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Antimicrobial Properties of Sisal (*Agave sisalana*) Used as an Ingredient in Petroleum Jelly Production in Swaziland

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Abstract: A petroleum jelly product containing sisal extract (*Agave sisalana)* used to improve the skin integrity has been claimed to have antimicrobial properties, however, these claims have not been scientifically proven. The aim of the study was to investigate the antimicrobial properties of the *Agave sisalana* containing petroleum jelly. A microbial inhibition test using disc assays and a Delvo test were used to determine the antimicrobial properties of sisal extract. The results indicated that sisal has anti-microbial substances. The sisal extract inhibited growth of *Escherichia coli* and *Bacillus stearothermophilus.* Results have also shown that the sisal's antimicrobial active ingredient is oil soluble. It was concluded that the petroleum jelly product contain antimicrobial properties but the sisal concentration in the product may not be sufficient for a complete growth inhibition of microorganisms.

Key words: *Bacillus stearothermophilus, Escherichia coli,* inhibitory substances, petroleum jelly, sisal

INTRODUCTION

Varied sources of substances, particularly plant extracts, have been reported to have antimicrobial properties (Yeung *et al.*, 2009; Modaressi *et al.*, 2009; Shobana *et al.*, 2009). Sisal, *Agave sisalana*, is a herbaceous monocotyledonous plant from the Agavaceae family that is also claimed to have antimicrobial properties. It originated from central America and Mexico but is now found in many tropical countries including Brazil, Tanzania, Uganda, Mozambique, South Africa and Swaziland. Sisal fibers are widely used for many applications including ropes for the marine and agriculture industry, as well as composites (Lock, 1969; Zwane, 1997). There are many varieties of sisal but *Agave sisalana* and *Agave fourcroydes* are the most commercialized varieties.

Several researches have been done on the effect of chemical properties of sisal fibers on improving the pulping and bleaching processes of the raw material (Lock, 1969; Guti´errez *et al.*, 2008). Few reported studies suggest that sisal may have antiseptic properties (Guti´errez *et al.*, 2008), however, such claims, have not been verified.

In Swaziland, sisal extract is used as a main antiseptic ingredient in the petroleum jelly production by cottage processors. The basis for this is that sisal contains several chemical substances that have been individually studied and found to have antiseptic properties (Uretsky, 2002). Sisal extracts such as ferulic acid, have been claimed to have antioxidant properties that neutralizes free radicals and this could cause oxidative damage of cell membranes and DNA (Sahalean, 2004). The aim of this study was to determine the antimicrobial properties of sisal extract when used as an antiseptic ingredient in petroleum jelly production.

MATERIALS AND METHODS

Plant material and sisal extract preparation: The leaves of the *Agave sisalana* were collected from the Malkerns Valley in Swaziland during the months of November 2008 to January 2009 located at altitude 754 M, longitude E 31.18° and latitude S 26.56°. Sisal extract substances were obtained from fresh leaves using either water or oil as follow:

- One kilogram of the sisal leaves was weighed, and washed in running cold water.
- The leaves were then crushed in a blender.
- Crushed leaves were boiled in 500 mL of either water or petroleum ether until the leaves turn brown, to obtain sisal extract.
- The extract was filtered using a clean cotton cloth and stored in sterilized 250 mL screw capped schotts bottles.

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Table 1: Inhibition properties of sisal on *Bacillus stearothermophilus* as detected by the Delvo SP-NT method

Treatment	n	Inhibition (%)	Lack of inhibition (%)
Milk only	7	0	100
Milk plus 10% (v/v) sisal extract	7	100	0
Milk plus 10% (v/v) petroleum jelly	7	0	100

Source of bacteria cultures: The *Escherichia coli* (*E. coli*) were isolated from cow dung by enrichment on McKonkey broth and streaking on Eosin Methylene Blue (EMB) agar. Sheen green colonies were confirmed as *E. coli* by biochemical tests as described by Singleton (1995). The source of the *Bacillus stearothermophilus* was the Delvo Test SP-NT kit (Girst-brockades Delft, The Netherlands). As described before, Stead *et al.* (2008), the Delvotest SP-NT ampoules contain a standardized number of *Bacillus stearothermophilus* spores, nutrient medium and a bromocresol purple pH as indicator.

Bacterial inhibition tests: Growth inhibition of *Escherichia coli* was determined using the disc diffusion technique as described before (Singleton, 1995; Kelman *et al.*, 2001; Hussain and Ananthan, 2009). Bacteria were grown in Nutrient broth (Oxoid Ltd., England) at 37°C for 18 h and then 1 mL of appropriately diluted cultures were spread on Muller - Hinton agar. Discs impregnated with 50 to 150 µL of the test suspensions were put on the agar surface using sterilized forceps. Plates were incubated at 37°C for 48 h and the zones of growth inhibition were measured in mm as the clear halos excluding the paper discs diameters.

