# EFFECT OF SOME ANTIBIOTICS AND MEDICINAL PLANTS ON BACTERIA ISOLATED FROM DENTAL CALCULUI[+]

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## Abstract

**Fifty three samples of dental calculi were collected and speculated for bacteria colonizing them. Only twenty two calculi were harbouring bacteria. *Streptocccus mutans* was isolated from 18 samples while *Staphylococcus aureus* from 4 calculi. Most of used antibiotics were effective against both bacterial species. The antimicrobial activity of aqueous and oil extracts of clove (*Eugenia caryophyllus*), nigella (*Nigella sativa*), chamaemplum (*Anthemis mobils*), and lemon grass (*Cymbopahon schoenanthus*) were tested. It was found that *S.aureus* was more efficient than *Streptococcus mutans* in resisting the oil and aqueous extracts of medicinal plants used in study. Oil extract of lemon grass was the most efficient extract in the inhibition of growth of both isolates**

## المستخلص

**جمعت ٥٣ عينة من تكلسات الاسنان و تم التحري عن البكتريا المستعمرة لها. ووجدت البكتريا في ٢٢ عينة فقط. عزلت بكتريا *Streptocccus mutans* من ١٨ عينة و بكتريا *Staphylococcus aureus* من ٤ عينات. و كان كلا النوعين حساس لاغلب المضادات الحياتية المستعملة في الدراسة. كما اختبرت الفعالية ضد الجراثيم للمستخلصات المائية و الزيتية للنباتات الطبية الاتية القرنفل *Eugenia caryophyllus* وحبة البركة *Nigella sativa* والبابونج *Anthemis mobils* وعشبة الليمون*Cymbopahon schoenanthus*، ووجد ان *Staphylococcus aureus* كانت أكثر كفاءة من *Streptocccus mutans* في مقاومة المستخلصات المائية و الزيتية للنباتات الطبية قيد الدراسة. و كان المستخلص الزيتي لحشيشة الليمون الاكثر كفاءة في تثبيط نمو كلا النوعين.**

## Introduction

Dental calculus, also referred to as tartar, is a hard deposit on the tooth that may lie above and/or below the gum margin [1]. This deposit results from calcification (hardening) of plaque [2]. It is removable by professional scaling only [3]. Whereas calculus deposits do not cause disease, their presence offers further surface for growth of plaque which also acts as mechanical interference in daily tooth cleaning activities [3]. When it forms below gum margins, calculus may increase the risk of developing periodontal disease [4].

Dental calculus is classified according to its relation to the gingival margin as supragingival or subgingival:

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Supragingival calculus is located coronal to the gingival margin and therefore is visible in the oral cavity. [1,2].

Subgingival calculus is located below the crest of the marginal gingival and therefore is not visible on routine clinical examination [1,2,3]. Research suggests that subgingival calculus, at a minimum, may expand the radius of plaque - induced periodontal injury [4].

Supragingival and subgingival calculus generally occur together, but one may be present without the other [1].

Dental calculus is composed of inorganic and organic components. The inorganic portion consists of calcium phosphate, calcium carbonate, traces of magnesium phosphate, and other metals. On the other hand, the organic portion consists of protein-polysaccharide complexes, desquamated epithelial cells, leucocytes, and various types of microorganisms [1,2,3].

Stagnation and accumulation of bacterial plaque at the gingival margin leads in turn to calculus formation [4].

Calculus formation is attributed to several bacterial species, but none of which is isolated from the calculus itself, so our study is aimed to isolate bacteria from the calculus to elucidate their part in dental calculus formation.

## Materials and Methods

Fifty three dental calculus samples were collected (with the aid of dentists in their private clinics) from patients aged 20 – 59 years in a period of time from December,2$^{nd}$ 2003 to March, 3$^{rd}$ 2004.

The patients were instructed to use chlorohexidin mouth wash five times just before the sampling. The selected area was cleaned until no visible surface deposits or integuments remained and after a desired period the teeth were air dried and calcification sample was obtained with a sterile instrument by scraping. Thereafter the sample was transported in sterile phosphate buffer to the laboratory, treated with alcohol for 15 minutes, in order to reduce the contamination with mouth normal flora, washed three times with the sterile phosphate buffer. The cleaned samples were crushed in brain heart infusion medium with a sterile stainless steel rod, incubated for 24 hours at 37°C. A loopful drop was streaked on blood agar, Mannitol Salt agar and macConkey agar and incubated for 24 hours at 37°C. The grown colonies were identified according to Bergy's manual s[5].

