Determination of the inhibitory activity of some biological extracts agaiast multi rrsistans antibiotic Staphylococcus specis which and isolated from different sources of infechtion

Raheb salman kurdy[1] , Mohammed Nadhier Maroff[2]
[1]*Department of Biology , College of Science , Tikrit University , Tikrit , Iraq*
[2] *Dept. of Biology , College of Education for pure science , Tikrit University , Tikrit , Iraq*

Abstract
The study include 225 specimens that were collected from patients attended Tikrit General Hospital from mid of Septembar to mid December 2016. The specimens included the swab from: burn, wound, otitis media ,pharyngitis, and urine The specimens were incubated on to mannitol salt agar which is considered as selective and differential media for the genus Staphylococcus the results showed that 100 isolate (44,5%) grown on the medium. According to the phenotypic characteristics and tests biochemical they were *Staph. aureus ,Staph. Saprophytis , Staph. sciuri, Staph. Xylosus Staph. lentus Staph. haemolyticusi Staph.c aseolytic*
The ability of isolates to produce nitrate reductase, hemolysin, urease, DNAas were test . The antiboiotics were tested for resistance to 10 antibiotics. Some spceics showed high resistance to PenicillinG (94), Tobramycin (40%), Amikacin (39%), NitroFurantion (38%) Tetracyclin (36%), ,Novobiocin (24%), Trimethoprim (23%), Gentamycin (13%), Chloromphenicol (10%), Imipenem (3%).,
The inhibitory effect of biological extract of the Staph. species the pylogenetic effect has built of the concentrations of 100,150,250 mg / ml showed an inhibitory effect against types of Staphylococcus bacteria.
It was also found that the higher the concentration of the extract, the greater the inhibitory effect.
The results showed that Staph.aurea bacterial was the most affected by fungal compounds if the highest inhibition dimeter in concentration was 250 mg / ml 26 mm in the case of extract of *Trichoderma koningii,* whereas the inhibition rate in the same case the concentration was 24 ml. in the case of extract of *Trichoderma viride.*

Introduction
Antibiotics are biochemicals produced by microorganisms that can be industrially produced and inhibit the growth or killing of other organism[1].
Antibiotic resistance is one of the biggest health problems in the world, prompting researchers to investigate modern antibiotics to overcome bacterial resistance, which increases mortality and epidemics [2].
The resistance of bacteria to chemically manufactured antibiotics is dangerous in human health [3]. This has made the need for continuous detection of diverse resources, especially from natural sources used in the treatment of human diseases. Natural and all its wild and marine components are a treasure and an important source of many vital elements of natural importance and the most important of these biological elements are fungus and its derivatives of secondary metabolites, which have been proven by previous studies to be important in the biological control of many pathogens If many secondary metabolites are isolated, It is a chemical compounds have the ability To inhibit growth or kill human pathogens(4). Recent studies show that.
Multiple types fungal species can be considered as promising projects in the production of metabolic compounds that are important in their use to meet the increasing threats of bacterial resistance to therapeutic antibiotics due to the diversity of secondary metabolites resulting from them, which vary depending on the quality of fungus or food medium or the develop environmental conditions of those fungi (5).

Material and Methods
Collection of samples
included collection of 225of specimens that were collected from the patients attended Tikrit General Hospital. The specimens included the swab of: burn, wound,uirn, chronic otitis media**,** pharynx infections and urine sample.
Culture media
Blood Base agar, Muller hinton agar, mannitol salt agar and Nutrient agar (Oxoid, England),were used for isolation, identification and determination of antibiotic sensitivity used 10 types of antibiotics All these media were prepared according to manufacturer instructions, sterilized by autoclaving at 121ºCfor 15 minutes.
Identification of the Isolated *Staphylococcus*
The isolates were identified according to the Bergey's Manual (6), as follows:
Gram stain
All the bacterial isolates were examined under light microscope after
Staining with Gram stain to notice their response to the stain, sizes, shapes and arrangement of the cells (7).
Biochemical tests
The following biochemical test were used for the identification of staph.species
Catalase test, Oxidase test, DNase test . Hemolysin production Urease production Nitrate
Antimicrobial Susceptibility Test
This test performed by modified Kirby-Bauer method as the following(19)

