

Antibacterial Effect of Gum Arabic on Streptococcus Mutans in Vitro

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Abstract

Background: Nowadays a great need for an anti-bacterial agent can be used on a daily basis without side effects of antimicrobial chemicals as chlorhexidine. This study aims to evaluate the microbiologic effect of Gum Arabic against streptococcus cultures. **Materials and Methods:** Aqueous of Gum Arabic Hashab (Acacia Senegal) and Talha (Acacia Seyal) with different concentration (10%-100%) were used to evaluate the antimicrobial activity by the agar well diffusion method. *Streptococcus mutans* was placed on the agar. Gum Arabic solution was propelled in the wells made in the agar, after incubation at 37°C - for 24 hours in anaerobic incubator. The diameter of inhibition zones formed around wells were measured and compared with control (chlorhexidine 2%). Minimum inhibitory concentration for Hashab and Talha was determined. **Results:** No significant difference was found between Gum Arabic and chlorhexidine. The antimicrobial effect of Gum Arabic Hashab and Talha was similar to that of chlorhexidine (p-value > 0.05). Gum Arabic Hashab and Talha had same effect against *Streptococcus mutans* (p-value > 0.05). Minimum inhibitory concentration was 7% for Gum Arabic Hashab and 8% for Gum Arabic Talha. **Conclusion:** Gum Arabic inhibited the growth of *Streptococcus mutans*. It highly recommended to be used as preventive measure for cariogenic process.

Keywords: Antibacterial; Gum Arabic; Streptococcus Mutans; Dental Caries.

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Introduction

Dental caries is one of the most prevalent oral diseases worldwide. It affects the majority of individuals in all age groups during lifetime and characterized by the colonization and accumulation of oral microorganisms on dental surfaces, resulting in the formation of dental plaque (or bacterial biofilm) and demineralization of the tooth structure [1].

Dental caries is a disease that progresses when carbohydrates fermented by bacteria produce acid and lead to tooth demineralization [2].

Several bacteria have been described in association with the cariogenic process, especially the acidogenic and acid uric bacteria. *Streptococcus mutans* is the most important agent capable of demineralizing enamel. It possesses many virulent properties that support the role in the caries procedure and become pathogenic under certain conditions which lead to frequent and prolonged acidification of the dental plaque. *Streptococcus mutans* adapt to the low pH environment. Acid production rate increases and plaque PH decreases resulting in a cariogenic plaque [3].

Over the last decades, the uses of natural products as complementary for prevention and

treatment of dental caries has increased among different populations [4-6].

Gum Arabic is one of the most natural products widely applied in the medical fields. It is a dried exudate produced from the trunk and branches of the Acacia Senegal tree, known as Hashab or hard gum and the Acacia Seyal tree, known as Talha or flaky gum. Gum Arabic is pale white to orange brown in color. It is solid and breaks with a glassy fracture; it can stay unaltered for decades if stored properly. Sudan is the world's largest producer and exporter of gum Arabic [7].

Acacia Gum consists of a complex mixture of calcium, magnesium and potassium salts of polysaccharidic acid (Arabic acid). It contains tannins which are reported to exhibit astringent, haemostatic and healing properties. In addition to cyanogenic glycosides and several enzymes; oxidases, peroxidases and pectinases, all had shown to exhibit antimicrobial properties [8].

Antibacterial chemicals, as chlorhexidine have been used for elimination of oral bacteria. Discoloration of teeth and unpleasant taste can occur when these chemicals are prescribed for an extended period. The Gum Arabic has antibacterial and antiplaque agent that can be used on a daily basis with minimal side effect [9,10].

To our knowledge no published study concerning the effect of Gum Arabic on *streptococcus mutans* are available. Therefore, this study has been design to test the effect of Gum Arabic as an antibacterial agent on cultured *streptococcus mutans*.

Materials and Methods

First an ethical approval was obtained from the research committee, Faculty of Dentistry-University of Khartoum prior to the conduction of the study.

