

## EFFECT OF AQUEOUS EXTRACT OF EUCALYPTUS LEAVES ON SOME TYPES OF GERMS THAT CAUSE OF PERIODONTAL INFLAMMATION

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### Abstract

This study was conducted in order to identify the bacterial species that cause gingivitis, where 25 swabs were taken from the gums of gingivitis patients who were visiting specialized dental clinics in the city of Babylon between November 2022 and March 2023. To identify and isolate the bacteria that cause gingivitis, some microscopic and biochemical studies were carried out. This study revealed the existence of several types of bacteria that cause gingivitis, including: Streptococcus salivarius (2.59%), Streptococcus angiosus (2.59%) Staphylococcus aureus (7.78%), Escherichia coli (5.93%), Streptococcus mutans (9.25%), Streptococcus pyogenes (4.44%) Pseudomonas aeruginosa (3.33%), Pseudomonas monilli (1.85%). The effect of the aqueous extract of eucalyptus leaves was also tested on the bacterial isolates above. It was found through the test that this aqueous extract of eucalyptose leaves had an inhibitory effect on the isolated bacteria, and the concentration (100 mg / ml) showed the highest inhibitory effect on the growth of bacteria. Knowing that the bacteria isolated from patients with gingivitis were sensitive to varying degrees to the antibiotics that were used in this study for the purpose of comparison, which included: ampicillin, tetracycline, Amikacin, amoxicillin, trimethoprim. The study also showed that there is a synergistic activity between the aqueous extract of eucalyptus leaves with the antibiotic Amikasin for the growth of bacteria. The study concluded that the extracts of some medicinal plants should be used as an alternative to antibiotics.

**Keywords:** Amikacin, eucalyptus, ampicillin, bacteria.

### I. Introduction

The use of antibiotics such as Ampicillin, Clindomycin, Tetracycline, Kanamycin, trimetaprim and Erythromycin played an important role in treating bacterial infections, including gingivitis, for many years, but many problems arose in their use, such as the emergence of resistant strains. It is worth noting that bacteria have two types of resistance, natural resistance and acquired resistance. Gram-negative bacteria have a natural resistance to a large number of highly effective antibiotics affecting Gram-positive bacteria. This resistance is due to the presence of the lipopolysaccharide layer in their cell wall, which prevents the permeability of the inhibitory concentrations. Among the antigens into the bacterial cell. Brooks et al., (1998), and the problem lies in the acquired resistance, which has increased rapidly in recent years. Therefore, recent studies tended to use plant extracts in treatment, as they were characterized by their effectiveness and ease of obtaining them, in addition to being inexpensive and non-polluting to the environment. Yadav and Kumar., (2006). The use of a mixture of antibiotics has many benefits, including reducing the chances of emergence of resistant strains, increasing the effect of antibiotic efficacy, and reducing toxicity in the treatment of infections resulting from multiple microbes. Boukef ., (1986). Plants currently occupy an important place in agricultural and industrial production and are the main source of medical drugs and active substances that are used in the preparation of medicines or used as raw materials for the production of a number of chemical compounds that are used in the manufacture of a number of Among the important drugs. Hernandez et al., (1999), and it became known at the present time that plants contain basic substances such as carbohydrates and proteins that play a role in medicine. He mentioned that the active substances in plants are affected by many factors, including soil conditions, climate, method of collection and preservation. The eucalyptus leaves, is one of the important medicinal plants whose flowers were used. Its oil has been used worldwide and for



generations to relieve toothache. extract of eucalyptus leaves has anti-inflammatory properties, as it contains Eugenol, which acts as an analgesic and antibiotic Cowan., (1999). These are utilized in the pharmaceutical and cosmetic sectors to produce essential oils due to their high level of 1,8-cineole (more than 70%). Despite the fact that eucalyptus is widely planted for the production of pulp, plywood, and solid wood, its leaf aromatic oil has remarkably diverse biological activities, such as antimicrobial, antiseptic, antioxidant, chemotherapeutic, treating gastrointestinal and respiratory disorders, and healing wounds. Ashok. K et al (2017). Numerous studies have been conducted on the effect of microorganisms on teeth and gums. These studies have shown that there are streptococcus bacteria. Juergens et al., (1998). The eucalyptus oil slows the growth of some microorganisms due to the presence of some phenolic compounds that have the ability to ignite types of bacteria, including Thymol and Carvacrol. The terpene compound Eucalyptol (Cineole), which is a major compound in the volatile oils of the Eucalyptus plant (the compound responsible for its bactericidal activity), Elbanna, (2006).

