THE IMMUNOLOGICAL AND ANTIBACTERIAL EFFECT OF *Syzium aromatic* EXTRACT ON BACTERIA ISOLATED FROM TEETH

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ABSTRACT

Seventy five samples were collected from human teeth of ages about (25-65)years from both sex . Several type of bacteria were diagnosed namely *Staphylococcus .aurous* (33.33%), *E. coli* (13.33%) , and *Staphylococcus epidermis* (22.66%) . *Syzygium aromaticum* crud extract were used as two type grinding and non-grinding .The minimum concentration of grinding type was(0.6-2.6) mg/ml and the minimum concentration of non-grinding type was (0.533-2.1) mg/ml that inhibit bacterial growth of *S .aurous* and *E. coli*. Antibiotic sensitivity test was applied using discs diffusion method , the sensitivity was (92%) for *Staph. aurous* toward Kanamycin (K), and (80%) *for E.coli* toward Ciprofloxacin(Cip).The results showed that the resistance of *Staph. aurous* was 18(72)% toward Metromidazol(MET) , 22(88)% toward Bacitracin (B), *E. coli* was 8(80%) toward Streptomycin(S). The phagocytosis test or phagocytosis activity also included in this study.

INTRODUCTION

Syzgium aromaticum is a small medium sized ever green tree 8.30 in tall. *S.aromaticum* (Cloves) is aromatic dried flower buds of tree in the family Myrtaceae(1).Clove is reported as a natural source of food flavoring ,analgesic, antiemetic, toothache , anesthetic , antibacterial , antiviral , fungicide , fungi static , antiseptic , carminative , tonic , antihistamine , astringent ,also have anticarcinogenic property ,contraceptic in low doses (2,3,4,5) . In addition the antimicrobial activity of cloves essenatials oil have been studied against a large number of multi resistant *Staphylococcus epidermidis* (6) . Other studied against *Bacillus subtilis Compylobacter jejuni* and *S. aureus* also, *Salmonella enterides* and *E. coli* (7,8,9).Several component from *S.aromticum* have been found to posses

growth inhibition activity against oral pathogens , these component namely (5,7-dihydrooxy -2- methylchromone-8-C-B-gluco pyrano cide , biflorin , Kaempferol , aminocitrin, myricetin , gallic acid ellagic acid and Oleanolic acid) (10), but the main component of *S.aromaticum* is eugenol C10 H12 O2; Hallyl -2- methoxy phenol of deep decay , and when mixed with Zinc oxide used in dentistry (11). Phagocytosis the process by which foreign particles including bacteria are ingested by certain endothelial cells of body (12). Phagocytosis was destragde the microbes when ingested by Leukocytes and other , phagocytosis it is a normal function of body stimulated by invasion of pathogenic such as bacteria and was include many stage such as chemotaxis ,opsonization , attachment phagocytosis and intracellular killing the foreign body (12,13) . By ingestion of microbial pathogens , phagocytic leukocytes accomplish two essential immune function, firstly , they initiate a microbial death pathway, in part by ingested pathogen to lysosome , which are rich hydrolytic enzymes and also by targeting the phagocyte oxidase complex to the phagolysosome , Secondly phagocytosis to direct antigens to both MHCI and MHCII compartments (14). The aim of the study is to evaluated the activity of grinding and non-grinding *S. aromatic* against *S. aurous* and *E.coli* and maintance of the activation of phagocytosis in presence of extraction and their effect together against bacteria .

MATERIAL AND METHODS

1.Sampling

Swabs were collected from human teeth of age (25-65) years from students, worker and lecturers of Basrah veterinary medicine college . The swabs were dipped in nutrient broth and incubated at 37 C^{o} for 24 hrs. (15).

2. Laboratory diagnosis:

A . Culturing:

Mannitol Salts agar and Eosin methylene blue agar were prepared for bacteria growth

B . Microscopy:

Staining of bacteria grew on nutrient broth were done by gram staining procedure as describe by (15).

3. Biochemical tests: catalase and coagulase tests were done as describe by (15).

Extraction of *Syzygium aromaticum*

Flowers of *Syzygium aromaticum* (clove) were purchased from markets . The plant washed by distilled water to remove dust and then further dried in an oven at 50°C for 48 hrs. (16). Some of plant were grinded into fine powders using electric blender and other non- grinded and prepared by dessolving of 125 gm of each sample separately into 500 ml solvents (80% ethanol) using conical flasks plugged with cotton plugs . The mixtures were still in the room temperature for 24hrs. ,in sterile flask covered with aluminum foil in order to avoid evaporated and prepared to infiltration in the sterile whatman No. 1 filter paper. After filtration the mixture evaporated in water bath until 40 ml for the grinded plant and 30 ml for non-grinded extract and was left in container (17).