The study was carried out in the department of microbiology, Central laboratory (minister of Higher Education and Scientific Research), and in vitro experimental study on microbiological analysis of Gum Arabic against *streptococcus mutans*.

Test materials

- Control: chlorhexidine mouth rinse 2%
- Experimental solution: Gum Arabic Hashab and Gum Arabic Talha.

Test organism

Streptococcus mutans ATCC25175 suspension 1.5x10⁸ cfu/ml

Methodology

Aqueous of Gum Arabic Hashab (Acacia Senegal) and Talha (Acacia Seyal) with different concentration (10%-100%) were used to evaluate the antimicrobial activity by the agar well diffusion method. One hundred microliter of the inoculums of *Streptococcus mutans* was placed on the blood agar and Mueller Hinton agar. 8mm wells made with a sterile cork borer, 100 µl volume of each extract of Gum Arabic and chlorhexidine 2% were propelled directly in to the wells. Petri dishes were incubation at 37°C - for 24 hours in anaerobic incubator. The diameter of inhibition zones formed around wells were measured and compared with control (chlorhexidine). Minimum inhibitory concentration for Hashab and Talha was determined.

Results

Antimicrobial activity (assessed in terms of inhibition zone) of Gum Arabic Hashab and Gum Arabic Talha of different concentration tested against *Streptococcus mutans* (ATCC25175) in Blood Agar and Muller Hinton Agar (Table 1 & 2).

Table 1: Measures of inhibition zone by Gum Arabic Hashab and Gum Arabic Talha in Blood Agar

Concentration (%)	Inhibition zone			
	Hashab		Talha	
10	2 mm	2 mm	2 mm	2 mm
20	4 mm	2 mm	4 mm	2 mm
30	4 mm	2 mm	4 mm	4 mm
40	2 mm	4 mm	4 mm	4 mm
50	4 mm	4 mm	4 mm	4 mm
60	4 mm	6 mm	6 mm	4 mm
70	6 mm	4 mm	6 mm	6 mm
80	6 mm	6 mm	8 mm	6 mm
90	8 mm	6 mm	8 mm	6 mm
100	8 mm	6 mm	8 mm	6 mm
Chlorhexidine (2%)	10 mm	10 mm	10 mm	10 mm

Table 2: Measures of inhibition zone by Gum Arabic Hashab and Gum Arabic Talha in Muller Hinton Agar.

Concentration (%)	Inhibition zone			
	Hashab		Talha	
10	2 mm	2 mm	4 mm	2 mm
20	2 mm	2 mm	4 mm	4 mm
30	4 mm	4 mm	4 mm	6 mm
40	4 mm	6 mm	6 mm	6 mm
50	6 mm	6 mm	6 mm	6 mm
60	6 mm	6 mm	6 mm	6 mm
70	8 mm	8 mm	6 mm	6 mm

80	8 mm	8 mm	6 mm	8 mm
90	8 mm	8 mm	8 mm	8 mm
100	8 mm	8 mm	8 mm	8 mm
Chlorhexidine (2%)	12 mm	12 mm	12 mm	12 mm

Table 3&4 shows comparison between two types of Gum Arabic and chlorhexidine. It cleared that no significant different between Gum Arabic and chlorhexidine was noted.

Table 3: A comparison of inhibition zone by Gum Arabic Hashab and Gum Arabic Talha in the Blood Agar with inhibition zone by chlorhexidine (10 mm)

Concentration (%)	Hashab Mean \pm SD	P-value	Talha Mean \pm SD	p-value
10	2 \pm 0	-	2 \pm 0	-
20	3 \pm 1.4	0.090	3 \pm 1.4	0.090
30	3 \pm 1.4	0.090	4 \pm 0	-
40	3 \pm 1.4	0.090	4 \pm 0	-
50	4 \pm 0	-	4 \pm 0	-
60	5 \pm 1.4	0.126	5 \pm 1.4	0.126
70	5 \pm 1.4	0.126	6 \pm 0	-
80	6 \pm 0	-	7 \pm 1.4	0.205
90	7 \pm 1.4	0.205	7 \pm 1.4	0.205
100	7 \pm 1.4	0.205	7 \pm 1.4	0.205
Chlorhexidine (2%)	10 \pm 0	-	10 \pm 0	-

p-value \leq 0.05 statistically significant

Table 4: A comparison of inhibition zone by Gum Arabic Hashab and Talha in Muller Hinton Agar with inhibition zone by chlorhexidine (12 mm)

