A Study of Bioactivity of *Solanum incanum* L. Fruit Extracts on Microorganisms of the Oral Cavity

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Abstract

Tooth decay is an infection due to demineralization and destruction of the hard tissues of the teeth by oral microbes. It is as a result of production of acid by bacterial fermentation of food debris accumulated on the tooth surface. Depending on the extent of tooth destruction, various treatments can be used to restore teeth to normality, but there is no known method to regenerate large amounts of tooth structure. Instead, Dental Health Organizations advocates preventive and prophylactic measures, such as regular oral hygiene and dietary modifications to avoid dental caries. Herbal remedies have a long history of use for gum and tooth problem such as dental caries. *Solanum incanum* L. fruits are locally used to manage the tooth decay which is caused by mouth microbes. In this study, the effect of *Solanum incanum* fruit extracts on growth of oral microbes was investigated. When the microbes were treated with *Solanum incanum* fruit extract at concentration; 10 µl, 20 µl, 30 µl, and 40 µl, 50 µl, 60 µl, 70 µl, 90 µl and 100 µl respectively, the optimum concentration obtained was 70 µl. Similarly, laboratory observations showed that the betadine mouth wash used worked as best as *Solanum incanum* fruit extract with the optimum concentration attained at 70 µl. There was no significance difference on the inhibitory effects of both betadine mouth wash and *Solanum incanum* fruit extracts on oral microbes but on close observation of regression analysis models betadine had more inhibitory effects than *Solanum incanum* fruit extracts.

Key Words: Oral microbes, Dental caries, Solanum incanum, Growth inhibition

Introduction

The mouth harbors a diverse, abundant and complex microbial community, at times, the biodiversity occurring in time and space. Some cause tooth decay, or one to fall victim to one of the periodontal diseases (inflamed gums leading to loosening and loss of teeth). The highly diverse micro flora and fauna inhabits the various surfaces of the normal mouth causing various effects ranging from no effect to infection that could lead to complications that could eventually lead to major systemic issues including death.

Teeth provide a favorable habitat for plaque biofilm development. The development begins with the adsorption of host and bacterial molecules to the tooth surface. A pellicle, defined as a thin coat of salivary proteins begins to form within minutes of tooth eruption or cleaning [1]. The pellicle acts like an adhesive by sticking to the tooth surface and encouraging a conditioning film of bacteria to attach to the pellicle [2]. This conditioning film directly influences the initial microbial colonization, and continues to adsorb bacteria to the tooth surface. Following pellicle formation, there is passive transport of oral bacteria to the tooth surface, which involves a reversible adhesion process [3]. By using weak, long-range physicochemical interactions between the pellicle coated tooth surface and the microbial cell surface, an area of weak attraction is formed that encourages the microbes to reverse their previous adhesion to the pellicle and come off the tooth surface (hence the term "reversible adhesion") [2]. This reversible adhesion then leads to a much stronger, irreversible attachment, as short-range interactions between specific molecules on the bacterial cells and the complementary receptor proteins on the pellicle surface occur. Because many oral microbial species have multiple adhesion types on their cell surface, they can thus participate in a plethora of interactions with both other microbes and with the host surface molecules [2]. The co-adhesion of the later colonizers to the already present biofilm continues to involve many specific interactions between bacterial receptors and adhesions. These interactions build up the biofilm to create a more diverse environment, which includes the development of unusual morphological structures like corn-cobs and rosettes [2].

Mature dental plaque consists of an extremely complex microbial flora, containing perhaps as many as over 280 species of bacteria, many of which are found nowhere else in nature [4]. For the microbial ecologists dental plaque provides a wealth of fascinating microbial bio-interactions in life. Unfortunately, most people become aware of the existence of this complex biodiversity only when they have developed. Bacteria form the largest group of these microorganisms and accumulate on both the hard and soft oral tissues of the oral cavity in bio films. The oral micro biome harbors more than 25, 000 species of bacteria of which 1,000 species exist as part of the dental biofilm ecosystem [5], which causes tooth decay and other related periodontal problems such as gingivitis and chronic periodontitis [6]. These oral bacteria have evolved mechanisms to sense their environment and evade or modify the host, thus positioning themselves to cause damage to the host. Bacteria occupy the ecological niche provided by both the tooth surface and gingival epithelium. However, a highly efficient innate host immune defence system constantly monitors the bacterial colonization and prevents bacterial invasion of local tissues thereby maintaining a dynamic equilibrium between dental plaque bacteria and the innate host immune defence system [7].

