International Journal of Health Sciences and Research
www.ijhsr.org ISSN: 2249-9571

Original Research Article

Antimicrobial Activity of Hydrogen Peroxide, Sesame and Gum Arabic against *Streptococcus Mutans*

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Received: 23/11/2016 *Revised: 12/12/2016* *Accepted: 15/12/2016*

ABSTRACT

Background: Dental caries is one of the most common and costly diseases in the world and causes the tooth loss in adults by cariogenic microorganism and food debris, affecting more than 90% of the population, regardless of age, sex or race. One of the strategies for preventing the caries is inhibition of bacterial growth specially *S. mutans* and prevention of microbial colonization.
Objective and Methods: The present study was conducted to isolate, identify, studying the effect of sesame, gum Arabic (GA) and hydrogen peroxide (H_2O_2) against *S.mutans* in vitroand determination of minimum inhibitory concentrations (MIC) by using well diffusion method.
Results: We isolated 33 *Streptococcus* sp which were identified by morphological, biochemical characteristics and conformed by VITEK 2 system (version: 07,01) to 27.4, 24.3, 18.3, 12, 9, 6, 3% of *S. mutans, S. salivaries, S. mitis,S. sanguinis, S. downei, S.oralis and S. pneumonia* respectively. The H_2O_2 was highly active compound against *S. mutans* gave 24.0 $\pm$ 0.1. On the other hand, sesame and GA gave the moderate inhibition activity 20.0$\pm$0.2 and 17.0 $\pm$ 0.8 against *S. mutans*, respectively. The MICs of GA, sesame oil and H_2O_2 were used by agar well diffusion method against *S. mutans* gave 5 mg/ml for both GA and sesame, while gave 1.9% for H_2O_2.
Conclusion: The present research is a preliminary attempt to investigate the antibacterial activity against *S. mutans*. Based on the results of the present research, the sesame, GA and H_2O_2 can be used in toothpaste and medicine for the treatment of dental caries. However, further research is needed to identify and characterize the active molecules in sesame and GA responsible for their antimicrobial properties and to determine its potential for use in pharmaceutical manufacture.

Keywords: Dental caries, Sesame, Gum arabic, Hydrogen peroxide, minimum inhibitory concentration, *Streptococcus mutans*.

INTRODUCTION

Oral infectious diseases such as dental caries and periodontal are the most common diseases that affect almost everybody which caused by bacteria. [(1,2)] Dental caries is the localized destruction of susceptible dental hard tissue result from the interaction between acidic by product produced by bacterial fermentation of dietary carbohydrates, cariogenic flora and susceptible tooth structure. [(3)] The most common bacteria that cause dental caries are *S. mutans*, *Lactobacillus* sp and *Actinobacillus* sp. [(4,5)] The dental caries is the single most prevalent and costly oral infectious disease. [(6)] In Saudi Arabia, the dental caries is considered a major health problem, mostly among children. [(7)] Studies have shown alarming numbers for caries prevalence, achieving up to 96% among primary school children in the Western province. [(8)] In the similar study was carried