**Antibiogram** was done to all isolated bacteria by disc diffusion method with the following antibiotics discs (oxoid) tetracycline (30 µg/ml), penicillin (10 µg/ml), rifampicin (30 µg/ml), kanamycin (30 µg/ml), erythromycin (30 µg/ml), co-trimoxazole (25 µg/ml), vancomycin (30 µg/ml), amoxicillin (10 µg/ml), cephaloridine (30 µg/ml), and doxycyclin (30 µg/ml).

**Medicinal plant extracts:** The susceptibility of the bacteria isolated from the dental calculi to the aqueous and oil extracts of clove (***Eugenia caryophyllus***), nigella (Nigella sativa), chamaemplum (Anthemis mobils), and lemon grass (***Cymbopahon schoenanthus***) was tested. The aqueous extracts were used in concentrations of 5%, 10% and 20% for each plant [6]. Well method [7] was employed by making holes (5 mm in diameter and 4 mm in depth) in nutrient agar. The oil extracts were added to the medium in final concentrations of 500, 1000, 1500, and 2000 ppm [6] for each plant. All plates were incubated at 37°C for 24 hours.

## Results and Discussion

Out of fifty three dental calculi, only twenty two were harbouring bacteria. ***Streptocccus mutans*** was isolated from 18 samples while ***Staphylococcus aureus*** from 4 calculi.