Antibacterial Assay

Agar diffusion method

The agar diffusion method is the most wide spread technique of antimicrobial activity assessment. The appropriate solidified medium (Muller Hinton agar) was inoculated with bacterial inoculum (10^6 CFU/mL) and spread over the plates using a sterile cotton swabs . After inoculum absorb by agar, sterile filter discs (Whatman no 1, England, 6 mm diameter) were impregnated with 10 µl of stock solutions of two types of extraction placed on the agar surface using forceps dipped in ethanol and flamed. Positive control cultures with streptomycin solution (50 mg/mL) were used to assess the susceptibility of tested bacteria and to compare with them the essential oils efficiency. The dishes were incubated at 37^oC for 24h. After the incubation period, inhibition zone was measured in millimeters, for each disc and evaluated for susceptibility or resistance.

Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC)

Minimal Bactericidal Concentration (MBC) were carried out by the broth dilution method about 10^6 CFU/ml (18) . Concentration for grinding *Syzygium aromaticum* about (2.6mg/ml, 1.3mg/ml , 0.8mg/ml and 0.6mg/ml) for non-grinding about (0.53gm/ml, 0.71mg/ml ,1.061gm/ml and 2.1gm/ml) were used. MIC values were taken as the lower concentration that prevents visible bacterial growth after 24 hrs. of incubation at 37°C, and MBC as the lowest concentration that completely inhibited bacterial growth.

Antibiotics activity:

Antibacterial activity was carried out using a disc-diffusion method . Plates were prepared with 10 ml of sterile Mueller Hinton Agar we used ten type of antibiotics in order test the antibiotic action against *S. aureus* and *E.coli* , the antibiotic are (Ciprofloxacin , chloramphenicol, Streptomycin , Pencillin , Erythromycin , Enrofloxacin , Metronidazole , Tobramycin , Bacitracin , Kanamycin) these were produced by Jerusalem pharmaceutical Co. and Birzeit - pharamaceutical Co. and this producer according to the (19). The antibiotics were placed on the surface of the medium and left for 30 min at room temperature for compound diffusion. The plates were incubated for 24 hrs at 37°C. Zones of inhibition were recorded in millimeters and the experiment was repeated twice.

Laboratory animal:

Twelve laboratory animals(rabbit males) at age 10 weeks ,divided into two group :

First group four rabbits injected with grinded *S.aromaticum* extraction .

Secondly group: four rabbits injected with non-grinded *S. aromaticum* extraction and the other four animals remained as control .The dosage of extraction for each was 3ml for seven days .

Phagocytosis test or Phagocytosis activity:

The phagocytosis test was carried out to the two type of extraction grinding and non-grinding *Syzygium aromaticum* according to method of (20) and (21).

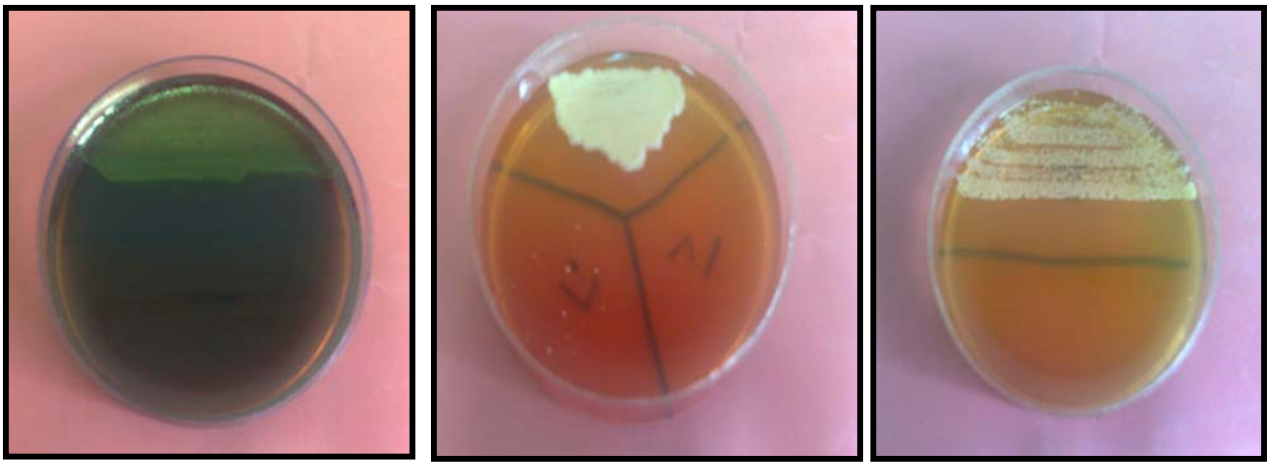
The blood were collected from two group of rabbits that injected with grinded and non-grinded *S. aromaticum* . The blood collected from cardiac venues in to test tube containing anticoagulant . culture of clinical isolated of E.coli growing at 37^{o} C was diluted by PBS to obtain the concentration of bacteria about 1×10^{3} CFL/ml . The blood samples from rabbits that injected by grinding *S.aromaticum* was diluted by PBS and divided into four test tubes , each one contained 0.5 ml of blood suspension (blood +PBS)added to each one 0.5 ml of bacteria suspension and then each tube putted in the routed incubator at 37°C. One test tubes incubated at zero time, second tube at 30 min. , third test tube at an hour and the fourth tube at two hours. After that 0.1 ml from each test tube were taken and cultured on Muller Hinton agar and incubated for 24hrs. in 37°C and then the bacterial number were counted for each plate.

