# CONTROLLING WOUND PATHOGEN USING STEVIA AND PEPPERMINT BASED MOUTH GEL

**Running Title:** Antifungal activity of grape seed mediated titanium dioxide nanoparticles


**Abstract**:

**Aim**:

To evaluate the antimicrobial activity of Stevia and peppermint based gel on streptococcus aureus, E. coli and pseudomonas.

**Background:**

In recent years, the research concerning the use of herbal products has been vastly analysed to assess their antimicrobial properties. It has been found that these herbs have certain phytochemicals which contributes to its antimicrobial property. The common oral wound pathogens are *Staphylococcus aureus, E. coli* and *Pseudomonas* hence our sample, Stevia and peppermint based moth gel was formulated to check its antimicrobial property on *S. aureus, E. coli* and *pseudomonas*.

**Materials and methods:**

The plants were collected, stevia and peppermint and an extract was prepared. 0.5g of carbopol was added to the extract to make it in a gel consistency. This gel was concentrated against the three wound pathogens, S. aureus, pseudomonas,E.coli. Through agar well diffusion method, the antimicrobial property of various concentrations of the gel was tested and compared with known antibiotic amoxicillin.

**Results:**

The current study revealed that stevia and peppermint based mouth gel had antimicrobial activity at all concentrations but was significantly less when compared with the standard and there was no significant difference in the antimicrobial activity between different concentrations of the gel used.

**Conclusion:**

From the present study, it may be concluded that stevia and peppermint based mouth gel have antimicrobial properties but are comparatively lesser when compared with the antibiotic.



**Keywords:** antimicrobial; Stevia; peppermint; Streptococcus aureus; E.coli; pseudomonas

## INTRODUCTION:

Wound infections are a significant health problem after trauma or surgery as it increases the mortality. Wounds are common in the oral cavity caused by either trauma, or surgery; these wounds heal by the same principle as the external wounds heal. Healing always starts with blood clotting that initially seals the wound(1–3). Platelet activation during the primary haemostasis releases a number of important cytokines that start the healing process via chemotactic signals to inflammatory and resident cells(4).If bleeding persists, wound healing is delayed as there is disturbance in the formation of granulation tissue. Wound healing is also impaired when the site is infected with bacteria(5,6).

The most common bacteria found in an infection are Staphylococcus aureus, pseudomonas and E. coli. Most of these bacteria have drug resistance, which has resulted in difficulties in curing the related infectious diseases(7). This brings about the screening of plant extracts of great interest to scientists in the search for new drugs for greater effective treatment of several diseases(8). Therefore, plant extracts and phytochemicals with known antimicrobial properties can be of great significance in therapeutic treatments (9,10). The antimicrobial action of the plant phytochemicals, the same "natural antibiotics" used in ecology, medicine and agriculture(11).

The greater part of the world population relies on traditional medicine for healthcare(12,13). This is also the case in the treatment of wound infections. In developing countries, formulation preparation from plants has been widely used for the treatment of soft tissue wounds and burns by medical personnel trained in western medicine as well as by traditional practitioners(14).

Stevia rebaudiana (commonly referred to as Honey leaf, Candy leaf and Sweet leaf), which is popular in the world not only as a low-calorie, medicinal, natural sweetener, also known for its antioxidant activity of leaf extract (15,16) It is rich in terpenes, flavonoids, phenols and flavonoids, which cause the antimicrobial properties of the plant (17) . The phytochemicals present in Stevia rebaudiana are austroinullin, β carotene, dulcoside, niacin, rebaudi oxides, riboflavin, steviol, stevioside and tiamin (18). Stevia is also proved to inhibit the growth of certain bacteria and other infectious organisms hence used against wounds sores and gum disease.

Our team has extensive knowledge and research experience that has translated into high quality publications.(19–31),(32–36) (37) (38). This study examined the effects of Stevia and peppermint based mouth gel on various pathogens. Since, this is a herbal formulation, side

effects of this formulation are low and hence large quantities can be applied. This may help in the future to be used as an attachment to other antimicrobial agents. Hence, through this in vitro study, we want to evaluate the potency of herbal plants of stevia and peppermint gel against the wound pathogen.

## MATERIAL AND METHODS:

### COLLECTION OF PLANT MATERIALS AND PREPARATION OF THE EXTRACT:

Stevia and peppermint powder were obtained readymade.The powdered stevia and peppermint powder was measured to 10g and was taken in a beaker. 100ml of distilled water was added into the beaker. The beaker was mixed, stirred well and was subjected to boiling and 90°C until the aqueous mixture was well concentrated. The concentrated mixture was then subjected to filtration. The obtained filtrate was subjected to more heating, till the volume of the filtrate was decreased to half.

