Antibacterial activity of oils extracts of *Brassica nigra* seeds on some bacteria isolated from plaque and healthy teeth in children (1-5) years

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Summary

In this study some bacterial species were isolated and identified from dental plaque and healthy teeth (control) in children (1 – 5) years that included pathogenic and non pathogenic staphylococci , *streptococcus* spp. Escherichia coli and *proteus* spp. The frequency of these isolates was in comparable values in males and females , also we studied the antibacterial activity of oils extracts from *Brassica nigra* seeds and some antibiotics by disk diffusion methods Results revealed that the isolates were more sensitive to oils alcoholic extracts compared with aqueous extracts and they were more sensitive to ciprofloxacin and Gentamicin compared with other antibiotics.

Key word: Children, dental plaque , *Brassica nigra*, Antibacterial Activity

Introduction

Dental caries is one of the most common and costly disease in the world and although rarely life threatening it is major problem for health service providers (Forrsten etal,2010) . In order to decrease the prevalence of caries an improved, understanding of the role of microorganism in dental disease is needed (Marsh,2003). Tooth surface is covered with a biofilm a slime layer consisting of millions of bacterial cells salivary polymers and food debris uncontrolled this biofilm can easily reach a thickness of hundreds of cells on the surface of an excellent adhesion site for the colonization and growth of many bacterial species (Davey and Otoole,2000). Over 700 bacterial taxa have been found in

the oral cavity however they are not all present in the same mouth(Aas etal,2005). Most of these microbes are harmless but under certain conditions some can cause oral infections like caries or periodontal disease (Sakamoto etal,2005) Usually the appearance of tooth cavities is followed by caries after 6-24 months (Mayooran etal,2000). The principle cause of dental caries is well understood the consumption of easily fermentable carbohydrates (in practice usually sucrose), stimulates the growth of oral microbes most notably *Streptococcus* and *Staphylococcus* due to the absence of oxygen and the species growth on sucrose lead to the fermentation of organic acids. Oral health is an integral part of the overall health of children (Marsh,2006). The prevalence of dental caries for the youngest of children has not decreased over the past decade despite improvement for older children (Beltran-Aguilar etal,2005). The most common oral disease encountered by children is dental caries (Tanzer etal ,2001). During the period of (1999-2002) 41% of US children 2-5 years ago had caries in primary teeth (Ramos-Gomez etal , 2002). Plaque can be defined a complex microbial community with greater than 10^{10} bacteria per milligram. It has been estimated that as many as 400 distinct bacterial species may be found in plaque (Hata andMayanagi,2003). Other researchers showed that oral flora contain >300 known species of bacteria (Wilson etal ,1997). In the search for drugs and new curse for human and animal ailments medicinal plants are definitely the most popular targets'. Consequently all parts of the plant body and in fact various plant products including their oils, juices and sop have been used in one form or the other in traditional and conventional medicine (Obi etal ,2009). This fact has been corroborated by (Newell and Linda, 1996) who reported that the antiseptic potentials of various products of plant are found in their essential oils. Consequently oil extract of seeds of common locally available medicinal plants brassica nigra that is a member of the Brassicaceae family. The seeds are globular black and about 1mm in diameter. They have a pungent taste and rich nutty odor (Erdogrul ,2002). It is a weedy plant cultivated for its seeds as a spice. The plant is believed to be native to the southern Mediterranean region of Europe and has been cultivated for thousands of years (Sokmann etal, 1999), it can grow from 2 to 8 feet tall with racemes of small yellow flowers that usually up to1- 3 across with 4 petals each. Its leaves are covered with small hairs and can wilt on hot days but recover at night (Larshini etal 2001). In addition its important as a food flavoring agent the seeds of *Brassica nigra* also have important medicinal uses

such as treatment of rheumatism and joint pains indurations of liver and spleen, tooth pain and throat tumors (Babpour etal ,2001).

Materials and Methods

Isolation and Identification of bacterial isolates

Sixty samples were collected from children teeth aged (1-5) years old included (30) male and (30) female. Forty samples were collected from tooth plaque and (20) from healthy teeth (control).All bacteria concerned in this study were isolated and identified from plaques and control samples using selective agar media like Mannitol salt agar,and Eosine methylene blue in addition to blood agar and MacConkey agar. These isolates were cultured and incubated in 37^{o}C for 24h. The colonies of bacteria were identified using conventional biochemical tests as described in (Colle etal,1996).