1- From an overnight culture plate, 4-5 colonies of bacterial isolate were picked up sterilized inoculating loop and emulsified in 5ml of sterile normal saline until the turbidity is approximately equivalent to that of the McFarland No. 0.5 turbidity standard .
2- A sterile swab was dipped into the bacterial suspension, any excess fluid was expressed against the side of the tube.
3- The surface of a Mueller-Hinton agar plate was inoculated by bacterial isolate as follows: The whole surface of the plate was streaked with the swab, then the plate was rotated through a 45º angle and streaked the whole surface again; finally the plate was rotated another 90º and streaked once more.
4- By a sterile forceps the antimicrobial disc was picked up and placed on the surface of the inoculated plate. The disc was pressed gently into full contact with the agar. All plate content 5 disc.
5- The step (4) was repeated to all antimicrobial discs under the test, spaced evenly a way from each other.
6- The plates were incubated at 35ºC for 18-24 hours.
7- After incubation, the plates were examined for the presence of inhibition zone of bacterial growth (clear rings) a round the antimicrobial discs, if there was no inhibition zone the organism was reported as resistant to the antimicrobial agent in that disc. If a zone of inhibition surrounded the disc, the diameter of the zone of inhibition was measured (by millimeters) and compared their sizes with values listed in a standard chart.

Isolate fungi

The used fungi were obtained from the college of Agriculture the University of Tikrit. The isolates were then placed on the sterile Potato dextrose agar medium and replicated for each sample. The dishes are incubators were then incubated at 25 ° C for 2-8 days. The developing colonies were then examined and a separate transfer was carried out Each on the center of Potato dextrose agar and Sabourod Potato dextrose agar.

Demonstration of colonies

The development of the colony on the flat medium (flat, high, and hawkish), and the color of the colony from the opposite side, and the development of the colony for a powdered ablution , Gonorrhea, or cotton, as well as the diameter of the colonies at the time of growth and formation of blackboards, the adoption of the above steps is a key factor in the diagnosis of fungi, based on [8].

Microscop of colonies

As a result of this examination to study the microscopic properties of fungi and their forms and their branches, and followed the two methods in the microscopy examination:

1- wet method

Take part of the colony by needle from a position between the center of the colony and its surroundings and then transfer it to a glass slide containing a drop of actophenol dye, then place the slide cover over it, and gently press the lid to spread the fungus and fungal structures and test the 40X magnification strength[9].

2- Adhesive Preparation

A transparent adhesive tape was applied after touching him to the surface of the fungal colony, and the tape was attached to a glass slide containing a drop of actophenol dye. The slide was then examined on a 40X magnification strength (10**)**

Preparation of the fungal extract

After the incubation period, a sterilized environment was performed to work on. The filtration fluid the myslem that prepared was then separated into the filter unit of 0.45Mm Nalgene and centrifugal the filter using the centrifuged 15 minutes in rpm1000. The filter was then collected again using the Nalgene filter 0.45Mm The fungal extract was placed in tubes, and the tube stopper was flattened with a waxy adhesive tape(11).

The lipholizerapparatus

After extracting the fungal extract, freeze drying was performed using the Lypholizer device. The test was carried out at 70 ° C for a period of time until dry powder was obtained(12**).**

Test the inhibitory activity of fungal metabolic compounds of Staphylococcus.