## II. Materials and Methods

**Sample collection and examination:** Twenty five swabs were collected from the gums using cotton swabs after the infection was diagnosed by the specialist doctor from the specialized dental clinics in the city of Babylon, and for the period from November 2022 to March 2023. The swabs were transferred to the laboratory of the College of Medicine for the purpose of developing them on Macconkey agar media and blood agar, then The dishes were incubated at a temperature of 37 °C for 24 hours, and after the end of the incubation period, the growing colonies were diagnosed based on the phenotypic characteristics of the colonies and the papokinemic characteristics mentioned in Collee et al., (1996). Chemical compounds of the Eucalyptus leaves: The eucalyptus plant has a 19% concentration of phytochemical compounds, a 30% concentration of pinene, and a 28% concentration of cineole, which contains monoterpene compounds, pinocarveola, aromadendrene, cuminaldehyde, bomeal, and globulet at terpenol. These compounds are biologically active. Aggregates were found in the aqueous extract of the Eucalyptus plant's roots, leaves, fruits, and seeds in various plant parts. Glycosides, anthraquinones, saponins, steroids, and tannins(Osawa et al., 1996 ; Zhao, 1997; Sani et al., 2014).

**Preparation of the aqueous extract:** Matkowski ., (2006), in 1994 adopted the process for making the aqueous extract. The portion of the plant under study was dried, then pulverized in a mill. In a glass beaker, 10 g of dry powder and 20 ml of sterile distilled water were combined.100 ml using a high-speed magnetic stirrer for a full day. After allowing the resultant solution to settle for thirty minutes, big particles were removed by filtering it through multiple layers of medical gauze. It was then centrifuged for ten minutes at a speed of three thousand rpm. To obtain the concentrated extract, the extract was concentrated using a rotating evaporation equipment at a temperature not to exceed 40 °C., then the product was placed in an oven at a temperature of 45-50 °C for the purpose of drying the material and obtaining a dry extract in the form of powder to be kept at a temperature of 20 °C until Usage. For the purpose of testing the sensitivity of the bacteria to the aqueous extract of the leaves of the eucalyptus plant, then follow the following: Sterilization of the extract and preparation of the concentrations: Mitscher et al., (1972), adopted the 1972 method for preparing and sterilizing the stock solution, whereby 1 gm of dry plant extract powder was taken and dissolved in 10 ml of sterile distilled water, so we had a stock solution with a concentration of 100 mg / ml. Sterilization of the solution by filtration using Millipore filter paper with a permeability diameter 0.45 Mm to get rid of bacterial contaminants present in it and obtain a sterile storage solution. Use this solution as a source for preparing concentrations (25, 50, 75,100) mg / ml. Preparation of the bacterial suspension: Nutrient broth medium was prepared according to the instructions of the prepared company, Himedia, India, and distributed in test tubes at an amount of (5) ml for each tube, then inoculated with (3) bacterial colonies growing on Nutrient agar medium at the age of 24 hours. The inoculated tubes were incubated for (18) hours at a temperature of 37 m) Then the suspension was diluted by physiological salt solution and compared with turbidity MacFarland tube No. (5) containing a standard stable turbid solution to obtain an approximate number



of bacteria cells from the diluted suspension approximately (1.5 x 810) cells/ml . Preservation at 4 C until use. Boron and Finegold., (1990).