out by Wyne AH et al., in Riyadh showing the caries prevalence sample achieved up 94.4%. [(9)] In the permanent dentition, Akpata ES et al., were observed caries prevalence to be 69-84% among 12-13 years old children in Riyadh. [(10)] A study was conducted by Amin TT and Al-Abad BM demonstrated decayed teeth clinically evident in 68.9% of male primary school children in Al- Hassa. [(7)] Antibiotics and antimicrobial agents provide an invaluable tool for a control of infection in modern dentistry and present in oral care products have been shown to be effective in inhibiting the growth of oral bacteria and the bio film development. [(11)] H_2O_2 one of the common chemical antimicrobial agents used to inhibiting *S. mutans* growth. [(12)] Several commensal oral streptococci such as *S. sanguinis* can be produced H_2O_2 under aerobic conditions and inhibit *S. mutans* growth. [(13)] Over the past few decades, there has been much interest in natural materials and traditional plants as source of new antibacterial agents, due to repeated use of antibiotics and increase pathogen resistance to synthetic drugs and antibiotics already in use, [(14)] and due to presence of 95% strains of *Staphylococcus aureus* resistant to penicillin and 60% resistant to Methicillin. [(15)] However, the use of these chemical agents (chlorhexidine) can cause undesirable side-effects such as dentine hypersensitivity, taste alternations or extrinsic tooth discoloration. [(11,16)] In previous years, natural extracts of plants have been used as antibacterial agents in traditional medicines, and have attracted considerable interest in the prevention of dental caries. [(17-21)] One of the effective antibacterial agents has a well-established history while searching novel anti-infective agents effective against *S. mutans* are the sesame and the gum Arabic. Sesame was known a Latin Sesamumindicum L. and belonged to the Family: Pedaliaceae. It was a folkloric claims on the therapeutic effect of leaves, seeds and oil for infectious diseases are popular in Africa, India, China and other Asian countries. [(22-25)] Sesame also had antibacterial activity, its main ingredient in antibacterial mouthwash in India and used to relief toothache and treat the gum disease (orally and topically) have been used for promoting healing in burns and other wounds in China, composed from a phenolic derivative with a methylenedioxy group and is known to be the main antioxidant. [(26,27)] Gum Arabic (GA) is the complex exudate of the *Acacia senegal* and *Acacia seyal* tree in Africa and Western Asia and composed from a polysaccharide based on branched chains of (1-3) linked β-D-galactopyranosyl units binding 4-O-methyl-β-Dglucuronopyranosyl, α-L-arabinofuranosyl, α-L-rhamnopyranosyl and β-D-glucuronopyranosyl units. It has found application in many foods. Current literature suggests its cardiac, renal, gut, and dental protective, satiety-inducing, antimicrobial, anti-inflammatory, biofungicide, and anticoagulant implications. [(28,29)]

MATERIALS AND METHODS

Sample Collection

The *S. mutans* were isolated from unstimulated saliva samples of thirty cariogenesis adult volunteers in sterile containers and immediately taken to the laboratory; all samples were collected from dental clinic patients Umm Al- Qura University Faculty of Dentistry. All volunteers must be non-smokers, nonalcoholic, non-pregnant, salivary glands free from any systemic disease, devoid of systemic diseases and did not use antibiotics before six months included in this study, no systemic diseases. Samples were isolated by the streak plate procedure on mitis salvaries agar (MSA), incubated in an aerobic conditions with 5% Co_2 for two days at 37°C. *S. mutans* isolates were maintained as frozen cultures in brain heart infusion (BHI) broth with 20% glycerol at - 20°C until use.

Streptococcus mutans identification

The *S. mutans* were identified by morphological, biochemical according to Bergey‟s Manual of Determinative Bacteriology [(30)] and conformed by VITEK 2 system (version: 07,01) microbial

identification system (BioMerieux) related to *S. aureus* ATCC 6538 as Gram positive control.

Sesame, GA and H_2O_2preparation

The study included GA, sesame oil and H_2O_2 were purchased from the commercial market. Forty g/100ml of GA was prepared by dissolving 40 g of GA powder in 100 ml purified water. The solutions were stirred with low heat (40 ºC) for 60 min using a hot plate magnetic stirrer and filtered using cheesecloth to remove the undissolved materials. Sesame oil (40 mg/ml) was dispersed in tween 80 and sterilized by filtration through 0.45µm membrane filter under aseptic conditions. H_2O_2(30%, v/v) was prepared according to manufacture. Serial dilution from GA, sesame oil and H_2O_2were prepared.

Inoculum preparation [31]

S. mutans were inoculated in BHI broth and incubated for 4-6 hours, to the point growth was considered in the logarithmic phase. The density of the bacterial suspension was adjusted with sterile phosphate buffer saline to match the density of McFarland‟s standard 0.5.

