EFFECT OF *NIGELLA SATIVA* (BLACK SEED), *SALVADORA PERSICA* (SIWAK) AND ALUMINUM POTASSIUM SULPHATE (ALUM) AQUEOUS EXTRACTS ON ISOLATED BACTERIA FROM TEETH ROOT CANAL

Mohammed I. Nader **Amina N. Al-Thwaini** **Ismail A. Abdul-Hassan** **Ali W. Ali**

Institute of Genetic Engineering and Biotechnology for Postgraduate Studies, University of Baghdad, Iraq

Received 16/8/2009 Accepted 2/3/2010

ABSTRACT

Swabs were taken from 20 patients from root canal (Endodontic Department, Al-Elwiya Specialized Centre for Oral and Dental Surgery), *Streptococcus oralis*, *Streptococcus anginosus*, *Enterococcus durans*, *Enterobacter cloacae* and *Staphylococcus epidermides* were identified according to their cultural properties, microscopic examination and biochemical *Api* and *miniapi* tests. Antibacterial action was evaluated on these bacterial isolates by using six different concentrations of *Salvadora persica*(Siwak), *Nigella sativa* (black seed), aluminum potassium sulphate (alum) and mixture of them, the concentrations are 3.125, 6.25, 12.5, 25, 50 and 100%. At 100% concentration of alum, *Salvadora persica*, *Nigella sativa* and the mixture of them, the maximum inhibition zone diameters were 42mm on *Enterococcus durans*, 27mm on *Enterobacter cloacae*, 26mm on *Enterobacter cloacae* and 25mm on *Staphylococcus epidermides*, respectively.

Key words: Extracts *Nigella sativa*, Siwak, Alum, Antibacterial

To whom correspondence should be addressed (E-mail: mohgen2000@yahoo.com)

تأثير المستخلصات المائية للحبة السوداء والسواك والشب في البكتيريا المعزولة من تسوس الأسنان

محمد إبراهيم نادر آمنة نعمة الثويني إسماعيل عبد الرضاعبد الحسن
علي وليد علي

معهد الهندسة الوراثية والتقنيات الإحيائية للدراسات العليا، جامعة بغداد، العراق

الاستلام 2009/8/16 القبول 2010/3/2

الخلاصة

أخذت مسحات من قنوات جذور الأسنان من 20 مريض من قسم حشوات الجذور– مركز العلويه التخصصي لطب وجراحة الفم والأسنان). تم تشخيص العزلات البكتيريه (*Streptococcus oralis* ، *Streptococcus anginosus*، *Enterococcus duran*، *Enterobacter cloacae* ، *Staphylococcus epidermides*) إستناداً لخواصها الزرعيه والفحص المجهري والاختبارات الكيموحيويه *Api* and *miniapi*. تم تقييم الفعالية المضادة للبكتيريا لمستخلصات الحبة السوداء والسواك والشب والخليط المكون من هذه المستخلصات ضد العزلات البكتيرية المعزولة من قنوات جذور الأسنان وذلك بإستعمال ستة تراكيز مختلفة لكل من هذه المستخلصات(3.125، 6.25، 12.5، 25، 50، 100%). عند استعمال مستخلصات الشب والسواك والحبه السوداء والخليط المكون منهما، كانت قيم الحد الأعلى لقطر منطقة التثبيط 42 مليمتر لبكتيريا *Enterococcus durans* و27 مليمتر لبكتيريا *Enterobacter cloacae* و26 مليمتر لبكتيريا *Enterobacter cloacae* و25 مليمتر لبكتيريا *Staphylococcus epidermides*، على التوالي.

INTRODUCTION

One of the aims of root canal treatment is to eliminate the bacteria, their products and substance from the root canal system. Mechanical debridment of the root canals plays a fundamental role in a chieving endodontic success. In spite of all attempts, attaining complete sterility of the canal system is still extremely difficult.

Many wonderful helping plants surfaced when the cultures of our world started to share trade and teach their herbal medicines to each other. One such plant was *Nigella sativa* commonly known as black seed. This mild aromatic herb is indigenous to the Middle East where it has been used as a traditional remedy for over 2000 years. It was used so extensively that it became known as the seed of blessing "Habbatul Barakah" (1).

One of such plants was *Salvadora persica* commonly known as Siwak, which was used as a chewing stick. Prophet Mohammed (peace and pray upon him) is considered by muslims the first dental educator in the oral hygiene. Since, he recommended them to use Siwak five times a day, as he said "if I had not found it hard for followers or the people, I would have ordered them to clean their teeth with Siwak prior to each pray" (2).

The chemical material as alum has many benefits as it has antibacterial effect on *Pseudomonas aeruginosa*(3), in which maximum inhibition zone reached was35 mm when it used as a solution in 80% concentration. Also, it has antiyeast effect that inhibits the growth of *candida albicans* in which its effect on the budding process (4).

The aim of this study is to determine the antibacterial action of black seed, Siwak and alum on bacterial isolates from root canals.