RESULTS AND DISCUSSION

Seventy five swabs from human teeth of age 25-65 years were collected in this study .Bacterial isolates were included in table (1) and figure 1 .

Table (1):Number and percentage of isolates bacteria

Type of bacteria	No. of positive bacterial Isolates	The percentage of bacterial Isolate
Staphylococcus aureus	25	33,33%
Eshericlia coli	10	13.33%
Staphylococcus epidermicus	17	22.66%
No growth	23	30,66%
Total	75	



A B C

Figure (1): growth of bacterial isolates on media .
A: *E.coli* growth on EMB media
B: *Staph . aureus* growth onMacConky agar.
C: *Staph epidermicus* growth onMacConky agar

There were relationship between oral disease and microbial species . (21).Over 750 species of bacteria inhabited the oral cavity (50%which were yet to identified) and numbers of these are implicated in oral disease . The results appear *S.aureus* ,*E.coli* and *S. epidermies* at percentage 33,33%, 13.33% and 22.66% . The scientist (22) isolated bacteria from mouth and found that bacteria was acidogenic , gram positive bacteria such as Streptococci *Streptococcus mutarns* and *Strep. Sobrinus , Lactobacillus* and *Actinomyces* .This bacteria metabolize the sucrose found between teeth converted it to acids that dissolve the calcium phosphate of teeth causing decalcification and evented decay .

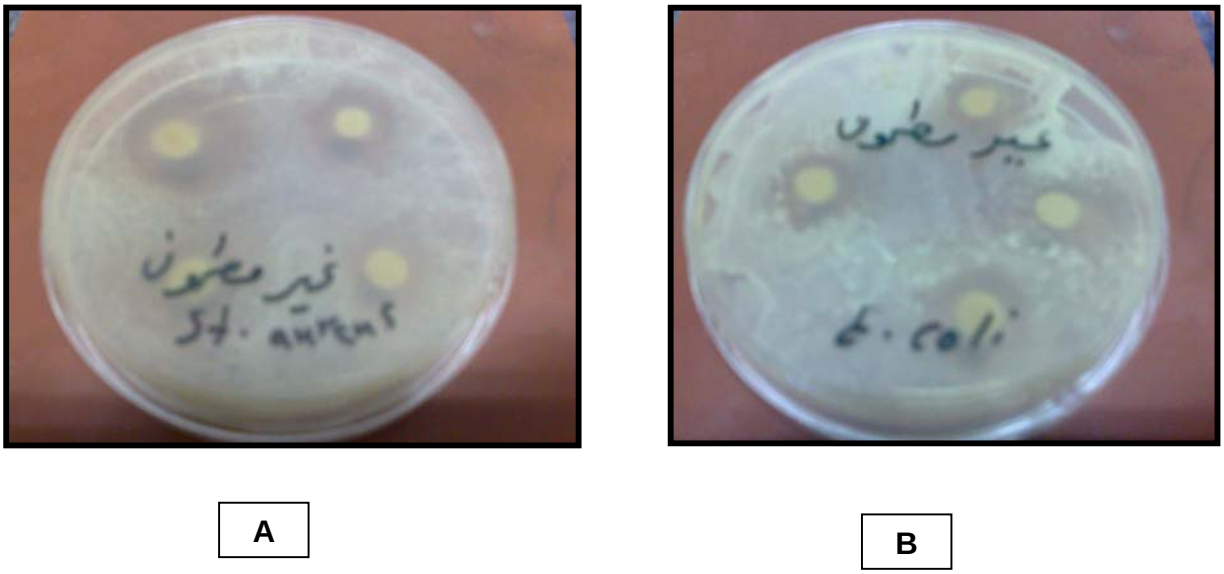
The results showed that the action of grinded against isolated bacteria more than non-grinded using of crud extraction against *E. coli* and *Staph. aureus* .The inhibition zone of *Staph. aureus* 24mm . *E. coli* 20.5mm. in present of grinded *Syzygium aromaticum* , and 18.7 mm in *Staph.aureus*, 18mm. in *E.coli* when used crud non-grinded . (table 2 , figure 2and 3).