Antibacterial activity of GA, sesame and H_2O_2 against *S. mutans* by well diffusion method [31]

The bacterial broth suspension was streaked evenly onto the Muller Hinton agar with a sterile cotton swab. After the inoculum had dried, an 8 mm wells were made using a cork borer. One hundred microliter of the GA, sesame oil and H_2O_2were added and incubated at 37ºC for 24h in an aerobic condition with 5% Co_2. The Mean Inhibition zone of the GA, sesame oil and H_2O_2 against *S. mutans* was measured by using the Fisher-Lilly antibiotic zone reader model 290 (USA).

MIC Determination

The MICs were determined as the lowest concentration of GA, sesame oil and H_2O_2 inhibiting the tested microorganisms on the culture plates. The MIC values were determined by agar well diffusion method. [32] Preparation of serially diluted of GA (0.6, 1.2, 2.5, 5, 10, 20 and 40mg/ml), sesame oil (0.6, 1.2, 2.5, 5, 10, 20 and 40mg/ml) and H_2O_2 (0.5, 1, 1.9, 3.8, 7.5, 15 and 30%) respectively. About 100 µ of each dilution of the GA, sesame oil and H_2O_2 were dispensed into the wells of inoculated plates with *S. mutans* and incubated at 37ºC for 24 hours in an aerobic condition with 5% Co_2. At the end of the period, the MIC means of the GA, sesame oil and H_2O_2 against *S. mutans* was measured by using the Fisher-Lilly antibiotic zone reader model 290 (USA) and recorded.

RESULTS AND DISCUSSION

Dental caries is one of the most widespread types of pathological diseases in the mouth. The formation of dental caries caused by the accumulation and colonization of oral microorganisms, especially *S. mutans*. Human pathogenic microorganisms have developed resistance to drugs due to the extensive use of commercial synthetic antibacterial drugs in large quantity without suitable medical prescriptions and tests. This condition has raised alarm in most developed and developing countries and the scientists are forced to search an alternative to these compounds and development of a new ecofriendly and effective drug against any type of infectious disease, often in the form of natural medicines from resources such as plants.

The design criterion for this study was to isolate, identify and study the effect of sesame, GA and $H_2O_2$to inhibition the cariogenic *S. mutans* in vitro.

This study focused on using H_2O_2, sesame and GA for several reasons:

- $H_2O_2$is considered as one of the effective salivary compounds or produced by various microorganisms as a metabolic end product and controlling of microorganisms growth via enhanced peroxidase effect. [33,34]
- Sesame oil had antibacterial activity and significantly reduced *S. mutans* counts in plaque and saliva of adolescents within one week. [35,36]

– Gum of Chios mastic (*Pistacialentiscus*) is a natural antimicrobial agent that has found extensive use in pharmaceutical products and as a nutritional supplement. Anti-inflammatory activities have been well described. [(37)]

We were Isolated 33 *Streptococcus* sp from 30 cariogenesis adult volunteers, 9 *S. mutans* (27.4%), 8 *S. salivaries* (24.3%), 6 *S. mitis* (18.3%), 4 *S. sanguinis* (12%), 3 *S. downei* (9%), 2 *S.oralis* (6%) *and* 1 *S. pneumonia* (3%) by streak plate method on MSA and incubated in an aerobic conditions with 5% Co_2 for two days at 37°C as shown Figure 1.

The *Streptococcus* isolates were identified by morphological, biochemical characteristics done related to *S. aureus* ATCC 6538 and conformed by Bergey"s Manual of Determinative Bacteriology [(30)] and VITEK 2 system (version: 07,01) microbial identification system (BioMerieux).All data were summarized in Table 1.