### PREPARATION OF THE GEL:

0.5 g of carbopol was taken in a sterile glass beaker to this beaker 50 ML of distilled water was added in small concentration and turned well until the solute was completely dissolved. This solution, 1ml of the extract (Stevia and peppermint) was added and stirred well.

Evaluation of antibacterial activity:
Three petri plates with Muller Hinton agar were taken. four wells on each plate were formed using a gel puncher. To three wells, Stevia and peppermint gel was loaded in the concentration range from 25µl, 50µl and 100µl. The fourth well was loaded with positive control, a known antibiotic (amoxicillin) . The plates were incubated at 37°C for 24 hours. After incubation, the Zone ofInhibition was measured in mm.

### STATISTICAL ANALYSIS:
All the data was analyzed statistically using SPSS version 23 (IBM).One way Anova test and post hoc test using Tukey's HSD were done to compare the standard and stevia and peppermint based mouth gel at different concentrations. Confidence limit was set at 95% and $p < 0.05$ was considered as statistically significant

## RESULTS:

The results showed that the prepared gel had anti microbial effect but the zone of inhibition for all three tested organisms ( S.aureus, Pseudomonas, E.coli) at all three tested concentrations was found to be significantly lesser than that of the standard antibiotic used($p < 0.05$). It was also found that there was no significant difference between the Zone of inhibition at different concentrations ($p > 0.05$) ( figure 1,table 2).