The role of bacteria in initiating tooth decay became clear when scientists raised animals under germ free environment and compared with those animals raised under a contaminated environment. It was found that animals raised under germ-free environment had healthy teeth even when fed on sugary food. Yet once these animals were inoculated with bacteria that normally live in the dental plaque, caries could develop rapidly. This phenomenon was first described by Frank Orland

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and his colleagues at the University of Chicago in 1995 [4] Dental caries (Latin, "rot"), also known tooth decay or a cavity, causes demineralization and destruction of the hard tissues of the teeth (enamel, dentin and cementum) [8]. It is as a result of the production of acid by bacterial fermentation of food debris accumulated on the tooth surface. If demineralization exceeds saliva and other remineralisation factors such as from calcium and fluoridated toothpastes, these once hard tissues progressively break down, producing dental caries (cavities or carious lesions, that is, holes in the teeth). Today, caries remains one of the most common diseases throughout the world [9].

Depending on the extent of tooth destruction, various treatments can be used to restore teeth to proper form, function, and aesthetics, but there is no known method to restore large amounts of tooth structure (*Figure 1*). Instead, dental health organizations advocate preventive and prophylactic measures, Such as regular oral hygiene and dietary modifications, to avoid dental caries (Oral Health Topics: Cleaning your teeth and gums, 2006).

Figure 1. *Destruction of a tooth by cervical decay from dental caries, this type of tooth decay is also known as root decay.*

Oral diseases are major health problems with dental caries and periodontal diseases among the most important preventable diseases. Oral health influences the general quality of life and poor oral health is linked to chronic conditions and systematic diseases. Of the more than 750 species of bacteria that inhabit the oral cavity, a number are implicated in oral diseases [10] the development of dental caries involves acidogenic and aciduric gram- positive bacteria (*Streptococcus mutans*, *Lactobacilli* and *Actinomyces*). Dental caries is an irreversible microbial disease involving the calcification of tissues of the inorganic portion and the destruction of the organic substance of the tooth, which often leads to cavitations. Although methods are known for prevention and management of dental caries, it has remained a major oral health problem with some of its manifestations persisting throughout life despite treatment [11]. These oral infections and their corresponding etiologic agents cannot be effectively and efficiently controlled, especially the older generation may not conduct mechanical plaque removal sufficiently as required, hence, the use of antimicrobial mouth rinse, conventional agents such as chlorhexidine and triclosan [12]. Tooth decay can be prevented, controlled and managed by frequent and appropriate methods of brushing and flossing oral cavity to remove dental plaque that cause tooth decay.

Brushing one's teeth has long been considered an important part of dental care in human health history. For instance, as long ago as 3000 BC, ancient Egyptians constructed crude toothbrushes from twigs and leaves of indigenous plants to clean and protect their teeth [13]. Similarly, other cultures such as Greeks, Romans, and Indians cleaned and protected their teeth with twigs from their own indigenous plants as well. Some people would fray one end of the twig so that it could penetrate between the teeth more effectively to help them remove the build-up of dental calculus near the gums of teeth [6]. The use of plants and plant products as medicines for various ailments is as old as human civilization [14]. Plants and plant products with medicinal values have been used in many different forms and states to complement the conventional form of healthcare provision since antiquity in many parts of the world [15].

In rural areas of many developing nations, it has been estimated that about 80% of the population still rely on ethno botanicals as their fundamental sources of medicines [16] and almost 80% of the human population in developing countries is dependent on plant resources for healthcare [17]. Several plants are used in oral health. For instance, Liquorice (*Glycyrrhizag labra*) is one of the most popular plants used in oral care property. It has been shown to have anti-plaque, antibacterial and anti-caries properties [18]. Scutellaria (*Scutellaria baicalensis*) is also effective against oral bacteria [19]. The plant has chemical components that combat gum-disease and caries. Marigold (*Calendula officinalis*) is also used in oral care; especially mouth washes [20]. The plant has antibacterial and anti-inflammatory properties. Balm Mint (*Melissa officinalis*) is used to treat oral infections and has soothing, antibacterial and antiseptic properties [21]. Paracress (*Spilanthes acmella/oleracea*) is used in traditional medicine to treat toothache as it tones gums and has anti-inflammatory property [22] for developing novel lead bioactive chemicals useful in pharmaceutical and agricultural industries in particular [22]. Furthermore, phytochemicals isolated from plants and are already in use as ethno medicines, have been known to serve as a good alternative to synthetic counterparts [23].