MATERIALS AND METHODS

Swabs were taken from root canal of 20 patients (Endodontic Department, Al-Elwiya Specialized Centre for Oral and Dental Surgery), thereafter, cultured on two blood agar plates and one MacConkey agar. One of the two blood agars incubated anaerobically at 37˚C for24hrs and the other blood agar and MacConkey agar were incubated aerobically at 37˚C for 24.hrs (5,6). Then subjected to identification according to their cultural properties, microscopic examination and biochemical *Api* and *miniapi* tests. The agar well diffusion method was used for the determination of antibacterial activity of *Salvadora persica* (Siwak), *Nigella sativa*(black seed), aluminum potassium sulphate (alum) aqueous extracts and the mixture of them by using bacterial isolates taken from the root canals to evaluate its effects on the isolated bacteria. Muller Hinton were inoculated with 0.1ml of bacterial inoculum (containing 1.5x 10^8cell/ml).Using cork borer, wells were made on the cultured media. Then, 0.1ml of *Nigella sativa*, *Salvadora persica* and alum aqueous extracts at all concentrations (3.125, 6.25, 12.5, 25, 50 and 100%) were added to wells, then the plates left for 30min in refrigerator at 4˚C, thereafter, they were incubated at 37˚C for 24hrs(7). The activity of aqueous plant extracts and alum was determined by measuring the diameter of inhibition zone (8).

RESULTS AND DISCUSSION

Effects of six different concentrations of aqueous alum extract on five bacterial isolates cultured in blood agar which isolated from root canal are presented in Table(1). The results show that the alum gives positive results on all bacterial isolates and the maximum inhibition zone (42mm) was observed for 100% concentration on *Enterococcus durans* and the minimum inhibition zone (9mm) was observed for 3.125% concentration on *Enterobacter cloacae*.

Table (1): Effects of different concentrations of aqueous alum extractson some bacterial isolates.

Concentrations bacterial / Bacteria	3.125%	6.25%	12.5%	25%	50%	100 %
Streptococcus oralis	15	17	24	25	30	35
Streptococcus anginosus	10	16	20	27	37	40
Enterococcus durans	14	17	19	27	32	42
Enterobacter cloacae	9	13	23	27	30	36
Staphylococcus epidermides	12	17	19	26	32	37

Values means inhibition zone diameter in millimeter

Mohammed(9) found that 60% alum concentration gives inhibition zone of 29mm diameter on *Pseudomonas aeruginosa*, while 50 and 40% alum concentrations were 25 and 22mm diameter, respectively. Therefore, alum is used in wound and burns disinfection and in treatment of ulcers in the oral cavity. Using 50% alum + 20% hydrogen peroxide mixture gives 42mm diameter inhibition zone on *Pseudomonas aeruginosa* (3).

Table(2) show the antibacterial actions of six different concentrations of *Salvadora persica* (Siwak) aqueous extract on five types of bacteria isolated from root canal. The results indicate that the maximum inhibition zone was 27mm diameter on *Enterobacter cloacae* at 100% concentration and the same concentration gives inhibition zones 22, 20, 14 and 11mm diameter on *Streptococcus oralis*, *Streptococcus anginosus*, *Staphylococcus epidermides* and *Enterococcus durans*, respectively.

Table(2): Effects of different concentrations of aqueous *Salvadora persica* extract on some bacterial isolates.

Concentrations / Bacteria	3.125%	6.25%	12.5%	25%	50%	100%
Streptococcus oralis	_	_	_	_	_	22
Streptococcus anginosus	_	_	_	_	13	20
Enterococcus durans	_	_	_	_	_	11
Enterobacter cloacae	_	_	_	_	22	27
Staphylococcus epidermides	_	_	_	_	10	14

Values means inhibition zone diameter in millimeter
(-)Means there is no inhibition zone.

Al-Shammary(10) found that 20% concentration of *Salvadora persica* aqueous extract led to inhibition zone 16mm diameter on *Streptococcus mutans*, whereas, Al-Nidawi (11) found that 20% aqueous extract of *Salvadora persica* led to inhibition zone17.2mm diameter on *Streptococcus mutans*.

Table(3) show the antibacterial actions of six different concentrations of *Nigella sativa* (black seed) aqueous extract on five types of bacteria isolated from root canal. The results show that the maximum inhibition zone was 26mm diameter on *Enterobacter cloacae* at 100% concentration and the same concentration gives inhibition zones 22, 21, 20 and 16mm diameter on *Streptococcus oralis*, *Streptococcus anginosus*, *Staphylococcus epidermides* and *Enterococcus durans*, respectively.

Table(3):Effects of different concentrations of aqueous *Nigella sativa* extract on some bacterial isolates.

Concentrations of *N. sativa* / Bacteria	3.125%	6.25%	12.5%	25%	50%	100%
Streptococcus oralis	_	_	_	10	20	22
Streptococcus anginosus	_	_	_	14	17	21
Enterococcus durans	_	_	_	_	_	16
Enterobacter cloacae	_	_	_	10	22	26
Staphylococcus epidermides	_	_	_	10	18	20

Values means inhibition zone diameter in millimeter.
(-)Means there is no inhibition zone.

