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The in vitro study on anti-microbial activity of aqueous extracts of internal shell of walnut against some oral microbial strains

Mohammad Ali Rouzegar[1,2], Amirreza Hashemian[1], Rasoul Yousefi[2], Jafar Panahi[3], Mohamad Reza Havasian[3] and Behzad Houshmand[4*]

1- Dep of Periodontology, Faculty of Dentistry, ilam University of Medical Sciences, Ilam, Iran
2- Dep. Microbiology, Faculty of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran
3- Student research of committee, Ilam University of Medical Sciences, Ilam, Iran
4- Dep. Periodontology, Faculty of Dentistry Shahid Beheshti University of Medical Sciences, Ilam, Iran

***Corresponding author*:** Behzad Houshmand

ABSTRACT: Microorganisms are main etiologic causes of periodontal disease and dental caries. Antimicrobial agent with mechanical debridement (as main treatment) can help elimination of microbial factor. Recently extreme usage and non-reasonable application of antimicrobial agent develop bacterial tolerance and side effect associated to existing agent, so it is essential to introduce new antimicrobial agent. Use of therapeutic herbs because of natural type has been considered as a strategy. The main aim of this study was evaluating antimicrobial effect of aqueous extract of internal shell of walnut against some bacterial strain. In this in vitro study, each eight strains of bacteria were cultured in blood agar and Mueller-Hinton media. Paper disk (with 6mm diameter) containing aqueous extract were located on this media, and measured inhibition zone following 24h. To compare effect of aqueous extract, data were analyzed statistically by one-way ANOVA and Tukey HSD. The antibacterial effect of aquatic extract of internal shell of walnut on A.viscuses, Staph.epidermis was high; on Stah.aureus was moderate; on L.acidophillus, P.gingivalis was low and on Strep.mutans,Strep.salivaris,Strep. sanguis not effected. This study showed that antibacterial effect of aquatic extract of internal shell of walnut on some microbial strain and suggested complementary studies for separation and detection of effective agents of this extract.

***Keywords*:** aqueous extract, antimicrobial effect, disk diffusion test .microbial strains, walnut.

INTRODUCTION

Periodontal diseases and caries are common mouth and teeth diseases and the common causes of losing teeth at any age. The main etiologic cause of these diseases is the microorganism of dental plaque. The important purpose for curing periodontal diseases is to eliminate these microorganisms (1, 2, 3). The most reliable way to have a healthy mouth for patient with periodontal disease is the mechanical method. However, this method is not always such a suitable way, because of its disability in removing the microorganisms completely (4, 5). There is a confirmed need to the new antimicrobial agent which does not have the defects of the former medicines because of the quick spread of the resistance of the medical material reduction of the efficiency of some medicines and the results from the usage of them on the receiver [(3, 6). History of medical herbs application has been known for the long time as the history of human creation. The oldest recorded document about disinfectant herbs goes back to the ancient Egypt. During this era Egyptians used herbs like cinnamon and tobacco to protect the crop (3). Walnut is one of the medical herbs .The anti-microbial effects of the extract of the internal shell of walnut were discovered accidentally by the professor of this thesis during a pilot study which has been done to examine the effect of this extract on the rat's blood pressure (5). Walnut is a mono-stock tree and its scientific name is "juglandregia". The most important compounds of the fruit include: tonine, vit C, juglandin, juglone and naphtokinone (6, 7). Studies show that regular use of walnut results in

reducing cholesterol and LDL and it plays a role as a protective factor against the cardiovascular disease. We can point to its usage against the disease: richet, tuberculosis, anemia, diabetes, diarrhea, etc (6, 8).

MATERIALS AND METHODS

Providing the aqueous extract

The liquid of the walnut shell was collected by pressure and passing the strainer. Then the resulted solution was dried in the low temperature by freeze-drier (Figure 1). The extract transformed in to dried powder. This powder was solved in the water, and then this solution got ready in the concentration of 20mg/dL (5, 6, 7).