Although there are a number of conventional agents already on the market and recommended by health professionals for use in oral health care, these agents contain chemicals that can alter oral micro biota and have undesirable side effects such as vomiting, diarrhea and tooth staining [24]. Search for alternative products continues and natural photochemical isolated from plants used as traditional medicines are considered as good alternatives. Since, in spite of great advances in health industry, oral infections are still considered some of the most serious public health problems facing humanity worldwide [25], there is need to search for new, safe and sustainably effective antimicrobial oral agents.

Materials and Methods

Preparations of the crude fruit extracts from *S. incanum* L

The ripe and unripe fruits of *S. incanum* L. were collected from several naturally growing fields of South Eastern Kenya University. The fruits were then washed with water thoroughly to remove the debris. The green viscous juice was obtained from these fruits through incisions on the pericarp. 1 ml of the fruit extract was made up to 10 ml by adding 9 ml of distilled water. Dose response bioassays involved exposing the test organisms to serially diluted fruit extracts and determining the minimum and maximum volumes that inhibits growth [26].

Preparation of culture media

104 grams of Macconkey agar was suspended in 2 litres of distilled water.

The solution was then boiled to dissolve completely after which it was sterilized by autoclaving at 120°C for 15 minutes.

The surface of the gel was then dried before incubation.

Preparation of oral microbes

The oral microbes were obtained from volunteered students at the University. This was achieved by swabbing through the teeth and crevices of the teeth. The microbes were cultured on Macconkey agar for 48 hours at 37°C after which they were sub-cultured onto the same media.

Screening of antimicrobial activities of the fruit extract

The test involved 'spot-testing' of extract by placing small quantities (in µl) on the surface of agar containing an overlay of bacteria. This was achieved by placing discs of filter papers soaked with diluted fruit extract (i.e., 10 µl, 20 µl, 30 µl, 40 µl, 50 µl, 60 µl, 70 µl, 80 µl, 90 µl and 100 µl concentrations at a constant volume of 50 µl) on the plates with overlay of bacteria. Each treatment level was done in four replicas. A similar experiment was set for oral microbes after which they were treated with betadine mouth wash at 10 treatment levels.

Statistical analysis

The comparison of mean percentage inhibition of fruit extract of *Solanum incanum* to oral microbes growth on Macconkey agar and mean percentage inhibition of betadine mouth wash at different concentrations i.e., 10 µl, 20 µl, 30 µl, 40 µl, 50 µl, 60 µl, 70 µl, 90 µl, 100 µl was analyzed using SPSS version 21.0 software. One way analysis of variance was used to compare growth inhibition at each concentration. At the level of significance of a=0.05, the entire data set of growth inhibition of both fruit extract and betadine, were subjected to pair wise comparison analysis using Post Hoc Test of *student-Neuewman- Kuels Test.*

To establish that the assumptions of homogeneity of variances of data fruit extract treatments and betadine mouth wash treatment was met, the Levene's test for the equality of variances was used. The test is similar to a T test in that the hypothesis that the variances in the two groups (fruit extract inhibition and betadine mouth wash inhibition) are equal (that is, the difference between the variances is zero). Therefore, if the Levene's test was significant at $p \leq 0.05$, it was concluded that the null hypothesis was incorrect and that the variances are significantly different and therefore, the assumption of homogeneity of variances had been violated, implying that the independent student's t test for equal variances was to be used. If , however, the Levene's test was non-significant ($p > 0.05$) then the null hypothesis is accepted and therefore the differences between the variances is zero, implying that the variances are roughly equal and the assumption is tenable, thus independent student's t test for equal variances was to be used.

