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ORIGINAL ARTICLE

Assessment of Antimicrobial Activity of Onion Extract (*Allium cepa*) on *Streptococcus mutans* and *Streptococcus sanguinis; in vitro* study

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ABSTRACT

Background: Dental caries is still remained as a major health problem. This problem along with the antibiotic resistance has created a renewed interest to search for new other antimicrobial substances from various sources including medicinal plants. Since limited data is available so far regarding the antibacterial effect of *Allium cepa* against *Streptococcus mutans,* and *Streptococcus sanguinis*, this study aims to assess this activity. **Materials and methods:** This experimental study was conducted in Shiraz University of Medical Sciences. The antibacterial activity of onion extract on *Streptococcus mutans,* and *Streptococcus sanguinis* was measured by disk diffusion and broth microdilution assays. Positive and negative controls were considered. Vancomycin was used as an index for antibacterial activity. The data was statistically analyzed by applying Fisher's exact test to compare the groups using SPSS software (version 17). **Results:** Onion extracts had more antibacterial activity against *Streptococcus mutans* than *Streptococcus sanguinis* ($p < 0.002$). The antibacterial activity of red onion was more than yellow and green onions ($p< 0.001$). Green onion had the least antibacterial activity. As the concentration of the onions increased, the antibacterial activity also showed increase. **Conclusion:** onion extract showed antibacterial activity when tested *in vitro*. However, pharmacological standardization and clinical assessment of this effect are essential before using onion extract as a preventive measure for human dental caries.

Key words: *in vitro*; *Streptococcus mutans; Streptococcus sanguinis; Allium cepa*

Introduction

Dental caries and periodontal diseases are considered to be the most common chronic diseases all over the world. Dental caries is defined as an infectious disease with microbial origin that results in destruction of the calcified tissues of the teeth. One group of the bacteria attributed to dental caries is *Streptococcus mutans*. All eight *S. mutans* serotypes are both acidogenic and aciduric and are strongly stimulated by sucrose (Roberson, T., H.O. Heymann, 2006). Pioneering animal model studies, as well as human epidemiologic researches, have strongly implicated *Streptococcus mutans* as the main etiological agents in human dental caries (Kuramitsu, H., 1993). *Streptococcus sanguinis* is recognized not only for its association with bacterial endocarditis but also because of its known antagonist role in dental caries. This microorganism competes with *Streptococcus mutans* for colonization sites on tooth surfaces (Caufield, P.W., 2000). Despite the recent decline in dental caries frequency among children in developed countries, tooth decay is still remained as a major health problem. Nevertheless, many populations are still in risk of developing carious lesions (Bauer, A.W., 1966).

Vancomycin is a narrow spectrum antibiotic which is highly effective against gram positive bacteria. It is commonly used for serious infections caused by penicillinase-resistant *Staphylococci* (Small, P.M., H.F. Chambers, 1990; Gonzalez, C., 1999). Recently the resistance of microorganisms has increased due to indiscriminate use of antimicrobial drugs commonly used for the treatment of infectious diseases (Cohen, M.L., 2000). This problem has created a renewed interest to search for new other antimicrobial substances from various sources including medicinal plants (Bauer, A.W., 1966).

Allium is the largest and an important representative genus of Alliacae family. Onion (*Allium cepa*) is a bulbous plant widely cultivated in almost every country. Traditionally it has been used to treat intestinal

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infections. It has been reported to have antibacterial, antiviral, antiparasitic, antifungal properties. It is also antihypertensive, hypoglycemic, antithrombotic, antihyperlipidemic, anti inflammatory, and antioxidant (8). *Allium cepa* extracts has been widely studied for its antimicrobial activity. Kim et al. reported its antibacterial activity against *Streptococcus mutans*, *Streptococcus sobrinus, Prevotella intermedia* and *Porphyromonas gingivalis* (Kim, J.H., 1997). Benkeblia *et al*. investigated antimicrobial activity of different concentrations (50, 100, 200, 300, 500 ml/l) of essential oil extracts of three types of onions (green, yellow and red) and garlic against two bacteria, *Staphylococcus aureus, Salmonella enteritidis,* and three fungi, *Aspergillus niger, Penicillium cyclopium,* and *Usarium oxysporum.* Green onion showed the lowest antimicrobial activity (10). Maidment *et al*. examined a total of twelve *Alliums* for antibacterial activity against the tested microorganism. *Allium cepa* did not show antibacterial activity (Maidment, D., 2001).