Figure 1. Freeze Drier

Antimicrobial examination of extracts on the bacteria

The bacteria; staph.aureus[1], staph.epidermis[2], strep.mutans[3], strep.salivaris[4], strep.sanguis[5], lactobacillus acidophilus, porphyromonas gingivalis and actinomyces viscosus (net strain in the microbiologic laboratory of the medical science of Hamadan)were selected .The standard paper disk (made of cellulose acetate) in 6mm diameter were incubated in the temperature of 37^{o}c during 20 min, and they were collected in sterilized dishes. Every bacteria was inoculated in the liquid cultures before the experiment and incubated in the temperature of 37^{o}c during 24 h to get the logarithmic growing stage .then the dark microbial suspension was examined along with the McFarland 0.5 (Figure 2) (9, 10, 11). While the number of the bacteria was 10 bacteria\mm. if the tarnish was increasing, the microbial suspension will be diluted by sterilized physiologic serum. After providing the standard concentration, the microbial suspensions were incubated by sterile swab in Mular- Hinton and blood agar media. Then these disks were put in the media in 25mm distance from each other. Cultures were incubating in the temperature of 37oc during 24h. The diameter of the inhibition zone was measured by rulers. In this study, the diameter 6mm considered non-effect, 7-10 mm considered low-effect, 11- 15mm considered moderated-effect and over15mm were consider high effect respectively (12, 13, 14). This action was reported for each bacterium confirm the efficiency of the extract, one for the aquatic extract in a blank disk so 3diskes were reduced for every plate in 8 cm diameter. The average of the non-growing halo diameter was recorded. The volume of samples equaled 24 disks in 8 plates (Figure 3) (11, 15, 16).

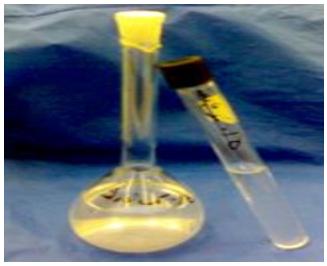

Figure 2. McFarland 0.5

1 Staphylococcus aureus
2 Staphylococcus epidermidis
3 Streptococcus mutans
4 Streptococcus salivarius
5 Streptococcus sanguinis

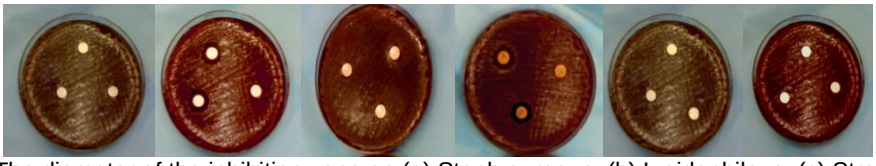

Figure 3. The diameter of the inhibition zone on (a) Staph.aureous, (b) L.aidophilous, (c) Strep.salivaris

RESULTS AND DISCUSSION

Results as seen the antibacterial effect of aquatic extract of internal shell of walnut on A.viscuses, Staph.epidermis was high; on Stah.aureus was moderate; on L.acidophillus, P.gingivalis was low and on Strep.mutans,Strep.salivaris,Strep. sanguis not effected (table 1 and Figure 4). Mouth is an ideal environment for growing and gathering the bacteria because of its particular characteristics such as: moisture, heat, etc. the probability of affecting periodontal disease and carries would increase, if the normal micro flora of mouth turns to pathogens (8, 17). When the mucus defensive barrier breaks down (for example by trauma) the bacteria inters blood and if there is the suitable condition, the systemic disease such as endocarditis show them (17, 18). Co-existence of microbes is complex phenomenon, and still there are some vague points. It is noticeable to clarify this point that antimicrobial agent are used which just affect some limited bacteria and do not affect the other ones and has the least effects on receiver (19, 20, 21). It is useful to use medicinal herbs and their applications. So the selected bacteria either the important factors of decay and periodontal diseases or the factors of causing systemic problems (staph.epidermis) (20, 22, 23).

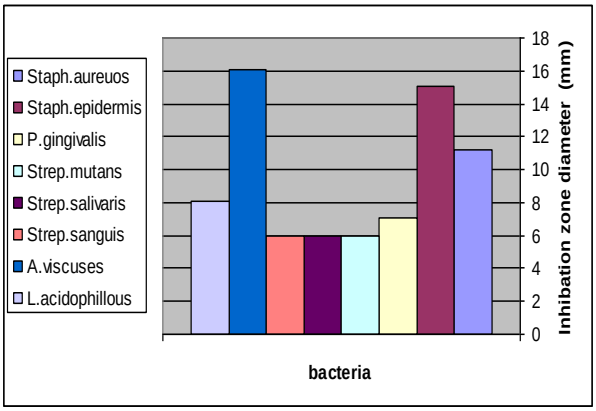

Figure 4. schematic diagram of the inhibition zone on bacterial strains

Table 1. The diameter of the inhibition zone on bacterial strains

P value	F	Inhabitation zone diameter (mm) Mean±SD	Bacterial strain
0.000	1781.23	11.25±0.35	Staph.aureus
		15.1±0.00	Staph.epidermis
		7.1±0.14	Porphyromonas.gingivalis
		6.00±0.00	Strep.mutans
		6.00±0.00	Strep.salivaris
		6.00±0.00	Strep.sanguis
		16.05±0.07	Actinomyses.viscuses
		8.05±0.07	Lactobacillus.acidophillus

CONCLUSION

Our study showed the anti-microbial effects of aqueous extract of the internal shell of walnut on some strain and we suggested further study in this field.

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