Growth inhibition data for fruit extract was subjected to simple regression in order to predict the outcome variable (inhibition) from predictor variable (concentration). The regression model was developed based on;

$\gamma i = \beta 0 + \beta 1 x + \varepsilon$ where,

$\beta 0$= Coefficient of the model representing y- intercept,

$\beta 1$= Coefficient of the model representing concentration 1,

X= various concentrations of fruit extract,

ε=error term representing the differences between the actual observed value and that predicted by the model (predictor regressor).

Results

Growth inhibition of fruit extract on oral microbes

The following results were obtained from an experiment carried in 4 replicates (blocks) i.e., A,B,C, and D with the goal of comparing 10 treatment levels of *Solanum incanum* fruit extract concentration from 10 ≥l to 100 µl (*Table 1*).

Table 1. *Growth inhibition of Solanum incanum fruit extracts against oral microorganisms.*

Concentrations of *Solanum incanum* fruit extracts (%)	Mean inhibitory effects (± SE)
10	1.1250 ± 0.96a
20	1.2000 ± 0.00b
30	1.3250 ± 0.03c
40	1.5250 ± 0.05d
50	1.6500 ± 0.03e

60	1.6500 ±0.03e
70	1.8000 ± 0.00f
80	1.8000 ± 0.00f
90	1.8000 ± 0.00f
100	1.8000 ± 0.00f
SE = Standard errors of the means	

Note: Following multiple post hoc comparative analysis, means with the same superscript of alphabetical letters are not significantly different from one another at α = 0.05 (Student-Newman-Keuls).

Growth inhibition of a commercial product, betadine mouth wash

The following results were obtained from an experiment carried in 4 replicates (blocks) i.e., A, B, C and D with the goal of comparing 10 treatment levels of betadine mouthwash concentration from 10 µl to 100 µl (*Table2*).

Table 2. *Growth inhibition of a co mmercial product, betadine mouth wash against oral microorganisms.*

Concentration of commercial betadine (%)	Mean inhibitory effects (± SE)
10	1.0750 ± 0.48a
20	1.3000 ± 0.00b
30	1.4000 ± 0.41c
40	1.5500 ± 0.29d
50	1.7000 ± 0.58e
60	1.8750 ± 0.03f
70	2.0000 ± 0.00g
80	2.0000 ± 0.00g
90	2.0000 ± 0.00g
100	2.0000 ± 0.00g
SE = Standard errors of the means	

Note: Following multiple post hoc comparative analysis, means with the same superscript of alphabetical letters are not significantly different from one another at α = 0.05 (Student-Newman-Keuls).

Table 3. *Effect of concentration of candidate products on the growth inhibition of oral microorganisms.*

		Sum of Squares	df	Mean Square	F	Sig.
EXTRACTS	Between Groups	3.001	9	0.333	121.263	0
	Within Groups	0.082	30	0.003		
	Total	3.084	39			
BETADINE	Between Groups	4.211	9	0.468	133.683	0
	Within Groups	0.105	30	0.004		
	Total	4.316	39			

For fruit extract, the F (9, 30)-ratio was 121.263 and indicated that the results were significant at $P < 0.05$. The P-value was far much below the α-value, 0.005, thus indicating that the results were highly significant. This therefore implies that the *Solanum incanum* fruit extracts had a significant inhibitory effect on the growth of oral microorganisms. While with betadine treatment, F(9, 30)-- ratio was 133.687 and also indicated that the results are significant at $P < 0.05$. The P-value is far much below the α-value, 0.005, thus indicating that the results are highly significant. This therefore implies that the commercial product of betadine had a significant inhibitory effect on the growth of oral microorganisms (*Table 3*).

Table 4. *Model summary for descriptive statistics for regression analysis: correlation due to concentration of Solanum incanum fruit extracts.*

R	R Square	Adjusted R Square	Std. Error of the Estimate	Sign.
0.958(a)	0.917	0.915	0.082	0

a Predictors: (Constant), Concentrations

The R (Pearson correlation coefficient), which ranges between 0 and 1, is a measure of multiple correlation between predictors and outcomes in models, showed that there was a very large positive correlation between concentrations of *Solanum incanum* fruit extracts and inhibitory effect (R = 0.958) (*Table 4*).

Given that $P < 0.05$, showed that the above correlation is statistically significant and this therefore implied that various concentrations of *Solanum incanum* fruit extracts predicted fairly well the growth inhibitory of the oral microorganisms.

