

CONTROLLING WOUND PATHOGEN USING STEVIA AND PEPPERMINT BASED MOUTH GEL

Running Title: Antibacterial activity of Stevia and peppermint based mouth gel

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 analyzed 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* and Stevia-peppermint based mouth gel was formulated to check its antibacterial property.

Materials and methods:

Stevia and Peppermint powder was bought from a local supermarket near Poonamallee. 1g of each stevia and peppermint was measured and dissolved in 100mL of distilled water. The mixture was boiled using heating mantle at 70°C for 15 minutes. Then the extract was filtered using Whatmann No.1 filter paper. The filtered extract was further condensed from 80mL to 10mL. 0.5g of carbopol was added to the 10mL extract to make it in a gel consistency. This gel was focussed to act 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- 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- Peppermint based mouth gel have potent antimicrobial properties.

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 pathogens.

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 and 50mL of distilled water was added in small concentration until the solute was completely dissolved. To 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 are prepared. The wound pathogens were swabbed on the agar surface and 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 of inhibition 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).

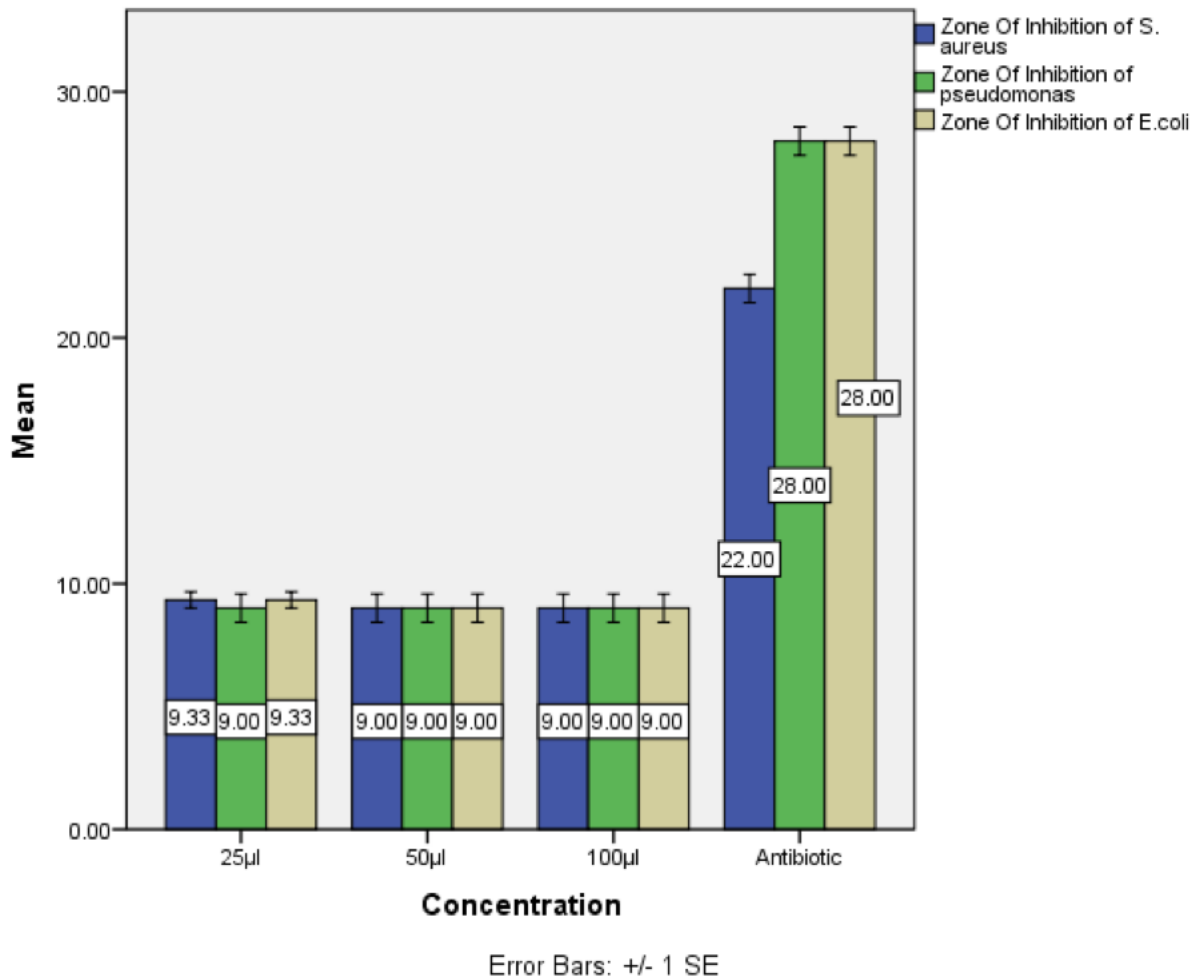


Figure1: Antibacterial activity of stevia-peppermint mouth gel

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 of Inhibition. 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 <i>S. aureus</i>	25µL	3	9.33	.577	.333	0.000
	50µL	3	9.00	1.000	.577	
	100µL	3	9.00	1.000	.577	
	Antibiotic	3	22.00	1.000	.577	
	Total	12	12.33	5.883	1.698	
Absorbance of <i>Pseudomonas</i>	25µl	3	9.00	1.000	.577	0.000
	50µl	3	9.00	1.000	.577	
	100µl	3	9.00	1.000	.577	

	Antibiotic	3	28.00	1.000	.577	
	Total	1 2	13.75	8.635	2.493	
Absorbance of <i>E.coli</i>	25µl	3	9.33	.577	.333	0.000
	50µl	3	9.00	1.000	.577	
	100µl	3	9.00	1.000	.577	
	Antibiotic	3	28.00	1.000	.577	
	Total	1 2	13.83	8.579	2.477	

Table 2 : 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 <i>S. aureus</i>	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
		50µl	100µl	.000	.745	1.00 0	-2.39	2.39
			Antibiotic	-13.000 [*]	.745	.000	-15.39	-10.61
			Antibiotic	-13.000 [*]	.745	.000	-15.39	-10.61
Absorbance of <i>Pseudomonas</i>	Tukey HSD	25µl	50µl	.000	.816	1.00 0	-2.61	2.61
			100µl	.000	.816	1.00 0	-2.61	2.61
			Antibiotic	-19.000 [*]	.816	.000	-21.61	-16.39
		50µl	100µl	.000	.816	1.00 0	-2.61	2.61
			Antibiotic	-19.000 [*]	.816	.000	-21.61	-16.39
			Antibiotic	-19.000 [*]	.816	.000	-21.61	-16.39
Absorbance of <i>E. coli</i>	Tukey HSD	25µl	50µl	.333	.745	.968	-2.05	2.72
			100µl	.333	.745	.968	-2.05	2.72

50µl	Antibiotic	-18.667 [*]	.745	.000	-21.05	-16.28
	100µl	.000	.745	1.00 0	-2.39	2.39
100µl	Antibiotic	-19.000 [*]	.745	.000	-21.39	-16.61
	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. This potential effect of the herbal mouth gel paves the way to use it as an effective agent in treating dental maladies and also by its enormous role in future dental field.

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