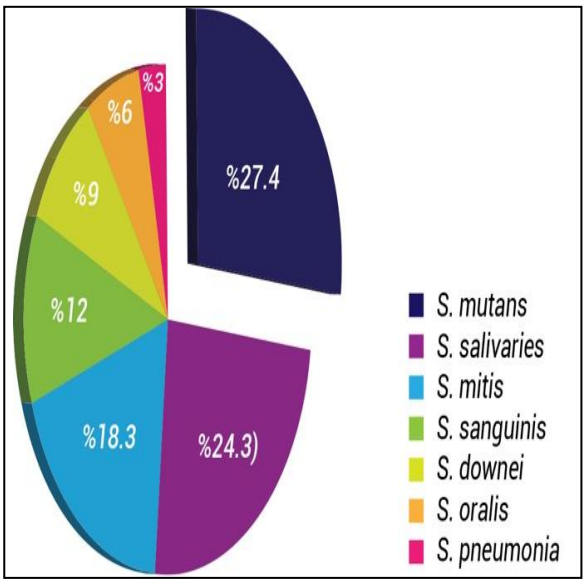

Figure 1: Percentage of *Streptococcus* sp isolates of cariogenesis adult volunteers.

Table 1: A summary of the cultural, morphological and biochemical characteristics *Streptococcus* sp isolates of adult cariogenesis volunteers

Characteristics		S. aureus ATCC6538	S. mutans (9Isolates)	S. mitis (6Isolates)	S. salivaries (8Isolates)	S. oralis (2Isolates)	S. sanguinis (4Isolates)	S. downei (3Isolates)	S. pneumoniae (1 Isolate)
Cultural	Aerobics	+	+	+	+	+	+	+	+
	Anaerobics	+	+	+	+	+	+	+	+
	Pigments production	–	–	–	–	–	–	–	–
	Nutrient agar	White	White	White	Creamy	Creamy	White	White	Whit e
Morphological	Gram stain reaction	+	+	+	+	+	+	+	+
	Cell shape	Spherical	Spherical	Spherical or ovoid	Ovoid	Spherical	Spherical	Spherical	Spherical
	Cell arrangement	Spherical	chains or pairs	chains or pairs	chains or pairs	chains or pairs	chains or pairs	chains or pairs	chains or pairs
	Spores formation	Non-S.	Non-S.	Non-S.	Non-S.	Non-S.	Non-S.	Non-S.	Non-S.
	Motility	Non-M.	Non-M.	Non-M.	Non-M.	Non-M.	Non-M.	Non-M.	Non-M.
Biochemical	Tolerance to 6.5% NaCl	+	–	–	–	–	–	–	–
	Acid aerobically production:								
	D-fructose	–	–	+	+	+	–	+	–
	Galactose	+	+	+	–	+	+	+	+
	D-glucose	+	+	+	+	+	+	+	+
	Lactose	+	+	+	–	+	+	+	+
	D-mannitol	+	+	–	–	–	–	+	–
	D-mannose	+	+	+	+	+	+	+	–
	D-Sorbitol	+	+	–	–	–	–	+	–
	Sucrose	+	+	+	+	+	+	+	–
	D- xylose	–	–	–	–	–	–	–	+
	Hemolysis on sheep blood agar	β	α	α	α	α	α	α	α
	Catalase test	+	–	–	+	–	–	–	–
	Urease test	+	–	–	+	–	–	–	–
VITEK 2system	Confidence	99%	99%	96%	98%	95%	98%	97%	99%

(+): Positive, (-): Negative, (Non-S.): Non-spore former, (Non-M.): Non- motile.

The antimicrobial activity of sesame, GA and H_2O_2against *S. mutans* in vitro was measured by using the inhibition clearing zone and the *S. aureus* ATCC 6538 used as a positive control. The mean values and ± SD of inhibition zone (mm) against *S. Mutans* was recorded in Table 2.