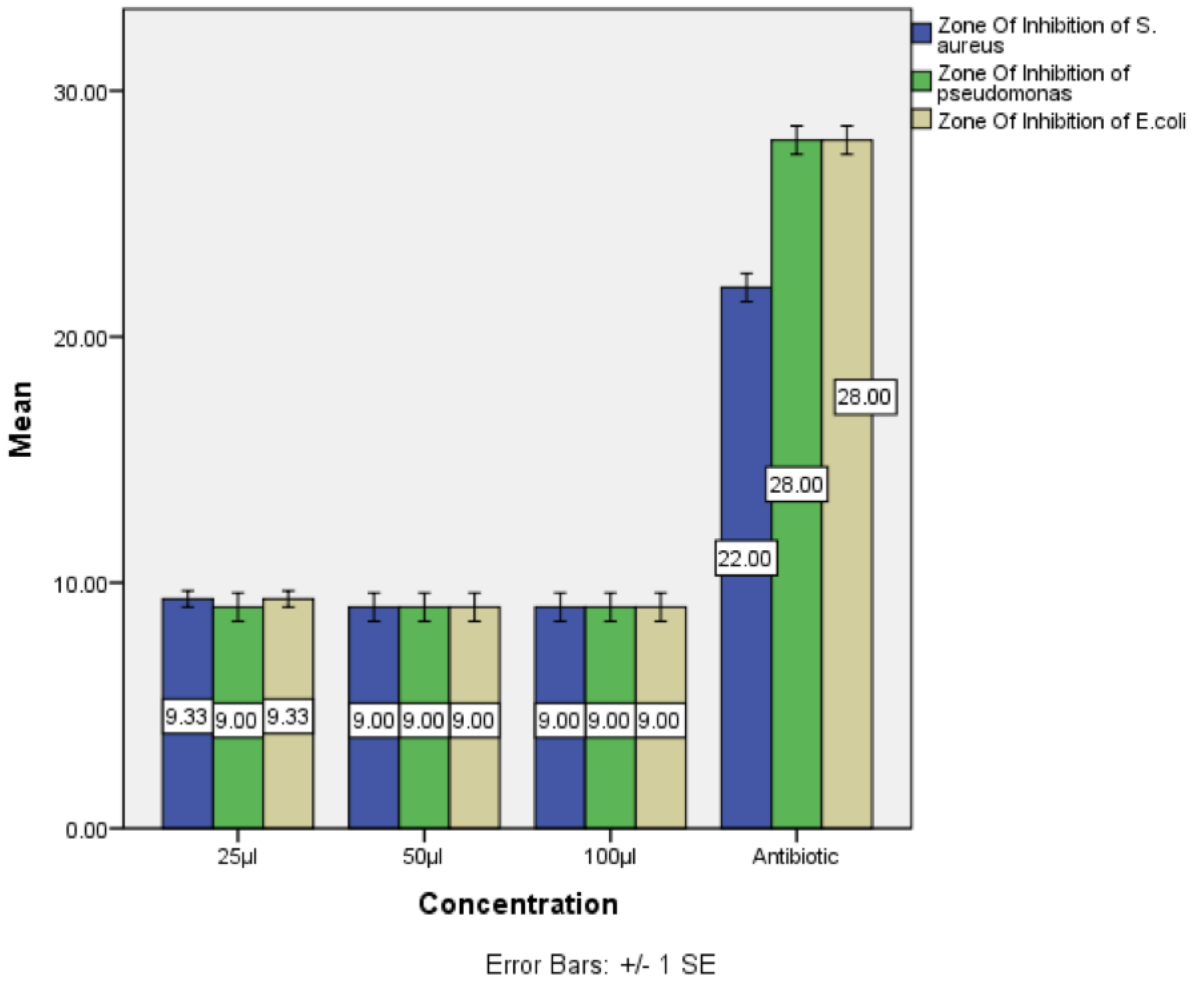


Figure1: The bar graph shows the comparison of the mean Zone of Inhibition of the standard and peppermint and stevia mouth gel at various concentrations. The X-axis represents the concentration in units of µL and antibiotic and the Y-axis represents the mean Zone ofInhibition. Blue represents the Zone of Inhibition of S. aureus, green represents Zone of Inhibition of Pseudomonas and beige represents the mean Zone of Inhibition of E.coli. The graph shows that the efficacy of Stevia and peppermint gel is significantly lesser when compared to the standard at all the concentrations($p<0.05$) and there was no significant difference in the zone of Inhibition between the different concentrations ($p>0.05$). One way Anova followed Tukey's HSD analysis was done for the statistical comparison.

Table 1: This table depicts the comparison of zone of Inhibition of Various concentrations of the Stevia and peppermint based gel and control group against S.aureus, E.coli and pseudomonas using One way Anova.

| | | N | Mean | Std. Deviation | Std. Error | significance |
|---|---|---|---|---|---|---|
| Absorbance of S. aureus | 25µl | 3 | 9.33 | .577 | .333 | |
| | 50µl | 3 | 9.00 | 1.000 | .577 | |
| | 100µl | 3 | 9.00 | 1.000 | .577 | 0.000 |
| | Antibiotic | 3 | 22.00 | 1.000 | .577 | |
| | Total | 12 | 12.33 | 5.883 | 1.698 | |
| Absorbance of pseudomonas | 25µl | 3 | 9.00 | 1.000 | .577 | |
| | 50µl | 3 | 9.00 | 1.000 | .577 | |
| | 100µl | 3 | 9.00 | 1.000 | .577 | 0.000 |
| | Antibioti | 3 | 28.00 | 1.000 | .577 | |

| | | | | | | |
|---|---|---|---|---|---|---|
| | c | | | | | |
| | Total | 12 | 13.75 | 8.635 | 2.493 | |
| Absorbance of E.coli | 25µl | 3 | 9.33 | .577 | .333 | |
| | 50µl | 3 | 9.00 | 1.000 | .577 | |
| | 100µl | 3 | 9.00 | 1.000 | .577 | 0.000 |
| | Antibiotic | 3 | 28.00 | 1.000 | .577 | |
| | Total | 12 | 13.83 | 8.579 | 2.477 | |

Table 2 showing the pair wise comparison of zone of Inhibition using Tukey's HSD test

**Multiple Comparisons**

| Dependent Variable | (I) Concentration | (J) Concentration | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|---|---|---|
| | | | | | | Lower Bound | Upper Bound |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Absorbance of S. aureus | Tukey HSD | 25µl | 50µl | .333 | .745 | .968 | -2.05 | 2.72 |
| | | | 100µl | .333 | .745 | .968 | -2.05 | 2.72 |
| | | | Antibiotic | -12.667* | .745 | .000 | -15.05 | -10.28 |
| | | 50µ | 100µl | .000 | .745 | 1.000 | -2.39 | 2.39 |
| | | | Antibiotic | -13.000* | .745 | .000 | -15.39 | -10.61 |
| | | 100µl | Antibiotic | -13.000* | .745 | .000 | -15.39 | -10.61 |
| Absorbance of pseudomonas | Tukey HSD | 25µl | 50µl | .000 | .816 | 1.000 | -2.61 | 2.61 |
| | | | 100µl | .000 | .816 | 1.000 | -2.61 | 2.61 |
| | | | Antibiotic | -19.000* | .816 | .000 | -21.61 | -16.39 |
| | | 50µl | 100µl | .000 | .816 | 1.000 | -2.61 | 2.61 |
| | | | Antibiotic | -19.000* | .816 | .000 | -21.61 | -16.39 |
| | | 100µl | Antibiotic | -19.000* | .816 | .000 | -21.61 | -16.39 |
| Absorbance of E.coli | Tukey HSD | 25µl | 50µl | .333 | .745 | .968 | -2.05 | 2.72 |
| | | | 100µl | .333 | .745 | .968 | -2.05 | 2.72 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | Antibiotic | -18.667* | .745 | .000 | -21.05 | -16.28 |
| 50µl | 100µl | .000 | .745 | 1.000 | -2.39 | 2.39 |
| | Antibiotic | -19.000* | .745 | .000 | -21.39 | -16.61 |
| 100µl | Antibiotic | -19.000* | .745 | .000 | -21.39 | -16.61 |

*. The mean difference is significant at the 0.05 level.