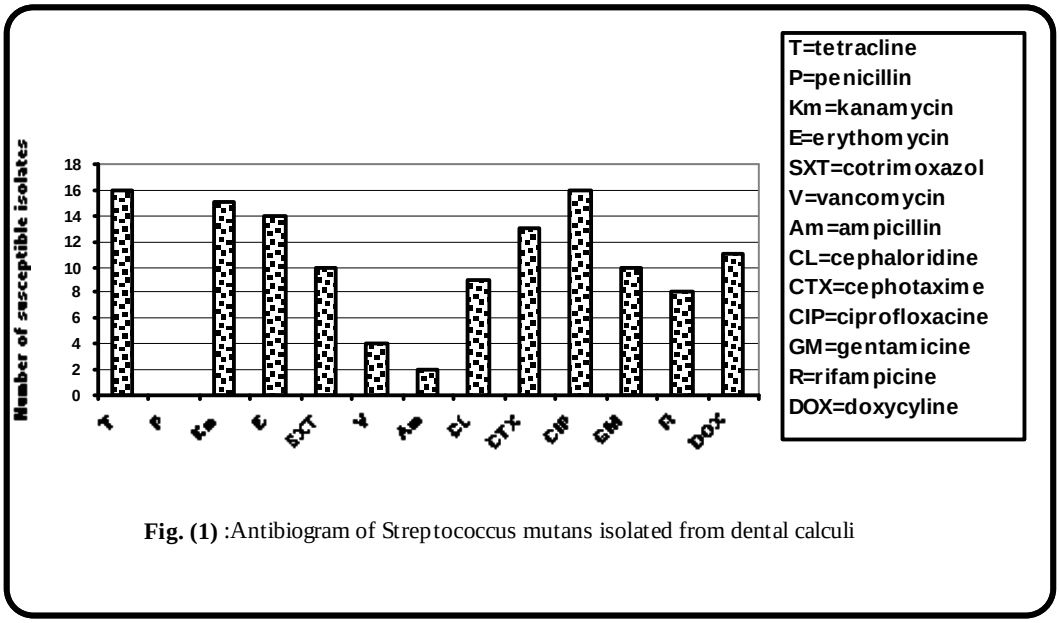


**Fig. (1)** :Antibiogram of Streptococcus mutans isolated from dental calculi

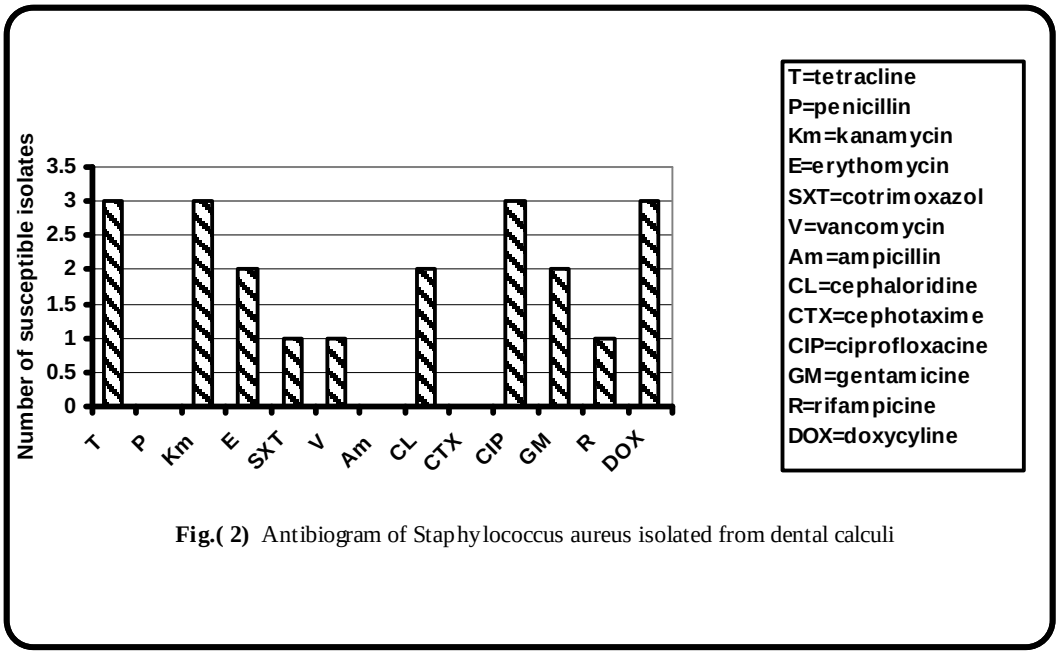


**Fig.( 2)** Antibiogram of Staphylococcus aureus isolated from dental calculi

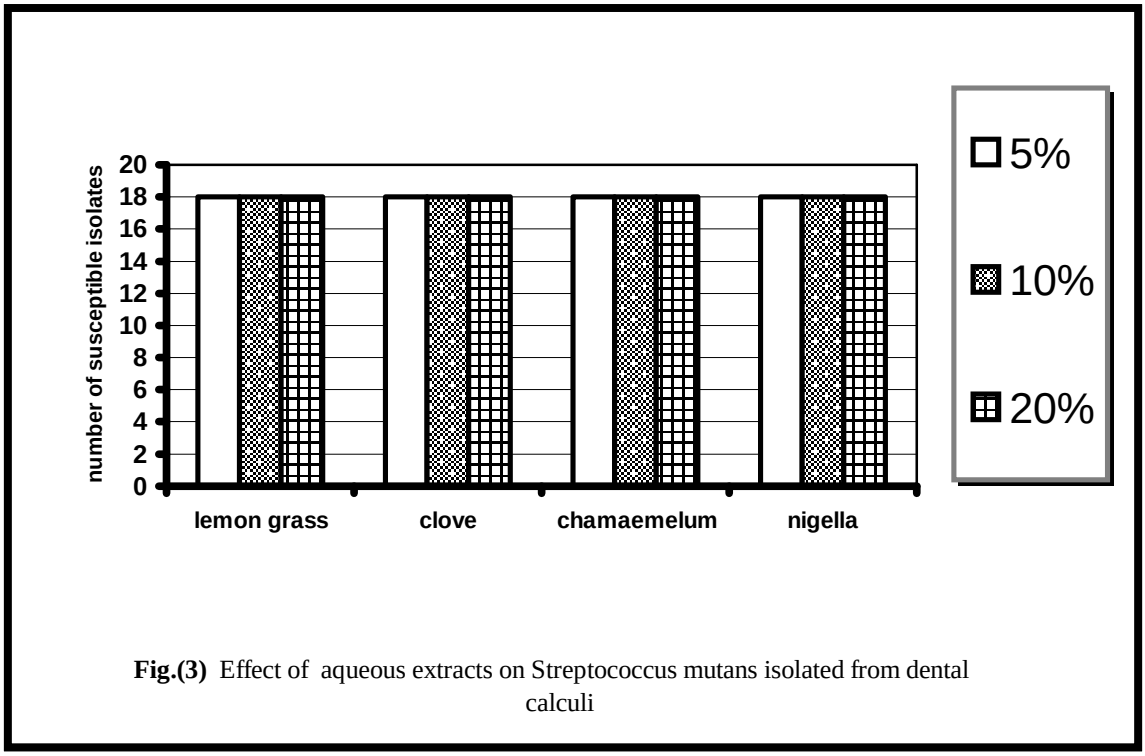


**Fig.(3)** Effect of aqueous extracts on Streptococcus mutans isolated from dental calculi

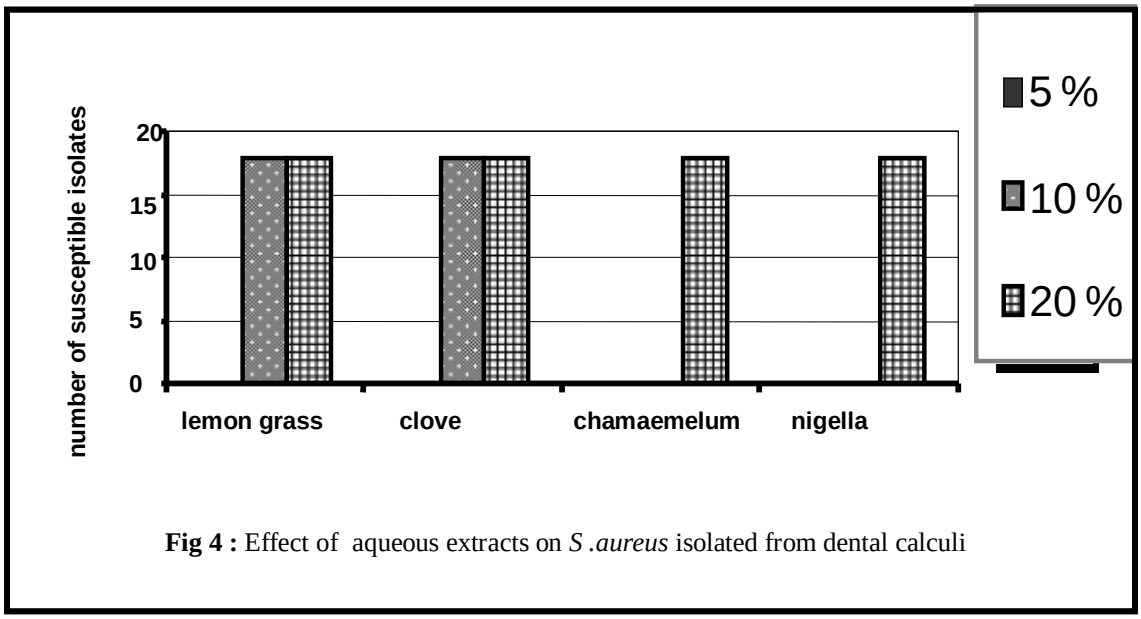


**Fig 4 :** Effect of aqueous extracts on *S .aureus* isolated from dental calculi

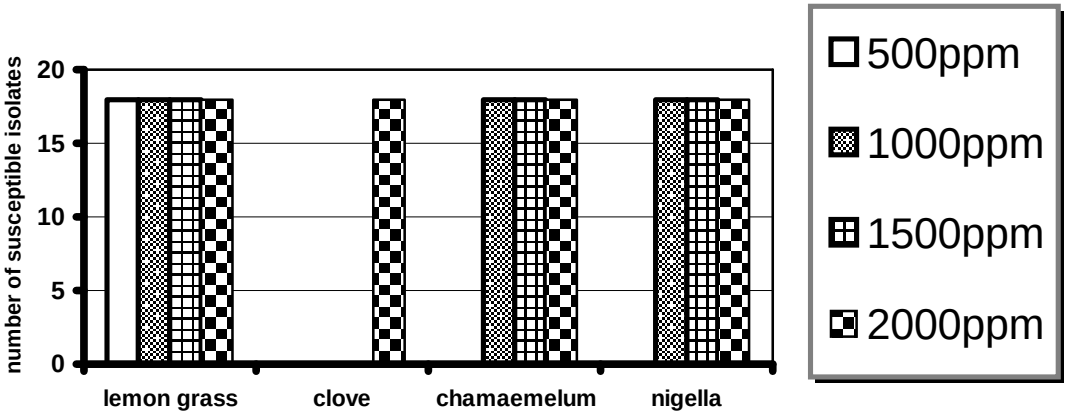


**Fig.(5)** Effect of oil extracts on Streptococcus mutans isolated from dental calculi

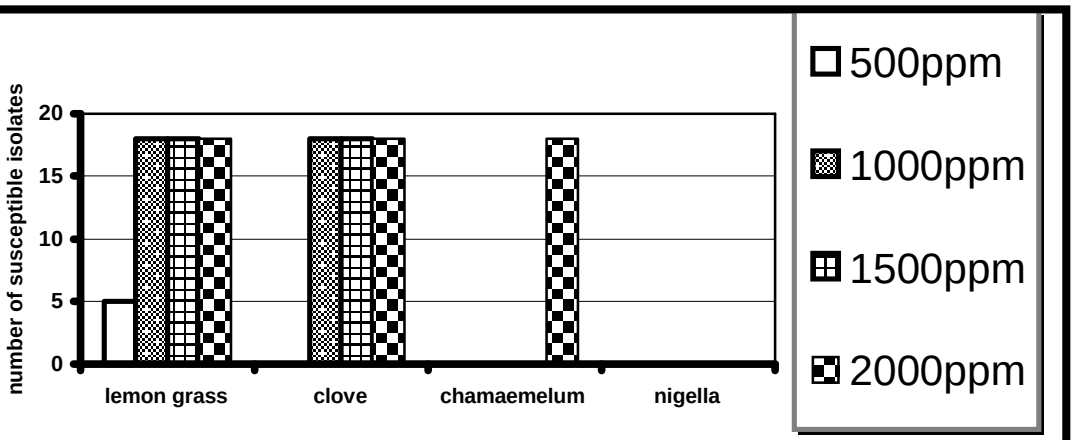


**Fig.(6)** : Effect of oil extracts on S .aureus isolated from dental calculi

Depending on such results, it can be said, that behaviour is so similar to crystal formation in other parts of body (e.g. kidney, joints, salivary glands, ….etc.). The bacteria act as a nucleation site for the formation of biogenic apatite structures under conditions of alkalinity [8]. In mouth, the plaque is the source of bacteria and alkalinity[1,4], in other words, bacteria found in plaque act as a nucleation center for biomineralization via depositing calcium phosphate on/in their cell wall matrix. It is also possible that some normal flora found in mouth (not plaque bacteria) are able to form calcification in similar mechanism [4], which mimics bacteria in nature; those form minerals in sea water.

