International Scholars Journals

Advanced Journal of Microbiology Research ISSN 2241-9837 Vol. 12 (7), pp. 001-004, July, 2018. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Evaluation of antimicrobial effect of "*Ammi visnaga*" against oral streptococci

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Accepted 14 April, 2018

Some species of "streptococci" have great role in dental caries. So control of their activities can promote prevention of dental caries. Use of herbal agents is a notable issue in recent researches. The aim of this study was evaluation of antimicrobial activity of aqueous and hydroalcoholic extract of seed and stem of *Ammi visnaga* against *Streptococcus mutans*, *Streptococcus salivarius* and *Streptococcus sanguis*. First step or screening was designed by determination of antimicrobial activity for each extracts using Disk diffusion method. For those extract which presented it; "no growth hallo" was evident around related paper disks. Next step was determination of MIC (minimum inhibitory concentration) and MBC (minimum bactericidal concentration) corresponding to "modified macrodilution" method for those extracts revealed antimicrobial activity. "No growth hallo" was obvious around *S. sanguis* and it was absent around *S. salivarius*; For *S. mutans*, only aqueous seed extract produced the hallo. MIC and MBC of aqueous and hydroalcoholic stem extract against *S. sanguis*, were 5 and 7%, stem extract against *S. sanguis* as well as hydroalcoholic seed extract against *S. sanguis*, 5 and 5%, aqueous seed extract against *S. sanguis*, 15 and >30% and aqueous seed extract against *S. mutans*, 20 and >30%, respectively. *A. visnaga* revealed antimicrobial activity against some species of oral Streptococci including *S. mutans*. So, we can use it for prevention of dental caries but further investigation is recommended.

Key words: *Ammi visnaga*, *Streptococcus mutans*, *Streptococcus salivarius*, *Streptococcus sanguis*, antimicrobial activity.

INTRODUCTION

Dental caries and periodontal disease are probably the most common chronic disease worldwide, Loesche introduced dental caries and periodontal disease as the most expensive infections that affected people (Niclaus and L-Michel, 1986). Dental caries developed under bacterial colonies which produce acidic material and then remove the mineral part of tooth structure (Niclaus and L-Michel, 1986). Oral "streptococci" presents the great part of oral microflora. They can be isolated from all parts of the mouth. These bacteria being the first ones to grow after birth and make 98% of all isolated microorganisms of infants mouth up to first month of birth but these percentage will be reduced up to 70% during end of first year (Marsh and Mikel, 1990; Nourozi, 1995). Three spices of *Streptococcus mutans*, *Streptococcus salivarius* and *Streptococcus sanguis* would be the great part of oral Streptococci and also Clarks was the first to isolate *S. mutans* from dental caries in 1924 but this activity confirmed at 1950 to 1960 in order to some experimental studies (Marsh and Mikel, 1990). Colonization of *S. mutans* was commonly presented during first to fourth years after birth (Jahanian, 2000) and epidemiologic studies revealed it as primary pathogen of enamel caries in adolescents, root caries in older people and rampant caries of infants (Marsh and Martin, 1999). *S. salivarius* preferred to colonize on epithelial surface such as

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mucosa and tongue and presented the dominant microorganism of saliva. This bacteria has been detected in bactriemia after dental procedures, especially extraction and infectious pulpitis (Ross and Holbrook, 1984). *S. sanguis* is colonized in the dental microbial plaque and play the great role to development of it. *S. sanguis* presents 20 to 50% of dental plaque Streptococci. It seems that, inhibition of these microbial activities would be an important way to the prevention of dental caries. Today, in order to some harmful side effect of chemical therapeutic agents, use of herbal ones would be a notable issue in the recent researches.

"*Ammi visnaga*" or "tooth pick plant" has been shown many important therapeutic effects including antimicrobial and antifungal ones (Abroush et al., 2001). Anrep was the first to face with this plant and introduce antispasmatic effect of it on Coroner artery. *A. visnaga* has two important ingredients: Khelin and Vistagin which present the recent activity. Also, in the ancient Egypt it was used to treatment of renal disease (Weiss, 2001). In our knowledge, all researches have confirmed the antimicrobial effect of this plant but the recent efficacy has not been evaluated specially against *S. mutans*, *S. salivarius* and *S. sanguis*. So, the present study was designed to the evaluation of antimicrobial effect of aqueous and hydroalcoholic extracts of seed and stem of *A. visnaga* in this manner.

MATERIALS AND METHODS

The standard species of *S. mutans* (PTCC 1601), *S. salivarius* (PTCC 1448) and *S. sanguis* (PTCC 1449) were obtained from microbial collection of "Industrial and scientific researches of Iran". The microbial suspension was prepared with normal saline 0.85% using equivalent concentration to No: 0.5 index of Nephlometry (Mc Farhand) tube. Also, the aqueous and hydroalcoholic extracts of seeds and stem of *A. Visnaga* was obtained from "Therapeutic plants research center". The liquid culture medium of "Mueller Hinton broth" (Merck company) and solid culture medium Blood Agar (Merck company) were used to evaluation of antimicrobial activity. Then, the methods described as follows were designed to primary assessment and determination of "minimum inhibitory concentration" or MIC and "minimum bactericidal concentration" or MBC of the extracts:

Determination of antimicrobial effect using "disk diffusion" method with paper disks by 6 mm diameter

This step was carried out to the evaluation of the presence or absence of antimicrobial activity for each extracts and also, evaluation of this inhibitory efficacy against microbial growth. The results of this section could find and exclude ineffective extracts and also insensitive bacterial species to them. In this step, paper disks with 6 mm diameter (Irandaroo Company) were used and 30 µl of each extracts were added to papers. Then, papers were inserted to blood agar culture medium which was incubated for 24 h in 37°C. In cases with antimicrobial activity against the mentioned bacterial species, "no growth halo" was evident around related paper disks. The halo diameter was also determined with millimeter ruler (Anhalt and Washington, 1985).