DISCUSSION:

Nowadays, due to the excessive usage of antibiotics, the common pathogens have become resistant to them, which is in turn decreasing the efficacy of antibiotics. Due to this reason, it is of utmost importance to find out new antimicrobial agents to combat antibiotic resistance and to aid in better wound healing following an infection(39). The results of this study reveals that the stevia and peppermint based gel is having some antimicrobial properties against staphylococcus aureus, pseudomonas and E. coli but the efficacy was significantly lesser than amoxicillin. The study also revealed that the antimicrobial effect of stevia peppermint gel was not concentration dependent.

The current study evaluated the antimicrobial property of stevia and peppermint based gel on various wound pathogens like Staphylococcus aureus, pseudomonas and E.coli at different concentrations like 25µl, 50µl and 100µl. Staphylococcus aureus is the most common organism associated with surgical wound infections. In a study done by Nwachukwu et al, it was found that 42.3% of infections were found to be caused by Staphylococcus aureus(40). Few other authors found that among the Gram-negative organisms, Escherichia coli were frequently isolated from the wounds(41,42).

In the current study, amoxicillin was used as a standard to compare the antimicrobial activity of Stevia and peppermint gel. Amoxicillin is the most commonly used antibiotic in treating wound infections. Amoxicillin belongs to the beta lactam group of antibiotics(43). Beta-lactams act by binding to penicillin-binding proteins that inhibit a process called transpeptidation (cross-linking process in cell wall synthesis), leading to activation of autolytic enzymes in the bacterial cell wall (44).

In the current study, It was noted that the antimicrobial activity of Stevia and Peppermint mouth gel was lesser than that of the standard (amoxicillin). It was also noted that the antimicrobial activity of stevia and peppermint mouth gel was concentration independent which means that with increase in the concentration, there was no change in the antimicrobial activity of the gel.

In a study done by Das et al. the antimicrobial activity of aqueous, methanolic, and ethanolic extracts of S. rebaudiana leaves was evaluated. All individual extracts showed potential antimicrobial activity compared to standard ampicillin, but the activities were lower than standard which was similar to the results found in our study(45).

In a study done by Gosh et al., evaluated the antimicrobial activity of S. rebaudiana leaf extracts (i.e., petroleum ether, cyclohexane, chloroform, water, acetone, and ethanol) against 10 pathogenic as well as food-spoilage fungal (Alternaria solani, Helminthosporium solani, Aspergillus niger, and Penicillium chrysogenum) and pathogenic bacterial (Escherichia coli, Bacillus subtilis, Enterococcus faecalis, Proteus mirabilis, Pseudomonas aeruginosa, and Staphylococcus aureus) isolates, using streptomycin and cotrimazole as controls. These authors found that petroleum ether extracts at 250 mg/mL (MIC) inhibit the growth of E. coli and S. aureus (by the plate dilution method) among bacteria and P. chrysogenum among fungi. Among all extracts, petroleum ether exhibited the best antimicrobial potential followed by water, chloroform, cyclohexane, acetone, and ethanol. This shows that extracts of S. rebaudiana act on a wide spectrum of microorganisms(46). The medicinal properties are attributed to the primary and secondary metabolites synthesized by the plant(47).

The principal constituents of peppermint include monoterpene alcohols mainly menthol (38-48%), ketones mainly menthones (20-30%), some monoterpenes and oxides. The metabolites of stevia includes, steviol and its glycosides, stevioside, rebaudioside (A to F), steviolbioside, dihydro isosteviol, rubusoside, and dulcoside A. these metabolites contribute to its antioxidant, antibacterial,anti-spasmodic and antiviral properties(48–52).

Even Though the present results reveal that the stevia and peppermint gel is not as good as the amoxicillin even at 100 microlitre concentration, further studies using higher concentrations or different extract preparation methods may add more value to this study. Moreover the other

medicinal properties of stevia such as its antioxidant and anti inflammatory effects will add additional benefits while using this gel(53) (54) (55) (56) (57) (58) (59) (60) (61) (62) (63) ((63,64) (65) (66). Due to the increase in the antibiotic sensitivity, herbal formulation will be a viable alternative to the conventional antibiotics. These herbal formulations can also be used as an adjuvant with the antimicrobials, increasing their efficacy Further research on isolating different components may be undertaken and may be incorporated into existing anti-inflammatory herbal compositions to improve their efficacy. Promising results regarding the antimicrobial effect of Stevia peppermint gel can be further validated with future in vivo studies to find the safe and effective concentration for clinical usage.

**Conclusion:**

From the present study, it may be concluded that stevia and peppermint based mouth gel have antimicrobial properties but are comparatively lesser when compared with the antibiotic amoxicillin .

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