Table 2: Growth inhibition zones (mm) against *S. mutans*isolates by the GA, sesame and H_2O_2 agents

Active agent	Inhibition zone (mm)	
	S. aureus ATCC6538	S. mutans
Gentamycin (32 µg/mL)	23.0 + 0.0	23.0 + 0.1
Distilled water	0.8+ 0.0	0.8+ 0.0
GA (40mg/ml)	8.0 + 0.6	17.0 + 0.7
Sesame (40mg/ml)	9.0 + 0.3	20.0 + 0.3
H2O2 (30%)	20.0 + 0.1	24.0 + 0.1

Mean + standard division (SD)

Gentamycin (32 µg/mL) used as positive control gave 23.0 ± 0.1 against *S. mutans*. Distilled water was not shown any inhibition zone as a negative control. H_2O_2 gave the highest inhibition zone 24.0 ± 0.1 against *S. mutans*. Silhacek KJ and Taake KR, 2005; Kreth J *et al.*, 2005; were observed that H_2O_2inhibited the *S. mutans* growth since this species does not express effective systems for metabolizing this toxic product. [12, 38,39]

Sesame gave the moderate inhibition clearing zone 20.0±0.3 against *S. mutans*. Lakshmi T *et al.*, 2013; was reported, the sunflower oil or sesame oil plays a necessary role in treating gingivitis, plaque and reduce dental caries. [39] Anand TD *et al.*, 2008; was shown a significant antibacterial activity of sesame oil against *S. mutans* and *L. acidophilus*. [40] Durai TA *et al.*, 2008; Asokan S *et al.*, 2009; Thaweboon S *et al.*, 2011 found a significant reduction in colony count of *S. mutans* in plaque sample and decrease in plaque, gingival index score in the study and control group. [35, 4142]

Sesame oil was demonstrated to have antibacterial activity against *S. mutans*. It contains high amounts of unsaturated fatty acids. Linoleic acid and oleic acid are the predominant compositions. Oil pulling therapy with sesame oil significantly reduced *S. mutans* counts in plaque and saliva of adolescents within one week. [35]

GA gave the moderate inhibition clearing zone 17.0 ± 0.7 against *S. mutans*. Antimicrobial activity of GA was reported to be concentrated either in leaves or bark and their extracts have been reported inhibitory against *S. viridans, S. aureus, E. coli, S. typhi, B. subtilis, B. creus, S. sonnei, H. pylori* and even against *C. albicans, C. glabrata* and *A. niger* and *R. solani*. [43-49]

The MICs of GA, sesame oil and H_2O_2 using the agar well diffusion method against *S. mutans* gave 5 mg/ml for both GA and sesame, while gave 1.9% for H_2O_2. The mean values and ± SD of MICs (mm) against *S. mutans* was recorded in Table 3.

Table 3: Minimum inhibitory concentration (mm) of GA, sesame and H_2O_2 against *S. mutans*isolates

GA Serial dilution(mg/ml)	Inhibition zone (mm)	Sesame Serial dilution(mg/ml)	Inhibition zone (mm)	H_2O_2 Serial dilution (%)	Inhibition zone (mm)
0.6	ND	0.6	ND	0.5	ND
1.2	ND	1.2	ND	1	ND
2.5	ND	2.5	10.0 ± 0.9	1.9	13.0 ± 0.6
5	9.0 ± 0.7	5	13.0 ± 0.6	3.8	16.0 ± 0.3
10	12.0 ± 1.2	10	16.0 ± 0.3	7.5	18.0 ± 0.9
20	15.0 ± 0.3	20	18.0 ± 0.7	15	21.0 ± 0.3
40	17.0 ± 0.7	40	20.0 ± 0.3	30	24.0 ± 0.1

ND: Not detected.

ACKNOWLEDGEMENT

The authors are grateful to Educational Dental Hospital staff for their valuable contribution in the work.

Approval of the study protocol: The Research Ethics Committee at the Faculty of Dentistry, Umm Al- Qura University has approved our study protocol from the ethical point of view in November 2016.

Ethical Disclosures: The authors announce that no experiments were performed on voluntaries or animals and no data were collected from patient in this research.

Source of funding: There was no external source of funding.

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How to cite this article: Banjar MM, Khafaji AM, Maher YA. Antimicrobial activity of hydrogen peroxide, sesame and gum arabic against *streptococcus mutans*. Int J Health Sci Res. 2017; 7(1):97-104.

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