Preparation of Plant Samples

Healthy seeds of *Brassica nigra* (Black mustard) were purchased from a local market in Basrah, then blended to a fine powder using electric blender and preserved for extraction. Oils extraction of the plant was prepared using three different methods; using (70%) ethanol, (70%) methanol (alcoholic extract), and by Distilled water (aqeous extraction). Extraction was made by placing each extract in a soxhelt reflux apparatus and completely evaporated by rotary evaporator (Reinhold,1992). The dried extracts were then weighed and stored in 40^{o}C for further tests .

Disk diffusion tests

To study the antibacterial sensitivity tests we select eight isolates of *Staphylococcus aureus*, three isolates from *Staphylococcus epidermidis*, four isolates from *Escherichia coli* and two isolates from *Proteus* spp. The level of sensitivity of bacterial isolates was determined by disk diffusion (Kirby Bauer)method as described by (Nester etal,2004). Circular paper discs measuring 7.0 mm were cut from wattmann No.1 filler paper using paper perforator and sterilized in an autoclave, then these soaked with oils extracts (Ethanolic, Methanolic and aqeous extracts), separately and allowed to absorb the extract sufficiently,Sterile Muller-hinton agar plates were prepared and inoculated with each of the test organisms (10^{6} cells / ml)using stick spreader. Finally, the discs impregnated with oils extracts were carefully placed into the culture plates, and incubated at 37^{o}c for 24h and examined for growth and signs of inhibition. A similar experiment was set-up but instead of the oils extracts, ethanol and methanol solution

(70%) were used as control. The zone of inhibition was measured with a ruler. Culture bacteria with halos equal to greater than 7mm were considered susceptible to tests extracts. (Brantner and Grein,1994). Also we determined the antibacterial activity of five types of antibiotics (Ciprofloxacin (CIP), Gentamicin (CN), Vancomycin (VA), Tobramycin (TOB) and Ampicillin (AM), and the results were interpreted according to Clinical and laboratory Standard Institute documents(CLSI,2010)

Results and Discussion

In the Present study from (60) dental samples 53 (88.3%) Bacterial isolates were obtained that included 37 (92.5%) from dental plaque teeth and 16 (40%) from healthy teeth (control). The recovered bacterial isolates included *Staphylococcus aureus* 20،(%33.3) *Staph. epidermidis* 10 (16.6%), *Streptococcus* spp. 8 (13.3%), *Escherichia coli* 10 (16.6%) and *Proteus* spp 5 (8.3%) (Table 1) . However, the outcomes of the present study were quite different from those of (Lira etal ,2001) who reported different prevalence of bacterial species isolated from children (2-5) years of Japanese preschool. In another study the occurrence of bacterial species in children teeth was approximately similar to our results (Smith etal ,2001) that concluded that dental plaque is an infection disease in which members of bacterial isolates has been implicated as major etiological agent and most humans harbor these bacteria in their mouths (Costa and Franco,2007). Studies of (Okada etal,2002) suggested that Staphylococcus and *Streptococcus* have a high cariogenic potential in experimental animals.Our results showed that male and female children had almost the same level of bacterial species (82.8% and 96 % respectively) (Table 2). In another hand Table (3) shows the relationship between the age groups of children and the occurrence of bacterial species which illustrated that the prevalence of these bacteria was higher in the age group (3-5) years (106.6%) compared with age group (1-3) years (70%). Differences between bacterial species acquisition in infants could reflect true differences in the biofilms formed on the dental tissue (Linossier etal ,2008) this could have important implication in diseases caused by these organisms which regard to the creation of reservoirs of infection re-infection (Okada etal ,2005) The *in vitro* antibacterial activity of oils extracts of *Brassica nigra* are shown in Table (4) and illustrated in figures (1,2,3) which revealed that the inhibition zones of bacterial species were only around discs impregnated with ethanolic and methanolic plant extracts. As can be seen from this

table the oils extracts by ethanol and methanol have higher antibacterial activity approximately to all bacterial species enrolled in this study compared with slightly inhibition zones that formed by aqueous extract and no inhibition zone formed by ethanolic, methanolic solution (control) this agree with (Satish etal,2008) who observed that Brassica nigra seeds have inhibitory effects on this bacteria involved alkaloid, tannin, flavonoides, which have been reported responsible for anti microbial properties and as result, it could serve as anti from Table (5) the inhibition zones formed by standard antibiotic discs that explaine the antibiotic ciprofloxacin and Gentamicin have the highest microbial agents for the treatment of microbial infection (Nascimento etal ,2000). As we showed effect on bacterial species concerned in this study compared with slightly effect of Vancomycin and Ampicillin as well as the intermediate effect of Tobramycin.The ethanolic, methanolic oils extracts of Brassica nigra found to be the most potent anti microbial agents also the activity against both gram (+ve) and gram (-ve) bacteria suggest their potential uses as abroad spectrum anti bacterial agents that could be used to treat some stubborn staphylococcus and streptococcus infection, (Ayfer and Erdogral,2003) Asimilar use of oils extracts could also be adopted in treatment of infection due to Escherichia coli , Further considering the cost availability and extractability percentage and used as cheap alternative to substitute antibiotics especially in bacterial infection also in animal and poultry feeds. (31, 32)