Concentration (%)	Hashab Mean \pm SD	P-value	Talha Mean \pm SD	P-value
10	2 \pm 0	-	3 \pm 1.4	0.070
20	2 \pm 0	-	4 \pm 0	-
30	4 \pm 0	-	5 \pm 1.4	0.090
40	5 \pm 1.4	0.090	6 \pm 0	-
50	6 \pm 0	-	6 \pm 0	-
60	6 \pm 0	-	6 \pm 0	-
70	8 \pm 0	-	6 \pm 0	-

Table 7: Minimum inhibitory concentration of Gum Arabic Hashab and Talha.

Concentration % (Talha)	Inhibition zone (mm)	Concentration % (Hashab)	Inhibition zone (mm)
10	2	10	2
9	1.5	9	1
8	0.8	8	0.9
7	0	7	0.7
		6	0

The MIC for Talha and Hashab

It clear that in table 7 the minimum inhibitory concentration inhibits the growth of Streptococcus mutans was 7% for Hashab and 8% of Talha.

80	8 \pm 0	-	7 \pm 1.4	0.126
90	8 \pm 0	-	8 \pm 0	-
100	8 \pm 0	-	8 \pm 0	-
Chlorhexidine (2%)	12 \pm 0	-	12 \pm 0	-

p-value \leq 0.05 statistically significant

Table 5: A comparison of Gum Arabic Hashab and Talha in the Blood Agar

Concentration (%)	Hashab	Talha	p-value
	Mean \pm SD	Mean \pm SD	
10	2 \pm 0	2 \pm 0	-
20	3 \pm 1.4	3 \pm 1.4	0.999
30	3 \pm 1.4	4 \pm 0	0.423
40	3 \pm 1.4	4 \pm 0	0.423
50	4 \pm 0	4 \pm 0	-
60	5 \pm 1.4	5 \pm 1.4	0.999
70	5 \pm 1.4	6 \pm 0	0.423
80	6 \pm 0	7 \pm 1.4	0.423
90	7 \pm 1.4	7 \pm 1.4	0.999
100	7 \pm 1.4	7 \pm 1.4	0.999

p-value \leq 0.05 statistically significant

Table 6: A Comparison of Gum Arabic Hashab and Talha in Muller Hinton Agar

Concentration (%)	Hashab	Talha	p-value
	Mean \pm SD	Mean \pm SD	
10	2 \pm 0	2 \pm 0	-
20	3 \pm 1.4	3 \pm 1.4	0.999
30	3 \pm 1.4	4 \pm 0	0.423
40	3 \pm 1.4	4 \pm 0	0.423
50	4 \pm 0	4 \pm 0	-
60	5 \pm 1.4	5 \pm 1.4	0.999
70	5 \pm 1.4	6 \pm 0	0.423
80	6 \pm 0	7 \pm 1.4	0.423
90	7 \pm 1.4	7 \pm 1.4	0.999
100	7 \pm 1.4	7 \pm 1.4	0.999

P-value \leq 0.05 statistically significant

It obvious that in table 5 & 6 no significant different was recorded between the antibacterial effect of Gum Arabic Hashab and Gum Arabic Talha in the Blood Agar and Muller Hinton Agar in all concentration percentage.

Discussion

The development of therapeutic agent aimed at disrupting the colonization of teeth by dental pathogens and the subsequent formation of dental plaque is one of the prime strategies to reduce the incidence of tooth decay [11].

Natural Product; Gum Arabic are receiving increased attention due to the wide range of its pharmacological activities, including prevention of oral diseases. Gum Arabic could inhibit the formation of plaque and improve dental remineralization, acting as a potential preventive agent in the formation of caries [12,13].