The R^2 indicates the variance in the outcome for which the predictor can be able to account, thus providing a good gauge of the substantive size of the relationship. The concentrations of *Solanum incanum* fruit extracts account for 91.7% of the variance in the growth inhibitory of oral microorganisms. There are also other variables that have influence on the growth of oral microorganisms, accounting for 8.3% of the variance in the growth inhibitory. The adjusted R2 gives some idea of how well the model generalizes and ideally, is supposed to be the same as and/or very close to the value of R2. However, the difference is small, only 0.2%. This shrinkage means that if the model were derived from the

population rather than the sample, it could account for less than 0.2% variance in the outcome (growth inhibitory effect).

Table 5. *Regression analysis of ANOVA on the model due to concentration of Solanum incanum fruit extracts.*

Model	Sum of Squares	df	Mean Square	F	Sig.
Regression	2.828	1	2.828	420.53	.000(a)
Residual	0.256	38	0.007		
Total	3.084	39			

Note: a Predictors: (Constant), Concentration, b Dependent Variable: Inhibitory effect

The F- ratio (1, 38) is 420.531, which is significant at $p < 0.05$. The analysis reveals that the model is indeed highly significant, given that $P < 0.05$. This regression model results is better prediction of growth inhibitory effect of oral microorganisms than if the mean value of growth inhibitory effect was used. In conclusion therefore, the regression model overall predict the growth inhibition significantly well (*Table 5*).

Table 6. *Evaluation of model parameters due to concentration of Solanum incanum fruit extracts.*

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	1.078	0.028		38.497	0
2	Concentration of *Solanum incanum* fruit extracts	0.009	0	0.958	20.507	0

Dependent Variable: Inhibitory effect

Model Parameters and the model due to concentration of *Solanum incanum* fruit extracts.

The *Table 6* provides details of the model parameters. From the regression analysis, both the constants and concentration of *Solanum incanum* fruit extracts are significantly important in the model in predicting the growth inhibitory effect of the oral microorganisms ($P < 0.05$). The model overall results in a significantly good degree of prediction of the outcome variable as both the $\beta 0$ and $\beta 1$ are statistically significant ($P < 0.05$) (*Table 6*) and also as ANOVA has already shown that the only variable, the concentration of *Solanum incanum* fruit extracts is a good predictor of the outcome (growth inhibitory of the oral microorganisms) (*Table 5*).

As the model was envisaged in the section of materials and methods, it was built alongside the following:

$\gamma i = \beta 0 + \beta 1 X + \varepsilon$,

Where, γ = outcome (growth inhibitory)

$\beta 0$ = a constant (y-intercept) and become the outcome in the absence of the variable, X (concentration of *Solanum incanum* fruit extracts).

$\beta 1$ = is a rate of change indicating a change in the outcome associated with a unit change in the predictor.

X = Concentration of *Solanum incanum* fruit extracts.

ε = the error term associated with the evaluation of the variable, X (concentration of Solanum incanum fruit extracts).

From *Table 6* analysis, $\beta 0 = 1.078$ (growth inhibitory in cm) when no *Solanum incanum* fruit extracts had been applied, the model therefore predicts that there was a growth inhibitory of 1.078 cm. The value of $\beta 1 = 0.009$. Therefore, if the predictor variable is increased by one unit, (concentration of *Solanum incanum* fruit extracts is increased by one unit), then the model predicts that 0.009 extra units in cm of growth inhibitory will be achieved. Given that from *Table 6*, $P < 0.05$, it implies that the two βs, $\beta 0$ and $\beta 1$ are not zeros (0) and it can therefore be concluded that concentration of *Solanum incanum* fruit extracts makes a significant contribution ($P < 0.05$) in predicting growth inhibitory effect, using the following model:

Growth inhibitory = 1.078 + 0.009 (concentration of *Solanum incanum* fruit extracts).

