# The effect of vanillin (2%) on the growth of cariogenic bacteria (*Streptococcus mutans* and *Lactobacillus*)

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## ABSTRACT

The purpose of this study is to evaluate the effectiveness of vanillin (2%) alone and with Amber dentifrice on the cariogenic bacteria (*Streptococcus mutans* and Lactobacilli).

The vanillin material was prepared in this study by the use of pure vanillin (2%). Two microorganisms were selected to examine the antimicrobial activity of the vanillin against dental caries. These microorganisms are *Streptococcus mutans* and Lactobacilli; both are isolated from the dental plaque on the carious surface and from saliva. For the assay the turbidity method was used as described by Abdul-Rahman (2001), using the mean value of optical density to record the growth of bacteria.

This study showed that the vanillin (2%) alone and with dentifrice reported significantly less mean value of optical density for both bacteria compared with the control read. That means it is effective in controlling and preventing dental caries by reducing the growth of cariogenic microorganisms.

***Key Words:*** Vanillin, Amber dentifrice, cariogenic bacteria.

## الخلاصة

إن الهدف من الدراسة هو لتقييم فعالية الفانيلين (٢%) على حدة أو مع معجون الأسنان (عنبر) على البكتريا المسببة لتسوس الأسنان (المكورات العقدية والمليفات).

تم تحضير فانيلين نقي (٢%)، وكذلك نوعين من البكتريا المسببة لتسوس الأسنان، تم جمع العينات من أسطح الأسنان المتسوسة ومن اللعاب. لتقييم فعالية الفانيلين المؤثر على نمو البكتريا، اعتمدت طريقة اختبار العكرة (Turbidity) باستخدام جهاز المطياف الضوئي لقياس المعدل.

أظهرت النتائج أن استخدام الفانيلين على حدة أو مع معجون الأسنان أدى إلى انخفاض كبير في معدل نمو البكتريا مقارنةً مع عينات مجموعة السيطرة (بدون فانيلين)، وكان الفارق بينهما معنوياً وهذا يدل على تأثير الفانيلين في السيطرة أو التقليل من تسوس الأسنان من خلال تأثيره على نمو البكتريا المسببة لتسوس الأسنان.

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## INTRODUCTION

The development of dental caries is a dynamic process of demineralization of the dental hard tissues by the products of bacterial metabolism, alternating with periods of remineralization. For caries to develop, acidogenic (acid producing) bacteria must be present, and a mean must exist to contain the metabolic acid at the point where caries is to develop.

Dental plaque fulfills both of these functions. In the dental plaque, there are more than (200) species of microorganisms. The great majorities are not directly involved in the caries process. The bacterial genera of special interest in cariogenicity are the *Streptococcus mutans* and Lactobacilli [1, 2].

Mutans streptococci are now considered to be the major pathogenic bacterial species involved in the caries process [3, 4]. Certain physiological characteristics of the mutans streptococci favor their position as a prime agent in caries. These traits include the ability to stick to tooth surface, production of abundant insoluble extracellular polysaccharides from sucrose, rapid production of lactic acid from a number of sugar substrates, acid tolerance and production of intracellular polysaccharide stores [5].

Lactobacilli are both acidogenic and aciduric. Lactobacilli, specifically *Lactobacillus casei*, have been shown to colonize white spot lesions prior to cavitation and associations have been demonstrated between the presence of Lactobacilli and lesion development [6].

Many methods used to attack the dental plaque and especially the bacteria that are responsible for caries initiation to prevent and control dental caries. As the use of fluoride topically, in addition to other actions of fluoride to prevent dental caries, it reported to have the ability to inhibit glycolysis by interfering with the enzyme enolase. Also in high concentrations, it has been shown to interfere with bacterial metabolism [7-9]. Although many studies used chlorhexidine to combat cariogenic bacteria [10, 11], currently there are some studies advocate to use vanillin to treat certain oral diseases [12] and reported to be effective against some Gram–positive and Gram–negative microorganisms [13].

So, the purpose of this study is to evaluate the effectiveness of vanillin (2%) on cariogenic microorganisms (*Streptococcus mutans* and Lactobacilli) *in vitro* study.

## MATERIALS AND METHODS

The vanillin material was prepared in this study by the use of vanillin (2%). Two microorganisms were selected to examine the antimicrobial activity of the vanillin against dental caries. These microorganisms are *Streptococcus mutans* and Lactobacilli; both are isolated from the dental plaque on the carious surface and from saliva. They were identified at the Department of Basic Sciences, College of Dentistry, University of Mosul. There were two samples, one pure vanillin (2%) alone, and the other (2%) vanillin mixed with Amber toothpaste product.