These results are in accordance with the results of Majid (12) who found that the use of 20% concentration of *Nigella sativa* oil extract led to inhibition zones 21, 20 and 19mm diameter on *E. coli*, *S. mutans* and *S. aureus*, respectively. Also, Khattab and Omar(13) found a marked decrease in the number of intracanal microbes when they used *Nigella sativa* oil extract. The results of antibacterial action of the alum, *Salvadora persica* and *Nigella sativa* aqueous extracts mixture on five types of bacteria isolated from root canal are presented in Table(4). The results indicate that the mixture of equal amounts of alum, *Salvadora persica* and *Nigella sativa* aqueous extracts mixture led to maximum inhibition zone 25 mm diameter on *S. epidermides* at100% concentration and the same concentration gives inhibition zones 24, 23, 20 and 17mm diameter on *S. anginosus*, *E. cloacae*, *S. oralis* and *E. durans*, respectively.

Table (4): Effects of different concentrations of alum, *Salvadora persica* and *Nigella sativa* aqueous extracts mixture on the bacterial isolates .

The mixture / Bacteria	3.125%	6.25%	12.5%	25%	50%	100 %
Streptococcus oralis	_	_	_	10	15	20
Streptococcus anginosus	_	_	10	17	20	24
Enterococcus durans	_	_	11	15	16	17
Enterobacter cloacae	_	_	12	14	19	23
Staphylococcus epidermides	_	_	10	13	20	25

Values means inhibition zone diameter in millimeter.
(-)Means there is no inhibition zone.

The alum, *Salvadora persica*, *Nigella sativa* and the mixture of them have antibacterial actions against bacterial isolates(from teeth root canal) and may be used as an endodontic irrigants.

REFERENCES

1- Randhawa, M.A. and Al-Ghamdi, M.S.(2002). A review of the pharmaco-therapeutic effects of *Nigella sativa*. *Pakistan J. Med. Res.*, 41(2): 77-83.

2- Al-Bukhari, M.I.(2004). Sahih Al-Bukhari. Kitab Al-Jumaa, Bab Al-Siwak Yaum Al- Jumaa, 1st ed., Cairo (In Arabic).

3- Al Ani, M.N.(2004). Evaluation of some prepared solutions from some medical plants and chemicals on *Pseudomonas aerugenosa*, Higher Diploma thesis; Institute of Genetic Engineering and Biotechnology for Postgraduate Studies, Baghdad University.

4- Al-Husainy, I. A.(2004). Effect of aqueous solution of aluminum potassium sulphate on *Candida albicans*, Higher Diploma thesis, Institute of Genetic Engineering and Biotechnology for Postgraduate Studies, Baghdad University.

5- Collee, J.G.; Franser, A.G.; Mmarmion, B.P. and Simmons, A.(1996). Mackie and MacCartney Practical Medical Microbiology 4th ed. Churchill living Ston, London.

6- Old, D.C.(1975). Practical Medical Microbiology *Vibrio, Aeromonas, Plesiomonas, Compylobacter. Aerobacter, Helico-bacter*, 14th ed. New York.

7- Crespo, M.E.; Jimenez, J.; Gomis, E. and Navarro, C.(1990). Antimicrobial activity of essential oil of *Thymus serpylloides subspecies gardorensis. Microbios*, 61:181-184.

8- Vignolo, G.M.; Suriani, F.; Holgado, A.P. and Oliver, G.(1993). Antibacterial activity of *lactobacillus* strains isolated from dry fermented sausages. *J. App. Ba*c., 75: 344-349.

9- Mohammed, B.(2004). Study effect mix of chemical(alum, hydrogen peroxide)with extract citrullus colocynthis on bacteria *Pseudomonas aeruginosa* isolated from contamination hospital, M.Sc. thesis, College of Dentistry, Baghdad University.

10- Al-Shammary, A.M.W.(2006). Assessment of *salvadora persica* (siwak) aqueous extract as anew endodontic irrigant in comparison to sodium hypochlorite (bacteriological, histopathological and immunological study, PhD thesis, Department of Conservative, College of Dentistry, Baghdad University.

11- Al-Nidawi, A.(2004). Effect of siwak extract on streptococcus mutans in comparison of selected antimicrobial agents (in vitro and *in vivo* study, M.Sc. thesis, College of Dentistry, Baghdad University

12- Majid, M.(2006). Assessment of the black seed oil extract as an intracanal medicament (a microbiological, histopathological and immunological study), PhD thesis, Department of Conservative, College of Dentistry, Baghdad University.

13- Khattab, N.M. and Omar, O.M.(2006). An investigation on the relative efficacy of *Nigella sativa* oil compared to formocresol as pulp medicaments in non-vital primary molar pulpotomies. *Egypt Dent. J*., 52(1): 169-177.