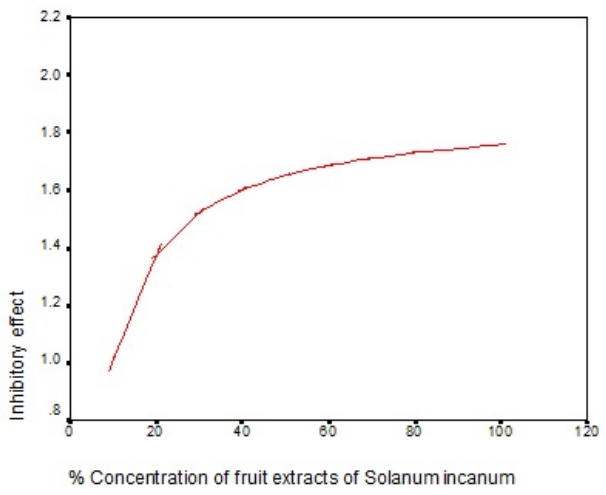

Figure 2. *The graph of inhibitory effects of fruits extracts (cm) against concentration (%).*

Evaluation of the effects of betadine

Table 7. *Model summary for descriptive statistics for regression analysis: correlation due to evaluation of betadine.*

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Change Statistics				
					R Square Change	F Change	df1	df2	Sig. F Change
1	.941a	0.885	0.882	0.11437	0.885	291.96	1	38	0
Predictors: (Constant), Concentration									

The R (Pearson correlation coefficient), which ranges between 0 and 1, is a measure of multiple correlation between predictors and outcomes in models, showed that there was a very large positive correlation between concentrations of betadine and inhibitory effect (R = 0.941) (*Table 7*). Given that P < 0.05 showed that the above correlation is statistically significant and this therefore implied that various concentrations of betadine predicted fairly well the growth inhibitory of the oral microorganisms (*Table 7*).

The R^2 indicates the variance in the outcome for which the predictor can be able to account, thus providing a good gauge of the substantive size of the relationship. The concentrations of betadine mouthwash account for 88.5% of the variance in the growth inhibitory of oral microorganisms. There are also other variables that have influence on the growth of oral microorganisms, accounting for 11.5% of the variance in the growth inhibitory. The adjusted R^2 gives some idea of how well the model generalizes and ideally, is supposed to be the same as and/or very close to the value of R^2. However, the difference is small, only 0.4%. This shrinkage means that if the model were derived from the population rather than the sample, it could account for less than 0.3% variance in the outcome (growth inhibitory effect) (*Table 8*).

Table 8. *Regression analysis of ANOVA on the model due to evaluation of betadine.*

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	3.819	1	3.819	291.96	.000b
	Residual	0.497	38	0.013		
	Total	4.316	39			
a. Dependent Variable: Length						
b. Predictors: (Constant), Concentration						
The F- ratio (1, 38) is 291.956, which is significant as p < 0.05. The analysis reveals that the model is indeed highly significant, given that P < 0.05. This regression model results is better prediction of growth inhibitory effect of oral microorganisms than if the mean value of growth inhibitory effect was used. In conclusion therefore, the regression model overall predict the growth inhibition significantly well.						

The *Table 9* provides details of the model parameters due to evaluation of betadine. From the regression analysis, both the constants and concentration of betadine mouth wash are significantly important in the model in predicting the growth inhibitory effect of the oral microorganisms (P < 0.05). The model overall results in a significantly good degree of prediction of the outcome variable as both the β0 and β1 are statistically significant (P < 0.05) (*Table 9*) and also as ANOVA has already shown that the only variable, the concentration of betadine mouthwash is a good predictor of the outcome (growth inhibitory of the oral microorganisms) (*Table 8*).

Table 9. *Evaluation of model parameters due to evaluation of betadine.*

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.	95.0% Confidence Interval for B	
		B	Std. Error	Beta	Lower Bound		Upper Bound	
1	(Constant)	1.098	0.039		28.116	0	1.019	1.177
	Concentration	0.011	0.001	0.941	17.087	0	0.009	0.012
a. Dependent Variable: Length								