These results were similar to that found by other worker (16,23). They found the separation and purification of grinding *Syzygium aromaticum* might more bioactivity than non-grinding , because numerous compounds ,chemical bonds act together strongly in grinded *Syzygium aromaticum* , also eugenol substances in high percentage in grinded.

Table (2): The inhibition zone against the Isolated bacteria using *Syzygium aromaticum* as grinded and non-grinded .

Clove oil as	Inhibition zone of isolated bacteria (mm)	
	E. coli	Staph. Aurous
Grinded	20.5	24
Non-grinded	18	18.7

Figure(2):The inhibition zone of the isolated bacteria using *Syzygium aromaticum* as grinded *A:S .aureus B: E.coli*



Figure(3):The inhibition zone of the isolated bacteria using *Syzygium aromaticum* as non grinded A:*S .aureus* B*: E.coli*

The concentration value of non-grinding *S . aromaticum* that inhibited the bacteria was (0.533-2.1mg/ml) and the MIC that inhibited growth of bacteria *E.coli* 0.71 mg/ml, *Staph. aureus* 2.1mg/ml .The inhibition of microorganism due to present of active substances such as eugenol (16) , table 3and 4.

These crude extracts had many different phytochemical and this is agreement with (3) that indicates to these point and ensured in his research that different target sites effected on bacteria could theoretically lead to either an additive or a synergistic effect (3,25) .In the studied of the MIC that defined as the lower concentration of ethanolic extraction of *S .aromaticum* that inhibited the bacteria (26,27) , So the concentration value of grinding *S.aromaticum* that inhibited growth of *Staph. aureus* and *E.coli* was (0.6- 2.6mg/ml) . The lower of concentration that caused inhibited growth of *E.coli* was 0.8mg/ml and *Staph.aureus* 2.6 mg/ml .

Table(3):The minimum inhibition concentration of grinding *S . aromaticum* against bacteria.

Bacteria Isolates	Concentration S .aromaticum mg/ml				MIC
	0.6	0.8	1.3	2.6	
E.coli	+	-	-	-	0.8
Staph.aureus	+	+	+	-	2.6

Table(4): The minimum inhibition concentraction of non-grinding *S . aromaticum* against bacteria

Bacterial isolates	Concentration of S .aromaticum mg/ml				MIC
	0.533	0.71	1.06	2.1	
E.coli	+	-	-	-	0.71
Staph. Aureus	+	+	+	-	2.1

The antibiotic sensitivity by disc agar diffusion method against bacteria *Staph. aureus* and *E.coli* . The high sensitivity was shown by *Staph. aureus* against Kanamycin (92%) and the *E.coli* against Ciprofloxacin (80%) . (Table 5) .

The study showed the increased resistance of *Staph. aureus* to conversional antibiotic which include metronidazole 72% and bacitracin 88%, *E.coli* to streptomycin 80% (Table 6) . These antibiotic recommended to be an effective drug for anaerobic infection , its´ frequent used for treatment of gingivitis as well as amoebiasis might have resulted in development of resistanse strain (28)

Table(5):The antibiotic sensitivity test results against *Staph .aureus*

Antibiotics	No. Sensitive	No. Resistance	Moderate growth
Ciprofloxacin(CIP)	12(48%)	6(24%)	7(28%)
Chloramphenicol(C)	10(40%)	8(32%)	7(28%)
Streptomycin(S)	7(28%)	14(56%)	4(16%)
Penicillin(P)	22(88%)	0(0%)	3(12%)
Erythromycin(E)	20(80%)	2(8%)	3(12%)
Enrofloxacin(ENR)	19(76%)	0(0%)	6(24%)
Metronidazol(MET)	4(16%)	18(72%)	3(12%)
Tobramycin(TOP)	17(68%)	6(24%)	2(8%)
Bacitracin (B)	3(12%)	22(88%)	0(0%)
Kanamycin(K)	23(92%)	0(0%)	2(8%)