Table (1) Percentage Frequency of bacterial species in Dental plaque & control of children (1-5) years

Children samples	**Number Of samples**	***Staph. aureus***	***Staph. epidermidis***	***Strept. Spp.***	***E. coli***	***Proteus***	**Total Number of Bacterial species**
Dental plaque	40	n=15 (37.5)	n=6 (15)	n=8 (20)	n=5 (12.5)	n=3 (7.5)	n=37 (92.5)
Control	20	n=5 (12.5)	n=4 (10)	n=0 (0)	n=5 (12.5)	n=2 (5)	n=16 40)
Total	60	n=20 (33.3)	n=10 (16.6)	n=8 (13.3)	n=10 (16.6)	n=5 (8.3)	n=53 (88.3)

n= Number of bacterial isolates

Table (2) Percentage Frequency of bacterial isolates in male and female of children

Children samples	*Number Of samples*	*Staph. aureus*	*Staph. epidermidis*	*Strept. Spp.*	*E. coli*	*Proteus*	*Total Number of Bacterial species*
Male	35	n=12 (34.3)	n=6 (17)	n=3 (8.5)	n=6 (17)	n=2 (5.7)	n=29 (82.8)
Female	25	n=8 (32)	n=4 (16)	n=5 (20)	n=4 (16)	n=3 (12)	n=24 (96)
Total	60	20	10	8	10	5	53

n= Number of bacterial isolates

Table (3) Percentage Frequency of bacterial species according to the age group of children.

Age of children samples	**Number Of sample**	***Staph. aureus***	***Staph. epidermidis***	***Strept. Spp.***	***E. coli***	***Proteus***	**Total Number of Bacterial species**
(1-3)	30	n=4 (13.3)	n=7 (23.3)	n=8 (26.6)	n=2 (6.6)	n=0 (0)	21 (70)
(3-5)	30	n=16 (53.3)	n=3 (10)	n=0 (0)	n=8 (26.6)	n=5 (16.6)	32 (106.6)
Total	60	20	10	8	10	5	53

n= Number of bacterial isolates

Table (4) Inhibition zones in millimeter induced by *Brassica nigra* seeds extracts on bacteria isolated in the study

Bacterial species	Ethanol Extract (mm)	Methanol Extract (mm)	Aqueous Extract (mm)	Ethanol control	Methanol control
Staph. aureus 1	……	……	……	……	……
2	……	8	……	……	……
3	……	10	……	……	……
4	12	8	……	……	……
5	13	……	……	……	……
6	……	12	……	……	……
7	10	9	12	……	……
8	12	11	……	……	……
Staph. epidermidis 1	13	12	……	……	……
2	……	12	13	8	……
3	……	……	……	……	……
Streptococcus. 1	10	……	……	……	……
2	9	……	……	……	……
3	14	……	……	……	……
E. Coli 1	13	12	7	……	……
2	13	14	……	……	……
3	14	15	……	4	……
4	12	11	……	……	……
Proteus 1	12	15	12	……	……
2	……	13	……	……	……

Table (5) Antibiotic sensitivity test in millimeter against study isolates

Bacterial species	CIP	CN	VA	TOB	AM
Staph. aureus 1	20	**18**	……	15	10
2	21	16	……	12	12
3	23	20	8	18	……
4	34	22	10	20	……
5	20	20	8	……	15
6	**23**	25	7	……	13
7	26	22	6	10	8
8	27	20	10	15	13
*Staph. epidermidi*s 1	25	26	……	17	10
2	27	25	……	15	13
3	26	23	……	10	8
Streptococcus. 1	27	22	……	12	12
2	25	23	6	10	15
3	26	20	7	11	13
E. Coli 1	24	22	……	8	16
2	27	23	5	7	13
3	27	28	……	25	8
4	28	27	……	23	9
Proteus 1	29	25	……	20	20
2	**22**	23	……	18	10