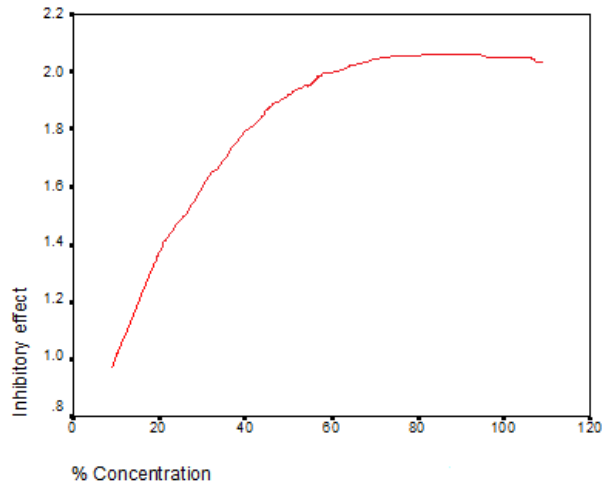

Figure 3. *Model of inhibitory effects of betadine mouthwash (cm) against concentration (%)*

As the model was envisaged in the section of materials and methods, it was built alongside the following:

$\gamma i = \beta 0 + \beta 1X + \varepsilon$,

Where, γ = outcome (growth inhibitory)

β0 = a constant (y-intercept) and become the outcome in the absence of the variable, X (concentration of betadine mouthwash).

β1 = is a rate of change indicating a change in the outcome associated with a unit change in the predictor.

X = Concentration of betadine mouthwash

ε = the error term associated with the evaluation of the variable, X (concentration of betadine mouthwash).

From *Table 9* analysis, β0 = 1.098 (growth inhibitory in cm) when no betadine had been applied, the model therefore predicts that there was a growth inhibitory of 1.098 cm. The value of β1 = 0.011. Therefore, if the predictor variable is increased by one unit, (concentration of betadine mouthwash is increased by one unit), and then the model predicts that 0.011 extra units in cm of growth inhibitory will be achieved. Given that from *Table 9*, P < 0.05, it implies that the two βs, β0 and β1 are not zeros (0) and it can therefore be concluded that concentration of betadine mouthwash makes a significant contribution(P < 0.05) in predicting growth inhibitory effect, using the following model:

Growth inhibitory = 1.098 + 0.011 (concentration of betadine mouthwash).

Table 10. *Independent samples t test for comparative analysis of the growth inhibitory effects of betadine mouthwash and Solanum incanum fruit extract against oral microbes.*

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
INEFFECT	Equal variances assumed	3.35	0.071	-1.488	78	0.141	-0.103	0.0689	-0.24	0.0346
	Equal variances not assumed			-1.488	75.895	0.141	-0.103	0.0689	-0.24	0.0347

The comparative analysis between the inhibitory effects of betadine and that of *Solanum incanum* fruits extracts showed that there was no significant difference between the two (P = 0.071) (*Table 10*). Nevertheless, on close observation of two models (*Figures 2 and 3*) betadine had more inhibitory effects than *Solanum incanum* fruit extracts.

Discussion

The results of this study provide evidence that fruit extracts of *Solanum incanum* inhibit growth of oral microbes. Although the inhibition is shown to increase with increase in concentration of the extract, the inhibition raises up to the optimum level 70% fruit extract (*Table 1*). Regression model analysis showed that for every one unit increase in concentration, 0.009 cm extra inhibition length was observed. However, 1.078 (growth inhibitory in cm would be realized when no solution of fruit extracts was applied (*Table 6*). The analysis of variance revealed that the concentrations of fruit extract are not equally effective as level of significance (.000) was less than p value (p = 0.05).

Similarly, the study showed betadine mouth wash (commercial product) to inhibit growth oral microbes with the inhibition rate increasing with the increase in concentration. For every one unit increase in concentration, 0.011 cm extra inhibition length was observed (*Table 9*). Optimum inhibition was achieved at 70% betadine (*Table 2*). In addition, 1.098 (growth inhibitory in cm would also be realized when no betadine was applied (*Table 9*). The Levene's test for equality of variance between betadine and fruit extract was non-significant (p > 0.05) implying that the differences between the variances is zero. However, on close observation of two models (*Figures 1 and 2*) betadine had more inhibitory effects than Solanum incanum fruit extracts.