For the assay the turbidity method was used as described by Abdul–Rahman (2001) [14]. The procedure is done by dissolving (1) gram of the new compound (for each sample) in (9) mL distilled water, then the ($^1/_{100}$) concentration were prepared from the first preparation. Then, (0.1) mL for each sample were added to small vials containing (4) mL of the Tryptic Soya Broth medium and incubated by (0.1) mL of

the bacterial suspension, incubated for (18) hours at (37)°C and the optical density of the culture were measured at (595) nm wavelength by the spectrophotometer (using spectrotonic 21), and the average were taken for triplicates of each sample. Also prepared a sample as control using distilled water instead of active ingredient to compare with the results of vanillin.

## RESULTS

Table (1) showed the mean value of optical density for vanillin (2%) alone. The results revealed the effect of pure vanillin (2%) on the cariogenic bacteria (*Streptococcus mutans* and Lactobacilli). There were very high significant reductions in bacterial growth of *Streptococcus mutans* compared with control (0.32 and 0.94), respectively, and significant reductions in bacterial growth for Lactobacilli compared with control (0.79 and 1.19), respectively.

Table (1): Mean and standard deviation of optical density value for cariogenic bacteria for vanillin (2%) alone

| Microorganisms | Control | Vanillin (2%) | t | Level of Significance |
|---|---|---|---|---|
| Streptococci | 0.941 ± 0.017 | 0.325 ± 0.042 | 16.61 | 0.004 |
| Lactobacilli | 1.190 ± 0.050 | 0.795 ± 0.052 | 4.313 | 0.050 |

Table (2) showed the mean value of optical density for vanillin (2%) with Amber toothpaste. The results indicated that there is high effect of new formula on the cariogenic bacteria. Also, there were very high reductions in both bacterial growth compared with control, although the mean value of the new formula was less than the mean value of the vanillin alone.

Table (2): Mean and standard deviation of optical density value for cariogenic bacteria for vanillin (2%) with dentifrice

| Microorganisms | Control | Toothpaste + Vanillin (2%) | t | Level of Significance |
|---|---|---|---|---|
| Streptococci | 0.941 ± 0.017 | 0.113 ± 0.058 | 19.95 | 0.008 |
| Lactobacilli | 1.190 ± 0.050 | 0.336 ± 0.020 | 15.90 | 0.004 |

## DISCUSSION

Vanillin or vanillinum is 4–hydroxy–3–methoxy benzaldehyde and may be obtained from Vanilla Planifolia Andrews, or other species of Vanilla (Fam–Orchidaceae), or prepared synthetically [12]. Vanillin occurs as fine, white or slight yellow crystals, usually needle like, having an odor and taste suggestive of vanillin [15].

Recently, many studies evaluate the effect of vanillin and reported to be effective against some Gram–positive and Gram–negative microorganisms (*Escherichia coli*, *Proteus vulgaris*, *Streptococcus puogens*, and *Staphylococcus*) *in vitro* [12] and in other clinical trials a double blind study reported to be effective in relieving pain associated with aphthus ulceration, hypersensitive cementum and ulcerative margins associated with pericoronitis [13].

From these findings we decided to evaluate their effect on other bacteria especially the cariogenic bacteria (*Streptococcus mutans* and Lactobacilli) to prevent the dental caries and use with ordinary dentifrice to evaluate their effect with dentifrice.

The reason for selection of these two microorganisms and especially *Streptococcus mutans* that there are many evidences *in vitro*, *in vivo* and animal experiments have shown that mutans streptococci, because of their metabolism, produce insoluble extracellular polysaccharides and acid production. It is considered among the oral microorganisms with the strongest cariogenic properties [1, 16]. The prevalence of caries and *Streptococcus mutans* has been reported in population from several countries [17–21].

This study showed that the vanillin (2%) alone reduce the growth of *Streptococcus mutans* (0.32) compared with the control reading (0.94) by using optical density value. These differences were very high significant. Also reported a significant reduction in the growth of Lactobacilli (0.79) compared with the control (1.19).

The results of the study showed that the high effect of vanillin when mixed with the toothpaste *in vitro*. It showed that the mean value of optical density were reduced for both bacterial growth compared with the control. For *Streptococcus mutans* the mean value reduced from (0.94) for control reading to (0.11) for new formula with very high significant difference between them. Also the mean value of Lactobacilli was reduced from (1.19) to (0.33), respectively, with very high significant difference between them.

From the findings of this study it is indicated that the vanillin (2%) alone and with toothpaste has a highly significant effect on the growth of cariogenic microorganisms that reflect the reduction in the mean value of optical density. That means the vanillin has a high effect on cariogenic microorganisms to prevent and control dental caries.

The increase of the effectiveness of vanillin with toothpaste is possible due to the presence of the fluoride in this toothpaste [22] or the effect of other ingredients. Many studies reported the effect of fluoride on the acidogenic bacteria, especially *Streptococcus mutans* and Lactobacilli because of its relationship to dental caries [23–25].

In conclusion, the vanillin reported high significant effect on cariogenic bacteria if used alone and increased their effectiveness when used with fluoridated dentifrice. So, further studies were needed to evaluate their effectiveness *in vivo* when

used in dentifrice and to study the reason for increase their effectiveness against these cariogenic bacteria when used with fluoridated dentifrice.

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