Determination of MIC and MBC by "modified macrodidution" method, under NCCLS (National Committee for Clinical Laboratory Standard, 1991)

This step was designed to antimicrobial evaluation of different concentration of the extracts which presence of this activity confirmed by "Disk diffusion" method. The count of vital bacteria was calculated after treatment by specific concentration of the extracts lasting 24 h and compared with the count of vital bacteria in control samples. The samples with bacterial count of 2.44×10^7 were considered as control samples. So, the minimum concentration of the extract which was treated by 20 µl bacterial suspension and showed equivalent bacterial count vs. control samples was considered as MIC. Also, the minimum concentration of the extract which was treated by 20 µl bacterial suspension and caused reduction of bacterial count to 1/1000 was considered as MBC.

RESULTS

Determination of antimicrobial effect using "Disk diffusion" method

Table 1 shows "no growth halo" diameter in different Streptococci species. This halo was obvious around *S. sanguis*. For *S. mutans* only aqueous seed extract produced the hallo and none of the extracts showed around *S. salivarius*.

Determination of MIC and MBC

These indexes for each extract against different bacterial species are present in Table 2. Comparing the bacterial count in different concentration with control groups, concentration of 5% for aqueous stem extract inhibited increasing of bacterial count and concentration of 7% causes bacterial count reduction. So, MIC of aqueous stem extract against *S. sanguis* was 5% and MBC of it was 7%. Respectively, MIC and MBC of hydroalcoholic stem extract against *S. sanguis*, were calculated 5 and 7%, hydroalcoholic seed extract against *S. sanguis*, 5 and 5%, aqueous seed extract against *S. sanguis*, 15 and >30%, aqueous seed extract against *S. mutans*, 20 and >30% in the same manner.

DISCUSSION

Oral Streptococci including *S. mutans*, *S. salivarius* and *S. sanguis* contributed in oral microflora (Javetz, 2002; Newman and Niseugard, 1999). Dental caries are common infections which is induced by these bacteria (Newman and Niseugard, 1999) some preventive and therapeutic instructions were recommended in this

Table 1. Antimicrobial evaluation of extracts against oral Streptococci by Disk diffusion method.

No growth halo diameter average (mm) in Streptococci			
Streptococci extract type	***S. salivarius***	***S. mutans***	***S. sanguis***
Aqueous stem	-	-	9±1.73
Hydroalcoholic stem	-	-	8±0.0
Aqueous seed	-	9.5±0.5	12.3±2
Hydroalcoholic seed	-	-	10±0.0

-: absence of No growth halo.

Table 2. Determination of MIC and MBC in bacterial species which antimicrobial activities of them were confirmed by Disk diffusion method.

Extracts	**MIC (%)**	**MBC (%)**
Aqueous stem against *S. sanguis*	5	7
Hydroalcoholic stem against *S. sanguis*	5	7
Hydroalcoholic seed against *S. sanguis*	5	5
Aqueous seed against *S. sanguis*	15	>30
Aqueous seed against *S. mutans*,	20	>30

manner including mouthwash using and due to some unsuitable effect of chemical mouthwashes, those herbal ones are introduced, recently, such as “toothbrush plants”(*Salvadora persica*). Some studies reported the antimicrobial activities of therapeutic plants against different oral Streptococci but there were few studies about *A. visnaga* in this situation. Bagherzadeh et al. (2003) studied antimicrobial activity of *Chamaemelum nobile* against *S. mutans*, *S. salivarius* and *S. sanguis* and concluded it. Pirveisi et al. (2003) studied the antimicrobial activity of *Zataria multiflora* and concluded it. These studies were designed as same methods as we were. Mahmood et al. (1999) studied the antifungal activity of *A. visnaga* and reported it. Abroush et al. (2001) studied antimicrobial activity of *A. visnaga* against *S. viridans* and reported it. Their method was based on Disk diffusion agar, Well and Point culture; so, their evaluation of antimicrobial activity was a qualitative one and determination of MIC and MBC were not considered. Khan et al. (2010) studied antimicrobial activity of *Trachyspermum ammi* seeds against *S. mutans* and confirm it. This study was designed to the evaluation of antimicrobial activity of *A. visnaga* against some species of oral Streptococci.

Results showed the aqueous and hydroalcoholic extract of this plant affects *S. sanguis* more than *S. mutans*, and *S. salivarius*. The notable point is the presence of screening step to evaluation of antimicrobial activity of each extracts by Disk diffusion method. Then, MIC and MBC was determined for these extract which produced “no growth halo” around the paper disks.

Limitations and suggestions

This study was designed to antimicrobial evaluation of seed and stem extracts of this plant. For further investigation it would be preferred to the examination of other parts of it, too. Also, other methods of antimicrobial evaluation are really recommended in this situation to confirm the results of this study.

Conclusion

It seems that, the extract of *A. visnaga* has no great antimicrobial activity against oral Streptococci. Aqueous seed presented greater antimicrobial activity against *S. sanguis* and *S. mutans* than others by Disk diffusion method; however hydroalcoholic seed extract showed greater antimicrobial activity referred to MIC of 5% and MBC of 5%. So, further researches needs to reach the practical usage of this herbal extracts on prevention of dental caries.

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