Considering the controversies in the antimicrobial activity of *Allium cepa* as reviewed above, the differences of microorganisms in each study reviewed above, the antibiotic resistance, and lack of enough investigations on onions cultivated in Iran, the purpose of this study was to examine in vitro antibacterial activity of Allium cepa extract against *Streptococcus mutans,* and *Streptococcus sanguinis.*

Materials and methods

This experimental study was conducted in Shiraz University of Medical Sciences. Three types of onion (green, red and yellow) were purchased from a local market in Shiraz. Vancomycin (Jaber ebne hayyan pharmaceutical company, Iran) was bought from a local pharmacy. Lyophilized ampoule of *Streptococcus mutans* (ATCC: 35668) and *Streptococcus sanguinis* (ATCC 10556) *was* purchased from the microbial collection of Persian Type Culture Collection (PTCC), Iran.

Preparation of onion extract:

1 kg of each type of onion was randomly selected, was washed carefully, and was blended (Panasonic Mx-x-61-W, Japan) with a moderate speed for 1 minute. 500 ml of distilled water was added and was left for 1 hour. The blended onions were then distilled using a vertical steam distillation unit. The extract produced was then kept in the refrigerator until use (Benkeblia, N., 2004).

Preparation of different concentrations V/V (50%, 25% and 12.5%) of onion extract:

5 cc of onion extract mixed with 5 cc of distilled water in a tube gives a 75% concentration. 2.5 cc of medicinal herb mixed with 7.5 cc of distilled water gives a 25 % concentration. 1.25 cc of medicinal herb mixed with 8.75 cc of distilled water gives a 12.5 % concentration.

Inoculation of culture media with reference strain:

The pure culture of *S. mutans* and *S. sanguinis* was sub-cultured in nutrient broth as follow: After cleaning the ampoule tip with alcohol, the lyophilized ampoule tip was broken. Its content was then added to Triptycase Soy Broth (TSB) media and was incubated in an appropriate atmosphere (H_2:CO_2:N_2 10:10:80) at 37°C for 24 hours. Bacteria cultivated were then inoculated onto the Blood agar media. The media was incubated for 24 hours. Bacteria that could grow under such circumstances were identified as reference strains. For antimicrobial susceptibility testing the turbidity must be adjusted equivalent to a 0.5 McFarland standard. A 0.5 McFarland standard is comparable to a bacterial suspension of 10^6 CFU/ml.

Disk diffusion method:

After preparing the different concentrations of onion extract, each sterile paper disk was impregnated with 30 µl of diluted onion extract. The disks were allowed to dry. Using a sterile forceps, the disks were placed on the inoculated blood agar medium. 1 paper disk on each plate was soaked in distilled water as a negative control. Then the plates were incubated in an appropriate atmosphere (H_2:CO_2:N_2 10:10:80) at 37°C for 24 hours and the diameter of the inhibition zone was measured by a caliper. Each test was done in triplicate and the average was reported (Murray, P., 1995).

Broth micro dilution assay:

For broth micro dilution, susceptibility panel in 96-well micro titer plates (12 columns and 8 rows) were prepared by dispensing 200 µl of TSB to the first column and 100 µl of TSB into the rest wells by an 8-channel pipette. The rows were labeled from A to H. Then, the two-fold serial dilutions of onion extract was made by

drawing up 100 µl of each solution from the second column wells into the third column and then move on to the next column until the eleventh column to achieve the final concentrations. This procedure was done for the rows C, D and E. the same method was used for vancomycin (32µg/ml) as an index for antibacterial activity for the rows F, G and H. Finally 100 µl of each bacterial suspension were inoculated into wells of micro titer plates to obtain the final volume of 5×10^5 CFU/ml in each well of the plate. The first and the last wells in each row were negative and positive controls, respectively. The positive control was inoculated with bacterial suspension only, while the negative control well was left blank without inoculation. The 96-micro well plates were sealed using a perforated plate seal and incubated in an appropriate atmosphere (H_2:CO_2:N_2 10:10:80) at 37°C for 48 h. The MICs were recorded as the lowest concentration where no viability is observed in the wells of 96-micro well plates after incubation period. Each test was done in triplicate.

Mean values and standard deviations of the diameter of inhibition zone in disk diffusion assay were calculated from the experimental data obtained. Differences between the mean values were considered significant when $p < 0.05$. The data was statistically analyzed by applying Fisher's exact test to compare the groups using SPSS software (version 17, Chicago, IL, USA).