Test of antifungal activity of plant extract: The agar diffusion method was followed by drilling wells Egorove., (1985). The method includes making (0.2) wells with equal dimensions in container dishes on Mueller-Hinton medium prepared according to the company's instructions (Himedia / India) with a diameter of 6 mm. To 4 of them (0.2 mm) was added. ) ml of each concentration of aqueous extract of eucalyptus leaves prepared in the above-mentioned paragraph. The fifth pit was left for control, or 0.2 ml of sterile distilled water was placed in it. The dishes were incubated at 37 °C for 24 hours. The results were compared by measuring the inhibition area by a ruler. Saxena et al ., (1995 ). Antibiotic susceptibility test: The usual procedure of Bauer et al., (1966), which used ready-made paper tablets and eight antibiotics for this purpose, was reportedly followed by Balows and Wandepitte., (1987). Cotton swab on Muller Hinton media that is solid. Next, using flame-sterilized forceps, evenly distribute the premade antibiotic tablets on the inoculation plates' surface and gently push the pills in place. Using a clear ruler to measure the diameter of the inhibitory zone on the other side of the plate, antibiotic-resistant and sensitive bacteria were identified. This measurement was then compared to the indices found in the standard tables NCCLS, (1999). Antibiotic mixing test using eucalyptus leaves extract: The following was tested using the disc method, which contained the mixture of the antibiotic and the extract together, against bacterial isolates, based on the outcomes of the sensitivity test for antibiotics and plant extracts. The antibiotic Amikacin was mixed with an aqueous extract of eucalyptus leaves at a concentration of 100 mg / ml. Preparation of the antibiotic solution amikacin: Dissolve 3 of it in 90 ml of distilled water and complete the volume to 100 ml. Keep at a temperature 4 m until use, as its concentration is 30 mg / ml. Paper discs impregnated with both materials were prepared, as mentioned in Almeida and Beava., (1982), when he cut (25) discs of filter paper (wattman No.1) with a diameter of one disc (6.25) millimeters, and they were sterilized in an oven at a temperature of (140 C) for (20 minutes). The tablets were saturated with solutions in the amount of (10) microliters (5) microliters of the antibiotic solution with 5 microliters of the plant extract solution (noting that each 5 microliter of the solution contains the same concentration of the tablet or its buffer solution used in the sensitivity test. The tablets were left to dry, Dishes containing Mueller-Hinton medium were prepared and cultured with the bacterial suspension prepared in paragraph 2. The discs were spread at the rate of (5) discs per plate and incubated at 37 °C for 24 hours. The areas of fixation formed around each disc were measured using a ruler. Results and discussio: Eleven types of bacteria that cause gingivitis, belonging to two sexes, were isolated from the gram-positive bacteria. 31.78%, and 4 genera of Gram-negative bacteria accounted for (16.92%), and Streptococci occupied (18.87%). Among Gram-positive bacteria with four types Streptococcus angiosus (2.59%), Streptococcus salivarius (2.59%) Streptococcus pyogens (4.44%), Streptococcus mutans (9.25%) Followed by Staphylococcus sp of both types Staphylococcus epidermidis (5.13%), Staphylococcus aureus (7.78%). As for the Gram-negative species, Pseudomonas of both types, Ps. aeruginosa (3.32%), Ps monilli (2.17) the lowest percentage being (1.21%) Proteus sp. And E.coli bacteria occupied the highest percentage (5.80%), followed by Klebsiella sp with (4.42%) .



Table (1) Bacterial species that were isolated from patients with gingivitis.

No	Gram positive bacteria	No of isolates	percentage
1	Streptococcus mutans	12	9.25
2	Streptococcus pyogenes	6	4.44
3	Streptococcus salivarius	4	2.59
4	Streptococcus angiosus	4	2.59
5	Staphylococcus epidermidis	9	5.13
6	Staphylococcus aureus	10	7.78
	Total No	45	31.78
No	Gram nagative bacteria	No of isolates	percentage
7	Escherichia coli	8	5.80
8	Klebsiella sp	6	4.42
9	Pseudomonas aeruginosa	4	3.32
10	Pseudomonas monilli	5	2.17
11	Proteus spp.	2	1.21
	Total No	25	16.92

Table(2) shows that the effect of the plant extract differs according to the concentrations used, as the effect started from the concentration of 50 mg / ml, and the most effective was the concentration of 100 mg / ml on E.coli bacteria, followed by the concentration of 75 mg / ml on the same bacteria, and the least effect was Concentration of 25 mg / ml on all test bacteria. It was also shown through the study that the antibiotic mixture it had a clear effect on both types of bacteria.

Table(2): Effect of concentrations of aqueous extract of eucalyptus leaves in inhibiting the growth of test bacteria

Concentration Mg/ml	types of bacteria	The diameters of inhibition zone (ml)			
		25%	50%	75%	100%
1	Streptococcus mutans	-	-	16	19
2	Streptococcus pyogenes	-	1	7	14
3	Streptococcus salivarius	-	-	7	13
4	Streptococcus angiosus	-	-	10	20
5	Staphylococcus aureus	-	-	13	16
6	Staphylococcus epidermidis	-	7	10	13
7	Escherichia coli	3	9	20	22
8	Klebsiella sp	-	3	7	10
9	Pseudomonas aeruginosa	-	3	7	10
10	Pseudomonas monilli	-	1	7	11
11	Proteus spp.	-	-	-	10