The present study attempted to elucidate the effect of two types of Gum Arabic, Hashab and Talha on oral bacteria *Streptococcus mutans*, aimed to prevent dental caries. *Streptococcus mutans* cultured in the Blood Agar and Muller Hinton Agar.

In the current study chlorhexidine was used as control agent because it is the most effective and widely documented substance in controlling cariogenic activity, due to its ability to control dental plaque, antimicrobial effective against periodontopathic bacteria which cause periodontal diseases and cariogenic bacteria in particular *Streptococcus mutans*. It produces long lasting suppression of *Streptococcus mutans* [14].

In this study pure culture of *Streptococcus mutans* code of ATCC25175 suspension 1.5×10^8 cfu/ml was inoculated in to nutrient broth (Central laboratory) Sudan. Then spread on to agar plate. Antimicrobial activity of Gum Arabic was evaluated by using agar well diffusion method. Clear inhibition zone was formed around the wells of Gum Arabic; they were compared with inhibition zone formed by chlorohixiden. There was statistically insignificant different between them.

The current results were in agreement with studies by Sekar et al tested in vitro, inhibitory activity of medicinal plant; Acacia Arabica, reported that Acacia Arabica showed significant activity against caries isolate biofilm (*Streptococcus sp*, *Lactobacillus sp*, *Staphylococcus sp* and *Klebsiella pneumonia*) [5].

The antimicrobial effect of Gum Arabic against *Streptococcus mutans* in the current study was supported by several authors, suggested that the antimicrobial activity of Gum Arabic was due to the high content of calcium, magnesium and potassium salts. It also contains tannins and several enzymes; cyanogenic glycosides, oxidases, peroxidases and pectinases [5,8,15,16].

Onishi et al concluded that the remineralization ratio of the Gum Arabic was significantly similar to that of the NaF. These findings suggest that Gum Arabic has caries preventive capacity due to its ability to enhance remineralization because its high concentration of Ca^{2+} , Gum Arabic had the capacity to prevent dental caries [13]. The previous study agreed with the results of the present study.

In present study antimicrobial effect of two type of Gum Arabic (Hashab and Talha) against *Streptococcus mutans* were compared. No significant different were recorded, which contradict with Ilham et al. [16] results, variable degree of antibacterial activity of AL mana (Hashab) and Tayebat (Talha) on different microorganism. All isolates were found to be more sensitive to Tayebat than AL mana.

Similarly Gazi et al. reported that the Acacia Arabica in chewing gum inhibited deposition of plaque, which was the causative factor of dental caries and gingivitis. The plaque control is the most acceptable line of treatment for caries and periodontal diseases [10].

In this study the results agreed with Tangadi et al finding, significant reduction in the gingival index and plaque index, brushing with Acacia Arabica-containing tooth paste inhibit the formation of plaque and gingivitis, It can be recommended for daily oral hygiene procedures [17].

Khalid et al reported that the extract of Acacia Arabica had antimicrobial activity against pathogenic bacteria isolated from dental biofilms and inhibited their growth. Thus it can prevent dental caries and periodontal diseases and attempts should be taken by pharmaceutical industries to utilize it in dental caring products [18]. The previous study was in agreement with the results of the current study.

This result is in accordance with Bora et al who found that the tooth pastes containing herbal component like Acacia Arabica had inhibitory effect on growth of *Streptococcus mutans*. The antimicrobial activity of the herbal pastes are due to the presence of secondary metabolites; alkaloids, flavonoids, polyphenols and lectins [19].

Comparison between the results of different studies investigating the effect of Gum Arabic as a cariostatic agent are hindered by the variation of Gum Arabic concentration and testing methods used. However, irrespective of the lack of standardization regarding the type of Gum Arabic, dosage and duration of therapy in the current study demonstrated that Gum Arabic maybe used

in caries prevention in accordance with several studies in vitro and vivo.

Conclusion

The result from this study claims that both types of Gum Arabic possessed an inhibitory effect on *Streptococcus mutans* growth.

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