From literature point of view, betadine is a stable chemical complex of polyvinylpyrrolidone (Povidine, PVP) and element of iodine [27]. The elemental iodine has very broad antimicrobial spectrum: bacteria, viruses, bacteria endosperm, fungi, and protozoans are destroyed through oxidative interactions and direct iodination of biological macromolecules. Povidine is a hydrophilic and act as a carrier of the iodine moiety to cell wall. The free iodine released is rapidly cytotoxic, killing the cell within 10 seconds [28]. The free iodine released from the Povidine- iodine complex is used up, until the available iodine is exhausted. Iodine penetrates the cell wall of microorganisms quickly and the lethal effects are believed to result from destruction of protein and nucleic acid synthesis. Povidine-iodine (PI) has anti-biofilm activity against *Staphylococcus epidermidis* and *Staphylococcus aureus*. Inhibition of biofilm by PI correlates with decreased transcription of the icaADBC operon, which in turn correlates with activation of the icaR transcriptional repressor in *Staphylococcus epidermidis* [29].

Previous studies have also shown that most of the plants of Solanaceae contain alkaloids, tannins, steroids, saponins, as well as reducing sugars [30]. The fruit extracts have been identified to have alkaloids, phenols, carbohydrates, tannins, triterpenoids, glycosides, steroids, resins and saponins. Flavonoids and chlorogenic acids have been documented for *Solanum incanum* [31].The observed antimicrobial activities of *Solanum incanum* have been shown to be caused by alkaloids. The Solasodine found in fruits may also be responsible for antimicrobial activity.

The antibacterial activity of flavonoids is being increasingly documented. Many other phytochemicals preparations with high flavonoids content have been reported to exhibit antibacterial activity [32]. Although there have been comparatively few studies into the mechanisms underlying flavonoids antibacterial activity, information from published literature indicates that different compounds within this class of phytochemicals may target different components and functions of the bacterial cell [33].The fruit extract may exhibit their action through inhibition of nucleic acid, protein and membrane phospholipids biosynthesis. For instance, flavonoids exhibit their action by inhibiting DNA, RNA, Proteins and Lipids synthesis in microorganisms. A study using radioactive precursors by Mori et al. in 1987 showed that DNA synthesis was most inhibited by flavonoids in Proteus vulgaris while RNA synthesis was most affected in *S. aureus*. Protein and lipids synthesis were also affected to a lesser extent. Mori et al. suggested that the B- ring of flavonoids may play a role in intercalation or hydrogen bonding with stacking or nucleic acid bases and that this may explain the inhibitory action on DNA and RNA synthesis.

From the literature of betadine and *Solanum incanum* fruit extract, it's revealed that both exhibit antimicrobial properties. This is achieved by inhibiting the synthesis of nucleic acid, proteins and lipids in microorganisms. From this study however, it was revealed that betadine had more inhibitory effects than *Solanum incanum* fruit extract on oral microorganisms. The difference observed could be attributed to differences in the rate at which they penetrate the target cells. Betadine has iodine that penetrates the cells quickly and acts in 10 seconds due to presence of povidine which act as a carrier. Povidine is also hydrophilic, thus facilitating the penetration. On the other hand, the phytochemicals in fruit extracts could have lower solubility to water than povidine thus reduced rate of penetration and consequently less inhibitory effects. This study conforms to these findings by ascertaining that the antimicrobial properties of this plant probably explain its traditional use for treating bacterial diseases.

Conclusion

Solanum incanum possess numerous biologically active compounds which could serve as source of herbal medicine, thus the antimicrobial activity of *Solanum incanum* fruit extracts could be attributed to the phytochemical constituents present in the crude extract. The purified components may have been even more potency with respect to inhibition of the oral microbes. *Solanum incanum* fruit extracts inhibited the growth of oral microbes with this inhibition increasing with the increase in concentration up to optimum level (70%). A similar inhibition of oral microbes was observed with betadine mouth wash. Comparison of the inhibitory effect of fruit extract and betadine mouth wash using independent t test revealed that there was no significant difference between the two ($P = 0.071$) (*Table 7*). Nevertheless, on close observation of two models (*Figures 1 and 2*) betadine had more inhibitory effects than *Solanum incanum* fruit extracts.

Recommendations

Since the fruit extracts have been shown to inhibit growth of oral microbes, further works on the types of phytoconstituents and purification of individual groups of bioactive components to reveal the exact potential of the plant to inhibit several pathogenic microbes should be done. Successive extraction and isolation of botanical compounds from plant material is largely dependent on the type of solvent used in the extraction. This study used water as the diluent. Maybe use of a different diluent such as alcohol could have provided a more antibacterial activity than using water. Thus appropriate solvent should be identified.

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