CIP = Ciprofloxacin ; TOP = Tobramycin ; VA = Vanocmycin; CN = Gentamycin; AM = Ampicillin

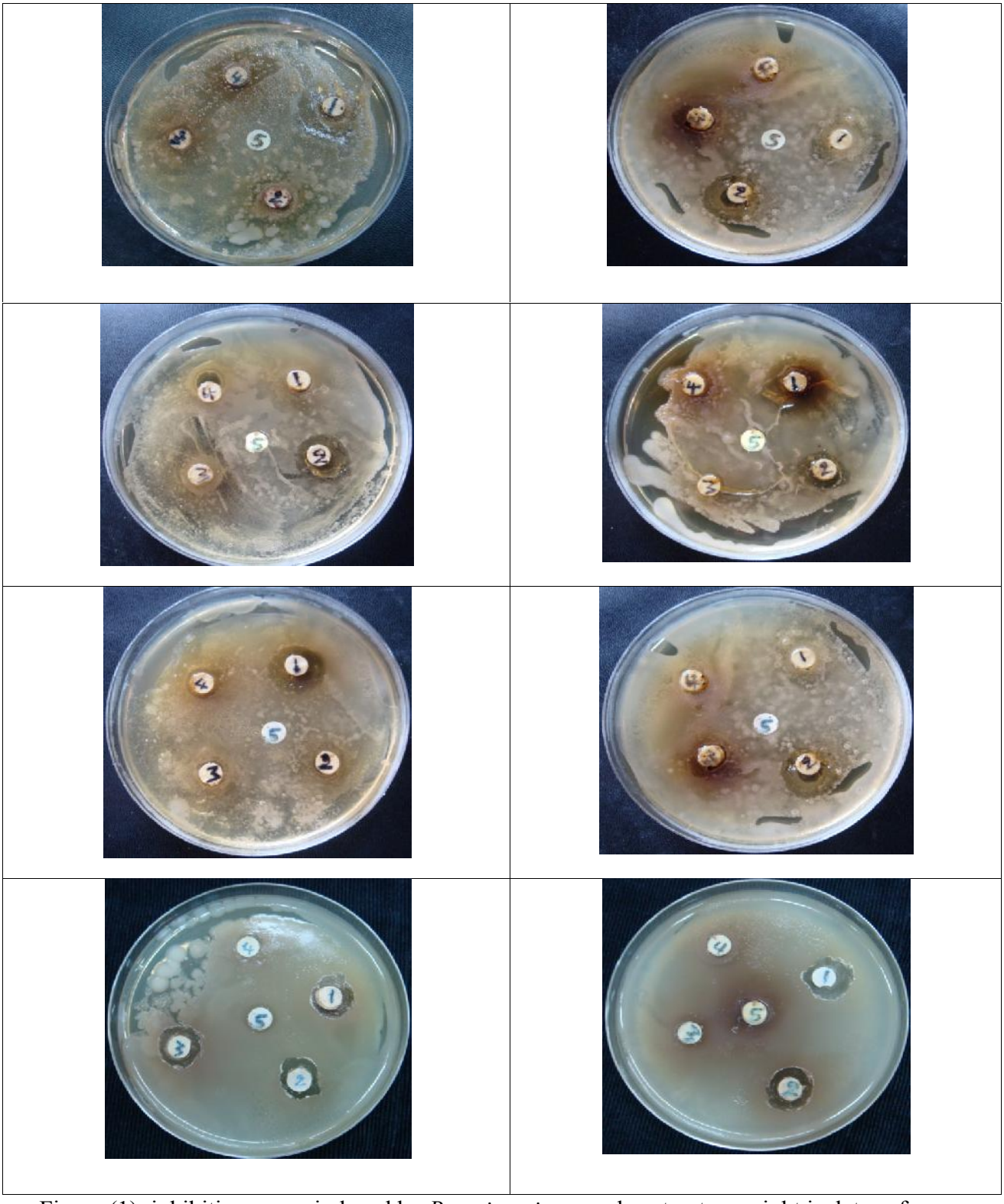
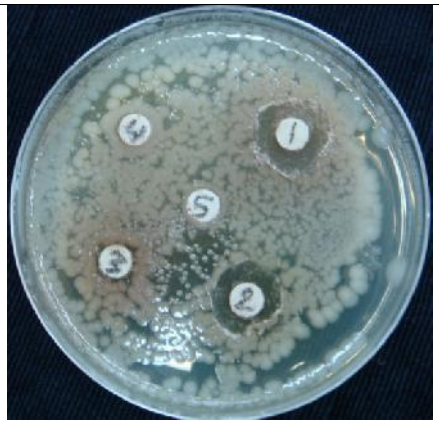