Growth inhibition of *Bacillus stearothemophilus* was determined using the Delvo test SP-NT kit (Girst-brockades Delft, The Netherlands). Sterilized milk samples were mixed with 10% (v/v) sisal extract solution and 100 µL of the mixtures were inoculated into the ampoules. The ampoules were sealed using a plastic film and were incubating in a Delvo ampoule incubator at 64°C for 2.5 h. Growth of *Bacillus stearothemophilus* was indicated by change of bromocresol purple to yellow and if the growth was inhibited, the ampoules remained purple.

Data analysis: Data were analyzed to meet all objectives using the statistical package MSTAT-C, V2.00 (Michigan State University) to obtain means. Statistical Package for Social Sciences V-7.5 (SPSS Inc., 1989-1996) was used to correlate the means. To find the significant differences amongst the means, the Duncan's Multiple Range Test (DMRT) was used. One way analysis of variance (ANOVA) was used to quantify the antimicrobial properties and for the comparative analysis of the results.

RESULTS AND DISCUSSION

In this study the susceptibility of *Bacillus stearothermophilus* to *Agave sisalana* extract was tested using the Delvo test SP - NT. The results presented in Table 1 shows that sisal extract completely inhibited the growth of *Bacillus stearothermophilus*. All samples containing oil extracted sisal retained the purple colour, indicating 100% inhibition. Samples containing petroleum jelly that had the sisal ingredient changed from purple to yellow. This indicated growth inhibition failure by the sisal in the petroleum jelly. The reasons for this are obscure, probably this could be attributed to low concentration of the sisal extract in the petroleum jelly or it could be attributed to absence of antimicrobial agents in the sisal. The latter, however, is unlikely because direct addition of oil extracted sisal completely inhibited growth (Table 1).

Fig. 1: Growth inhibition zone of Escherichia coli in samples treated with oil extracted sisal

The inhibition of *Bacillus stearothermophilus* by the oil extracted sisal suggests that sisal may contain some microbial inhibitory substances. The results (Table 1) however, have shown that these inhibitory substances may not be present in sufficient concentrations in the petroleum jelly to cause inhibition.

Growth inhibition of *Escherichia coli* by sisal extract was determined by the disc diffusion technique as suggested by Hussain and Ananthan (2009). Figure 1 shows the growth inhibition of *Escherichia coli* by oil extracted sisal.

Results presented on Fig. 2a, b show absence of inhibition by water extracted sisal and by sisal containing petroleum jelly. The observation that sisal extracted with water did not inhibit bacterial growth, yet oil extracted sisal had microbial inhibitory properties, may suggest that the antibacterial chemicals of sisal are mainly lipid

a b

Fig. 2: Lack of growth inhibition zone of *Escherichia coli* in samples treated with water extracted sisal and sisal enriched petroleum jelly, respectively

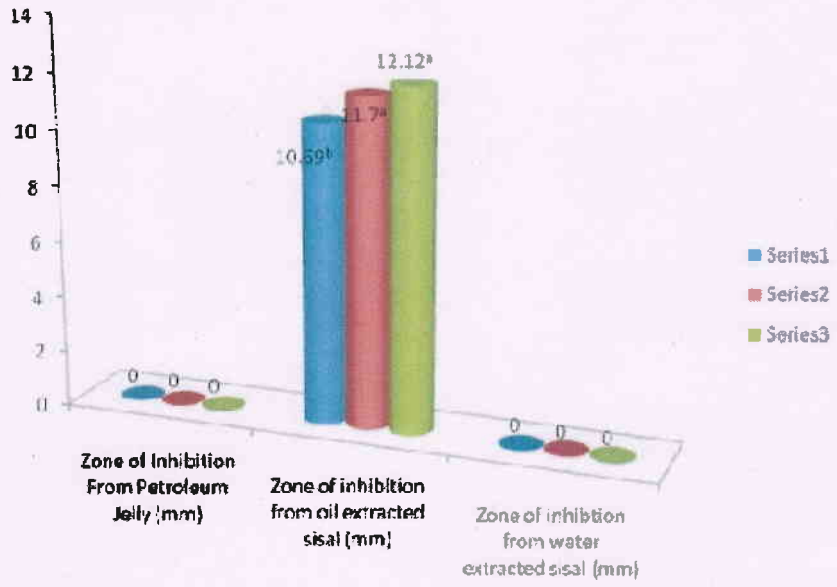


Fig. 3: Growth inhibition of *Escherichia coli* by sisal extracts of variable concentrations
Key: Amount of inhibitory substance used:
Series 1 = 0.05 mL; Series 2 = 0.1 mL; Series 3 = 0.15 mL.

soluble thus could not be dissolved in water (Guti´errez *et al.*, 2008).