Tan et al. [9] studied the ultrastructure of dental calculi with the aid of transmission electron microscope and found that non-mineralised channels were observed extending into the calculus, often joining extensive lacunae, both containing intact non-mineralised coccoid and rod-shaped microorganisms as viable bacteria within these lacunae may provide a source of re-infection

As it is shown in figures (1 and 2) , most used antibiotics were effective against both bacterial species.

In accordance with the results shown in figure ( 3 ) it can be concluded that the aqueous extracts are effective against ***Streptococcus mutans*** in all concentrations (5%, 10%, and 20%), while ***S.aureus*** has a different behaviour figure (4) since it resists the 5% solution of all medicinal plants in this study as well as the 10% solution of chamaemelum and nigella.

Figure (5) illustrates the results of effect of oil extracts on ***Streptococcus mutans***. It is clear that clove does not affect the growth of this bacteria up to 1500 ppm, but 2000 ppm concentration has an obvious effect. Otherwise other extracts seem to have a considerable effect on bacteria in the study.

As it is depicted in figure (6) , ***S.aureus*** has the ability to grow in presence of chameamelum (up to 1500 ppm) and nigella (up to 2000 ppm). It also is able to resist 500 ppm of all oil extracts in this study.

A noticeable notice may be seen from figures (4 and 6) that ***S.aureus*** is more effective than ***Streptococcus mutans*** in resisting the oil and aqueous extracts of medicinal plants under study, and this could be due to the disconcerting propensity to develop resistance to antimicrobial agents [10].

Dental calculus formation can be controlled by chemical mineralization inhibitors, applied in toothpastes or mouthrinses [4]. These agents act to delay plaque calcification, keeping deposits in an amorphous non-hardened state to facilitate removal with regular hygiene. Clinical efficacy of these agents is typically assessed as the reduction in tartar area coverage on the teeth between dental cleaning, rather than resolve the problem itself, since the calcification will form again. Therefore we suggest eliminating bacteria is the clue to prevent subsequent formation. Depending on the study results we strongly recommend performing culture and sensitivity tests for the dental calculus and employ the oil extract of lemon grass (***Cymbopahon schoenanthus***) in treatment policy.

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