Figure (1): inhibition zones induced by *Brassica nigra* seeds extracts on eight isolates of *staphylococcus aureus*

1 = Ethanol extract; 2 = Methanol extract; 3 = Aqueous extract; 4 = Ethanol solution; 5 = Methanol solution



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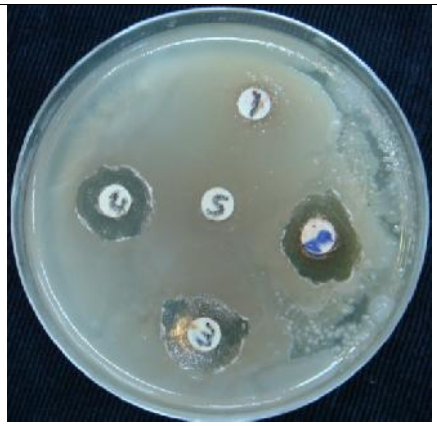

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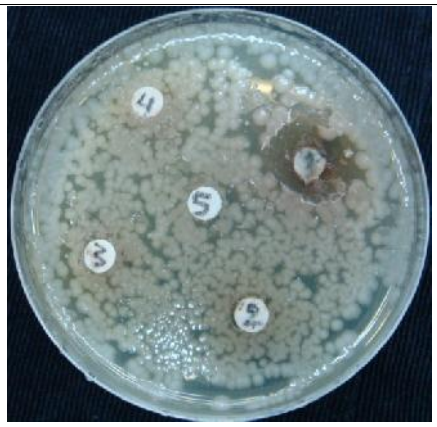

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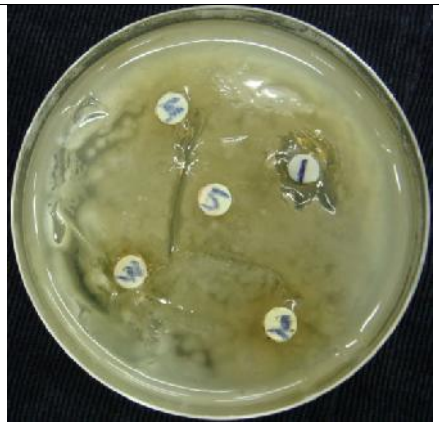

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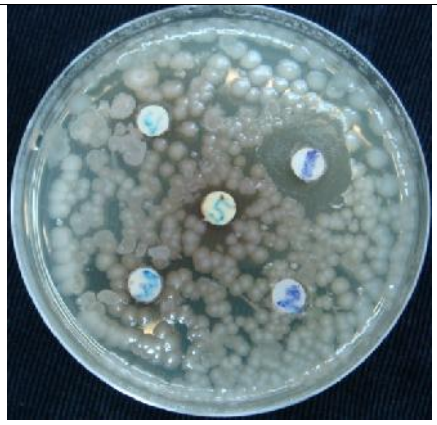

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Figure (2): inhibition zones induced by *Brassica nigra* seeds extracts on Three isolates of *staphylococcus epidermidis* (1-3) and three isolates of *streptococcus* spp. (4-6)

1 = Ethanol extract; 2 = Methanol extract; 3 = Aqueous extract; 4 = Ethanol solution; 5 = Methanol solution

