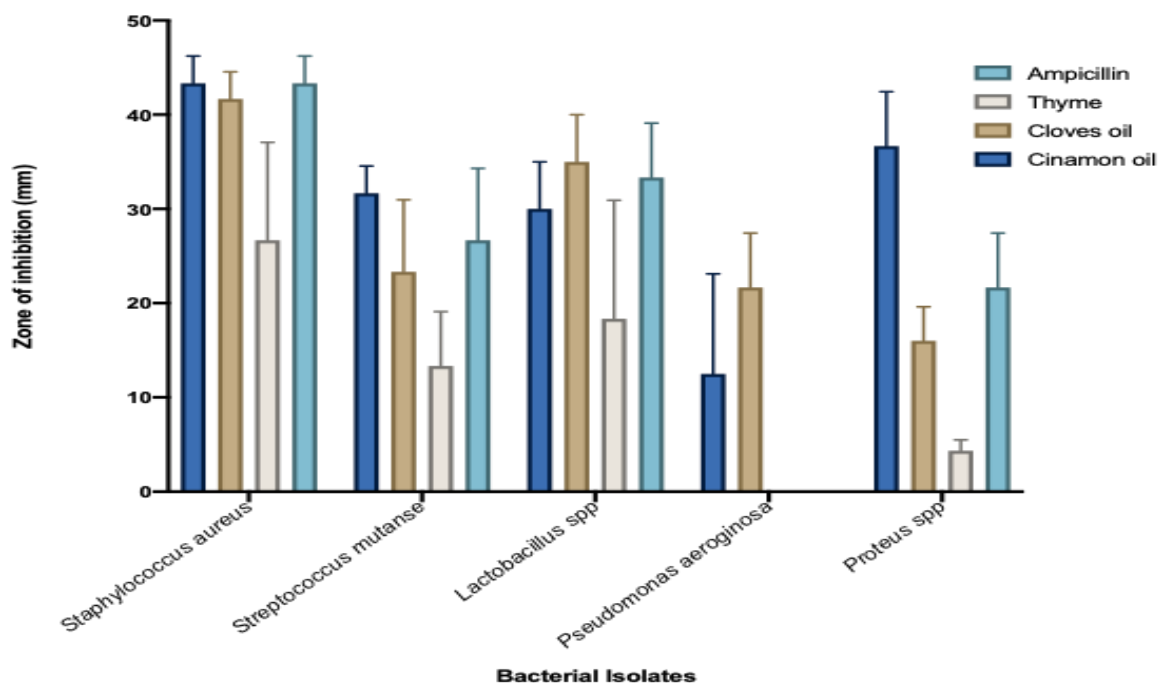


Fig. 1: Bacterial inhibition zone induced by cinnamon, cloves and thyme essential oil compared to ampicillin. (bar represent the triplicate mean \pm SD)

4- Discussion

Dental caries and periodontal disease are among the very frequent suffer of the mouth all over the world [17].

Dental plaque is characterized by diversity and complexity that why it is one of the frequently developed and propagated mouth disorders [18]. A lot of studies focused on using medicinal plant for treatment infectious disease [19-21].

Our findings showed that cinnamon oil is more effective as antibacterial than clove and thyme oil against several species of bacteria when compare the effect of these essential oils to the effect of ampicillin. Such effect of cinnamon essential oil was the most powerful against all the bacterial species investigated. These findings are consistent with those obtained by Rabuseenivasan and colleagues [22]. On the other hand, it was revealed that cinnamon, clove and rosemary oils have strong and consistent inhibitory activity against different pathogens [23]. The antibacterial effect of the oils may be attributed to the hydrophobic characteristics of essential oils and their constituents which allow them to penetrate the lipid parts of the bacterial cell wall, change the cell structure and increase membrane permeability [24, 25]. Hence, there will be extensive leakage of essential molecules and ions through the bacterial cell wall leading death [26].

The chief constituent of cinnamon, cinnamaldehyde, has antimicrobial activities on microorganisms, as it inhibited the biosynthesis of cell wall, vital functions of cell membrane, and activity of various specific enzymes. There may be more specific cellular targets of cinnamaldehyde that can be clarified in prospect research [27].

Clove (*Eugenia caryophyllata*) is one of the Myrtaceae family. It is extensively utilized in medicine as antiseptic against infectious disorders like periodontal infectious pathologies due to its antimicrobial effects against various bacterial species in the mouth [28].

Results recorded that thyme essential oil shows inhibition effect against each of *Staphylococcus aureus*, *Streptococcus mutanse*, *Lactobacillus* spp and against *Proteus* spp, however this effect was lower than the effect of ampicillin.

Thyme and clove oil extracts are recognized to have some antimicrobial effects. They are utilized in several food preparations to improve their flavor and also in alternative medicine [29, 30]. In spite of the fact that the exact mechanism of action of the extracts are not fully explained, the antimicrobial activity of ingredients such as thymol, terpenes, eugenol, flavones, glycosides of phenolic monoterpenoids and aliphatic alcohols among other components widely used [31, 32, 33] may be due to single or synergism with each other leading to a broad spectrum of antimicrobial effects against both bacteria and fungi. Thyme and cloves oil have antibacterial activity against *S. aureus* [34]. *Pseudomonas aeruginosa* possess percent of antibiotic resistance for wide range of antibiotics [35], in our study this bacterium recorded resistance to ampicillin.

Both cinnamon and cloves essential oil have antibacterial activity against *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli* [36]. So, our present study confirmed the other previous works.

5- Conclusions:

Most herbs and spices essential oil shows significant inhibition against oral bacteria, and these results may be have benefit for human health and may be resist development of antibiotic resistance bacteria due to access using of antibiotics.

Also cloves and cinnamon essential oil shows wide spectrum of inhibition against all types of bacteria were used in our study and it shows higher inhibition activities than ampicillin. And both clove and cinnamon essential oil recorded anti *Pseudomonas aeruginosa* activity which resist to ampicillin.

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