The results shown in Table (3) indicated that the Gram-positive isolates showed a variation in their resistance to the tested antibiotics (3), as the highest percentage of resistance shown by the Gram-positive isolates was against ampicillin, while it had the highest sensitivity towards the Amikacin antibiotic. Gram-negative, it had the highest percentage of resistance against an antibody Tetracycline, while less resistance to Amikacin, depending on the measurement of inhibition zones. Amikacin and the aqueous extract of eucalyptus leaves had an effect on the bacteria that cause gingivitis, and the highest inhibition diameter was (34) mm, while the lowest diameter was (29) mm. They are higher than the inhibition diameters that appeared in the sensitivity test, and this is an indication of the synergistic effect between the antibiotic and the plant extract.

Table(3): Diameters of zones of inhibition of gram-positive and negativebacterial species isolated from gingivitis against some antibiotics

Isolated Bacteria	Strep Mutans (5)	Strep Sens (20)	Staph aureus (10)	Staph aureus (9)	Staph aureus (6)	Staph aureus (2)	Staph aureus (1)	Staph aureus (4)	Staph aureus (3)	Staph aureus (4)	Staph aureus (2)	Staph aureus (3)
Ampicillin	-	8	-	6	9	7	-	-	-	-	-	-
Amikacin	24	21	20	22	23	21	23	21	24	22	19	
Amoxicillin	11	12	17	16	11	10	14	16	1	18	17	
Tetracyclin	12	11	9	14	14	17	9	10	8	10	8	
Trimethoprim	21	18	20	16	17	14	19	20	21	12	18	
Mix the extract with Amikacin	31	32	34	29	31	32	29	30	31	32	31	

The results showed that the species that cause gingivitis belonging to the genus Streptococcus are common, as they play an important role in tooth decay and are called the species that generate necrosis, in addition to that they predispose to the occurrence of opportunistic infections caused by the other group of Gram-positive bacteria represented by the genus Staphylococci due to their natural presence on the skin, nose, and gastrointestinal tract Jeane et al., (2003). Where the reason for its spread in the mouth is due to its possession of rapid resistance mechanisms and the ease of spread by means of plasmids through the conjugation and transformation processes between them, or to its possession of surface antigens and lysing enzymes that help it to penetrate body tissues, as in staphylococci. Trigg ., (1996). As for Gram-negative bacteria, they formed a smaller percentage compared to Gram-positive bacteria, and as confirmed by many studies, including the study of Waltimo et al., (1997), that most Gram-negative bacteria come from infections (the respiratory system or thegastrointestinal tract and appear in the mouth, and this is consistent with With the findings of the study of Trigg ., 1996 ; Pattnaik et al., (1996) and Sartorelli et al., (2007). The results also showed that the Gram-positive isolates gave a noticeable difference in their sensitivity to antibiotics, and the reason may be due to the production of beta-lactamase enzymes, and the genes encoding them may be carried chromosomally on plasmids. Hostack et al., (1997), or bacterial resistance may be attributed to one of these three mechanisms. Altering a target site for an antibody, decreasing its permeability, or producing an antibody Enzymes that are inhibited by it Sannders., (1992), or the cause of resistance may be due to the increase in indiscriminate use of antibiotics, which led to the emergence of resistance caused by bacteria in their favor due to the use of



sub-therapeutic doses, which leads to the emergence of mutant isolates Kalle et al., (1998); Nordmann et al., (1993). The results also showed that the test bacteria were sensitive to the aqueous extract of the leaves of the eucalyptus plant, and they may be greater or similar when compared to their sensitivity towards antibiotics. With the components of the cell or the presence of special receptors on the bacterial cell wall and suitable vectors that transport their molecules into the cell to stop the action of coenzymes and other active biological molecules. Hancock and Wong, (1984). This is consistent with what Dhakad et al., (2018), reached, in addition to containing. The eucalyptus plant contains Eugenol at a high rate, ranging between (80-90%) of its composition, which acts as an analgesic for toothache Khalid and Anfoka, (2005). Conclusion : We can conclude that the leaves of the Eucalyptus plant have an effective effect in inhibiting the activity of most types of bacteria that cause gingivitis diseases. It is possible to study extract many compounds from the leaves of this plant and reveal the effective ones against other types of bacteria that infect humans and use them instead of antibiotics.

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