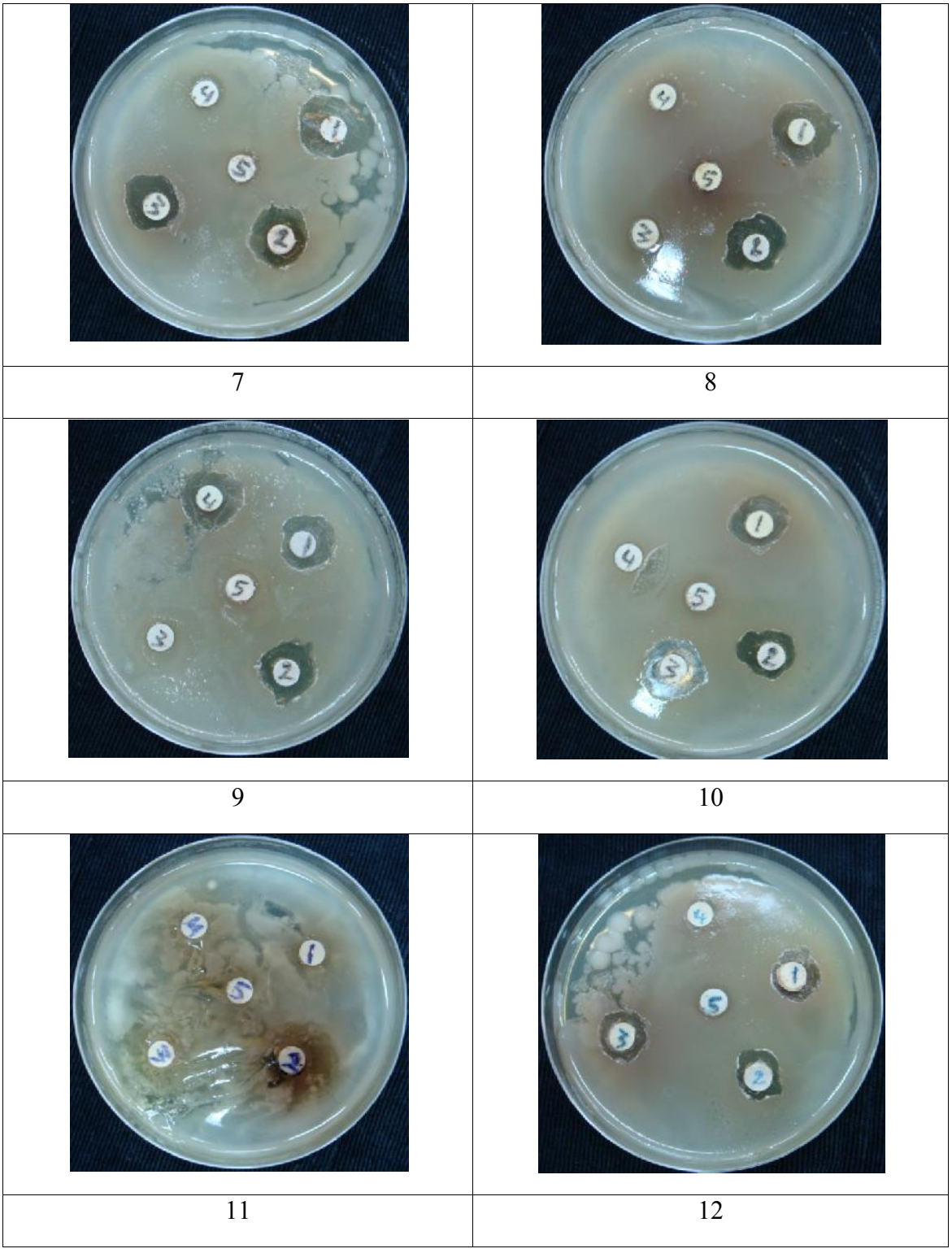

Figure (3): inhibition zones induced by *Brassica nigra* seeds extracts on Four isolates of *Escherichia coli* (7-10) and Two isolates *of Proteus* spp. (11-12)

1 = Ethanol extract; 2 = Methanol extract; 3 = Aqueous extract; 4 = Ethanol solution; 5 = Methanol solution

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دراسة الفعالية الضد جرثومية للزيوت المستخلصة من بذور الخردل الاسود على بعض البكتريا المعزولة من حالات تسوس الاسنان والاسنان السليمة للاطفال (1- 5) سنوات

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الخلاصـــــة

في هذه الدراسة تم عزل وتشخيص بعض الأنواع الجرثومية من حالات تســـوس الأسنـــــان والأسنان السليمة (السيطرة) عند الأطفال دون سن الخامسة والتي شملت *staphylococci* المرضية وغير المرضية ، *streptococcus spp* ، *Escherichia coli* وأيضـــــاً *Proteus* spp كما إن تردد هذه الأنواع كان بقـــيم متقاربة في الذكـــور والإناث وأيضا تم دراسة الفعالية الضد مايكروبية لمستخلصات الزيوت لبذور الخردل الأســـــود وكـــذلك بعض المضادات الحيـــاتية عـن طريق اختبار الانتشـــار ضمن القرص والتي بينت إن هذه العزلات أكـــثر حساســـية للمستخلص الكحولي الزيتي بالمقارنة مع المستخلص المائي بالإضافة إلى أنها أكـــثر حساسية للمضاد الحيـــويCiprofloxacin و Gentamicin بالمقـــارنة مـــع بقيـــة المضادات الحيوية .