Results:

This experimental study was conducted to assess the antibacterial activity of *Allium cepa* extract and vancomycin on *Streptococcus mutans* and *Streptococcus sanguinis*. The results of the disk diffusion method for three types of onion and vancomycin are illustrated in table 1. In this method no inhibition zones were measured for the 12.5% and 25% concentration of onion extract ($p < 0.001$). The diameters of inhibition zone of the 50% concentration of three types of onion (yellow, red and green) against *Streptococcus mutans* were (2.2 ± 0.2), (3.5 ± 0.3), and (1.2 ± 0.2) mm respectively. No inhibition zones were observed for the 12.5%, 25% and 50% concentrations of yellow and green onion against *Streptococcus sanguinis.* The diameter of inhibition zone of the 50% concentration of red onion was (1.5 ± 0.1) mm. Onion extracts had more antibacterial activity against *Streptococcus mutans* than *Streptococcus sanguinis* ($p < 0.002$). The diameters of inhibition zones for 32%, 16% and 8% concentrations of vancomycin against *Streptococcus mutans* were (10 ± 0.1), (8 ± 0.3), and (6 ± 0.2) mm respectively, and against *Streptococcus sanguinis* were (8 ± 0.2), (7 ± 0.2), and (5 ± 0.3) mm respectively. This showed that as the concentration of the vancomycin increased, the antibacterial activity also showed increase. The antibacterial activity of red onion was more than yellow and green onions ($p< 0.001$). Green onion had the least antibacterial activity. The results of the broth microdilution method for three types of onion and vancomycin are illustrated in table 2. The MIC values of yellow, red, and green onion, and vancomycin against *Streptococcus mutans* were 12.5 %, 6.25 %, 12.5 % mm and 4µg/ml, respectively. The MIC values of yellow, red and green onion and vancomycin against *Streptococcus sanguinis were* 25 %, 12.5 %, 50 % mm and 4µg/ml, respectively.

Table 1: Antimicrobial activity of various onion extracts and vancomycin determinate by disk diffusion assay.

variable	Concentration (%)	Inhibition zone (mm)	
		Streptococcus mutans	*Streptococcus sanguinis*
Onion			
Yellow	50	2.2 ± 0.2	N*
	25	N	N
	12.5	N	N
Red	50	3.5 ± 0.3	1.5 ± 0.1
	25	N	N
	12.5	N	N
green	50	1.2 ± 0.2	N
	25	N	N
	12.5	N	N
vancomycin	32	10 ± 0.1	8 ± 0.2
	16	8 ± 0.3	7 ± 0.2
	8	6 ± o.2	5 ± 0.3

N = no inhibition zone

Table 2: MIC values of various onion extracts and vancomycin determinate by broth microdilution assay.

Variable	MIC	
	Streptococcus mutans	*Streptococcus sanguinis*
Onion		
Yellow	12.5%	25%
Red	6.25%	12.5%
Green	12.5%	50%
vancomycin	4 µg/ml	4 µg/ml

Discussion:

Allium cepa has been widely studied for its antimicrobial activity. However a limited data is available so far regarding its antibacterial activity against *Streptococcus mutans,* and *Streptococcus sanguinis.*

The antibacterial activity of red type of *Allium cepa* extract was found to be better as compared to yellow and green types. Onion extracts had more antibacterial activity against *Streptococcus mutans* than *Streptococcus sanguinis.* The antibacterial activity of the extracts has been increased by increasing the concentrations.

In a study conducted by Benkeblia *et al*., red onion exhibited a better antibacterial activity than yellow one against *Staphylococcus aureus* and *Salmonella enteritidis*. The zone of inhibition increased with increasing concentration of extracts (Benkeblia, N., 2004).

Kim et al. reported antibacterial activity of onion extract against *Streptococcus mutans*, *Streptococcus sobrinus, Prevotella intermedia* and *Porphyromonas gingivalis* which is similar to our results (Kim, J.H., 1997).

Maidment *et al*. examined a total of twelve *Alliums* for antibacterial activity against *Escherichia coli*. *Allium cepa* did not show antibacterial activity (Maidment, D., 2001). This different result compared to our study is explainable. *Escherichia coli* is a gram negative bacterium while *Streptococcus mutans* and *Streptococcus sanguinis* are both gram positives. Gram positives are proved to be more sensitive to essential oils and extracts than gram negatives (Shelef, L., 1984; Mann, C.M., 2000; Zohri, A.N., 1995; Sikkema, J., 1994).

It is evident from our study that red onion has a better antibacterial activity than yellow and green onions. This might be due to the differences in their compositions, differences in their growth climate. It is also proved that the constituents of onion vary as the age of the onion increases (Briggs, W.H., 2002). The differences in the temperature while preparing agar plates may also affect the results.

It is hoped that this study would lead to the establishment of new and potent antimicrobial agent. However, further studies are recommended considering the age and the region of the onion against oral pathogens.

Conclusion:

It is concluded that Allium cepa extract has a more significant antibacterial activity against Streptococcus mutans that Streptococcus sanguinis. Therefore, Onion extract can be considered as an antibacterial agent to prevent human dental caries.

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