Table(6):The antibiotic sensitivity test results against *E.coli*

Antibiotics	No. Sensitive	No. Resistance	Moderate growth
Ciprofloxacin(CIP)	8(80%)	0(0%)	2(20%)
Chloramphenical(C)	4(40%)	3(30%)	3(30%)
Streptomycin(S)	2(20%)	8(80%)	0(0%)
Penicillin(P)	7(70%)	2(20%)	1(10%)
Erythromycin(E)	5(50%)	1(10%)	4(40%)
Enrofloxacin(ENR)	6(60%)	2(20%)	2(20%)
Metronidazol(MET)	7(70%)	0(0%)	3(30%)
Tobramycin(TOP)	4(40%)	4(40%)	2(20%)
Bacitracin (B)	7(70%)	3(30%)	0(0%)
Kanamycin(K)	7(70%)	0(0%)	3(30%)

Phagocytosis was done targeted to microbial function , microbes were initially engulfed into a plasma membrane – drives vacuole , the phagosome, which proceeds to acquired derivative properties by complex process termed maturation (29).

In the present study the count of bacteria of *E.coi* in present of non-grinding *Syzgium aromaticum* extraction of zero time about (78%) and was reach to percentage (37.0%) at two hour time . The count of bacteria *S.aureus* about (75.%) at zero time and was reach to the percentage(35.2%) after two hours' time .In gringding *Syzgium aromaticum* the count number of *E.coli* at zero time about (95 %)and was reach (23.5%)at two time hrs. , and count of *S.aureus* at zero time (90.2%) and was reach to the percentage (22.1%)at two hrs. time table (7).

Table (7): The count of bacterial of *E. coli* and *Staph.aureus* in present of grinding and non-grinding *Syzgium aromaticum* extraction with phagocytosis.

Bacteria	Grinding S.aromaticum				Non–grinding S.aromaticum			
	Zero time	1/2hrs. time	Hour time	2hour time	Zero time	1/2hrs. time	Hour time	2hour time
E.coli	190 95%	93 46.5%	75 37.5%	47 23.5%	156 78%	117 58.5%	83 41.5%	74 37.0%
Staph. aureus	180 90.2%	90 41.2%	70 35.1%	40 22.1%	150 75%	111 55.5%	80 40.2%	70 35.2%

التأثير المناعي والمضاد للبكتريا لمستخلص القرنفل على خمج الاسنان الجرثومي

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الخلاصة

تم جمع ٧٥ عينة مابين الاسنان لاشخاص تتراوح اعمارهم بين (٢٥-٦٥) سنة من كلا الجنسين وشخصت انواع من البكتريا وكانت النسب كالتالي (٣٣.٣٣%) *staph.aureus* (٢٢.٦٦%)*E.coli* (١٣.٣٣%) *Staph.epidermicus*.استخدم مستخلص نبات القرنفل *Syzgium aromaticum*بنوعين المطحون والغير المطحون حيث اظهرت النتائج ان فعالية النوع المطحون افضل من النوع العير مطحون واستخدم التركير الادنى للمستخلص بشكله االمطحون ml / mg(0.6-2.6) الذي ثبط نمو بكتريا *s.aureus* وبكتريا *E.coli* والتركيز الادنى للمستخلص بشكله الغير مطحون mg/ml(0.533-2.1) لتثبيط نمو بكتريا *s.aureus* ,*E.coli* .كما اجري اختبار المضادات الحياتية بواسطة الاقراص وكانت نسبية العزل عالية بالنسبة , *s.aureus* عالية وبلغت (٩٢%) بوجود المضاد الحيوي Kanamycin(K)و وبالنسبة لبكتريا *E.coli*حوالي (٨٠%) بوجود المضاد الحيوي Ciprofloxacin (Cip). واظهرت النتائج ان مقاومة بكتريا *Staph.aureus*المعزولة بوجود المضاد الحيوي Metromidazol(Met) وكانت النسبة حوالي ١٨(٧٢%) وحوالي ٢٢(٨٨%) بوجود المضاد

الحيوي(Bacitracin(B .اما بكتريا *E.coli* المعزولة كانت مقاومة للمضاد الحيوي Streptomycin (S) بنسبة ٨(٨٠%). كما استخدم هده الدراسة اختبار كفاءة عملية البلعمة .

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