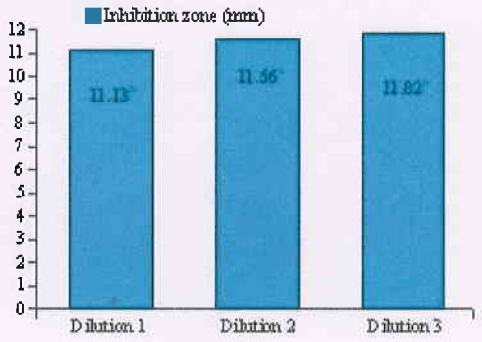
Results have also shown that there was no growth inhibition observed on the discs dipped in sisal containing petroleum jelly. This could be attributed to incorporation of sisal to petroleum jelly in insufficient concentrations to cause bacterial inhibition. These results are in line with the findings of Greenwood *et al.* (2002) that demonstrated that a substance is only able to inhibit the growth of micro-organisms if it contains special chemicals that have antimicrobial properties.

Results presented on Fig. 3 show that there were no zones of bacterial growth inhibition from water extracted sisal and from sisal containing petroleum extracts. Zones of *E. coli* growth inhibition were observed in oil extracted sisal treatments. The zones increased as the volumes of the inhibitory substances were increased. The lowest volume used, 0.05 mL produced the smallest zone of

Table 2: Pearson correlation test between sisal treatments, cell populations, and zone of microbial growth inhibition

	Correlation coefficient (N = 3)		
Treatment	Dilution factor	Extract volume	Inhibition diameter
Dilution factor	1.000	- 0.955	- 0.883
Extract volume	- 0.995	1.000	0.982
Inhibition diameter	- 0.883	0.982	1.000

**: Correlation significant at the 0.01 level (2-tailed).



Dilution 1 = 10^0; Dilution 2 = 10^{-1}; Dilution 3 = 10^{-2}

Fig. 4: Effect of microbial population on sisal growth inhibition of *Escherichia coli*

inhibition, 10.69 mm, whereas the highest volume, 0.15 mL produced the biggest ring, 12.17 mm and this difference was significant ($p<0.01$). Microbial growth inhibition by the sisal extract could be attributed to presence of high concentration of inhibitory substances in the oil extracted sisal (Uretsky, 2002).

The effect of microbial population on zone of inhibition diameter was determined by testing growth inhibition on different *E. coli* culture dilutions, 10^0, 10^{-1}, 10^{-2}. Figure 4 shows the mean inhibition diameters for the different culture dilutions used when inoculating the bacteria into the media.

The results have indicated that the higher the culture dilution, the bigger was the zone of inhibition obtained. This may suggest that if the bacterial population is lower the growth inhibitory properties of the sisal extract could be enhanced. These results have also shown that there were variations in the amount of growth inhibition due to the volume of sisal extract used and the dilution factor of the culture.

Table 2 indicates the relationship between means of the different treatments used. The results show a strong negative correlation (-0.883) between bacterial cell population and inhibition diameter, while on the other hand there was a strong positive correlation (0.982) between inhibition diameters and extract volume. The results confirmed that as the microbial population increases, the inhibition diameter decreases. Also when the sisal extract volume used was increased, the inhibition diameter was also increased. This could be attributed to a high concentration of the antimicrobial agents in the sisal extract in higher volumes and lower cell populations (Tortora *et al.*, 2007; Vanderzant and Splittstoesser, 1992).

The dilution factor is negatively correlated with inhibition diameter indicating that as dilution factor increases the degree of inhibition decreases. The more sisal extract is used the more effective it can be in decreasing the rate of microbial growth. On the other hand, there was positive correlation between inhibition diameter and extract volume indicating that as sisal extract (using oil) volume increases the area of inhibition also increases.

CONCLUSION

The active ingredients for inhibiting micro-organisms in sisal were found to be oil soluble and could not be extracted using water. Sisal extracted with oil contained anti-microbial substances in sufficient concentrations to inhibit the growth of micro-organisms. The amount of sisal extract used has direct impact on its effectiveness to inhibit microbial growth. The amounts of micro-organisms present also had direct influence on the effectiveness of the sisal extract in inhibiting the growth of micro-organisms. The petroleum jelly produced by rural Swazi women was found to contain low concentration of microbial inhibitory substances which was not a significant concentration to bring effective inhibition of the micro-organisms. It is recommended that producers should increase the amount of sisal added when making the product to prevent lower concentrations that do not inhibit microbial growth. Further work should identify the active ingredients of microbial growth inhibition in sisal and a study towards the development of standard recipe for preparing the petroleum product in Swaziland is also required.

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