The diffusion method was followed by drilling. Perez et al. (1990) as follows:

1. Pour 25 mL of nutritious into each dish.
2.In vitro fertilization was carried out 0.1 by a sterile diffuser of the bacterial culture containing 1.510 cells / ml, compared with a standard fixed turbidity solution, and then left to dry at room temperature
3-A 6 mm hole in the planted center was made using a sterile filament drill.
4-Add 0.05 mL of graduated concentrations prepared for fungal metabolic compounds using accurate sterile pipette.
5-Repeat each dish, then incubate the dishes at a temperature of 7 ° C for 24 hours in the incubator, determine the effectiveness of each concentration of the metabolite fungal compound with the measurement of inhibition diameter (zoon Inhibition).

Results and discussion

The results of the microscopic and biochemical tests showedtate ther were 100 isolates of thebelong to the genus *Staphylococcus aureus* fromn 225 samples.

The results showed that the number of samples showing bacterial growth on the mannitol salt agar100, which is a selective medium for Staphlococcus because it contains a high concentration of salts (7.5% NaCl) while growth of ther bacteria species was inhibits their growth (13) .. Staph. aureus has the ability to turn the color of the medium from pink to yellow because it ferments the mannitol sugar in the media, causing a chromatic change in the medium (14)**.**

Cultural characteristics

staph. aureus grow rapidly on most bacteriological media. Colonies on solid media are golden yellow,

ISSN: 1813 – 1662 (Print)
E-ISSN: 2415 – 1726 (On Line)

smooth, raised and glistening (13) Mannitol salt agar commonly used as a screening device for *S. aureus* (14). In the present study the samples obtained from pateints were cultured on mannitol salt agar to screen the presence of *S. aureus* among different clinical casesin, the formation of a yellow coloneis with a yellow area of fermentation a*staph*round it as shown in figure 4-1 is a presumptive identification of the genus. From the 100 samples, 73 samples gave growth of yellow coloneis with yellow zones which were selected for the for Also blood agar is frequently used as a universal enrichment media, for most human bacterial pathogens (15). *Staph. aureus* on blood agar appear as circular, smooth, yellow to golden colonies.

Microscopical examination

Prepared smears of *Staphyloccocus* isolates appeared as purple single, diplo, and grape like Gram positive cocci under light microscope. Young cocci stain strongly gram positive; on aging, many cells become gram negative [16].

Biochemical identification

Catalase test

Catalase test determines if the organism produces the enzyme catalase that breaks down hydrogen peroxide (H_2O_2) into water and oxygen. The staphylococci produce catalase, which differentiates them from the streptococci (17).

DNase test

DNase test is the base for detection of deoxyribonulease activity of microorganisms. Plates of DNase agar were inoculated with culture isolates, incubated at 37^{o}C for 24-48 hours. After incubation, the plates were examined for deoxyribonucleases (DNase) activity by flooding colonies of test culture isolates on agar with (1N) HCl to precipitate un-hydrolyzed deoxyribonucleic acid (DNA). Presence of DNase was indicated by clear zone surrounding a colony of culture DNase positive(17) mentioned that all of their *Staph. aureus* isolates were DNase positive.

Coagulase test

The enzyme coagulase produced by few *Staphylococcus* species is a key feature for pathogenic *Staphylococci.* This enzyme produces coagulation of blood, allowing the organism to "wall" itself from the host protective mechanisms(18). This test differentiates *S. aureus* from other species of *Staphylococc i.*

Antbiotic resistancs

The antibiotic were tested for resistance to 10 antibiotics. The bacteria showed high resistance to PenicillinG (94), Tobramycin (40%), Amikacin (39%), NitroFurantion (38%) Tetracyclin (36%), ,Novobiocin(24%), Trimethoprim(23%), Gentamycin (13%), Chloromphenicol (10%), Imipenem (3%).

ISSN: 1813 – 1662 (Print)
E-ISSN: 2415 – 1726 (On Line)

Table (1) The inhabition zoon of some antbiotic

bacterria	C		NIT		TM		TN		IMI		NIV		GEM		PG		T		AK		Nambar total
	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	Nambar resistance	
Staph.aurus	15	8	38	20	15	8	34	18	99	1	0	0	96	5	98	51	42	21	42	22	52
Staph.saprophytic	56	14	20	5	52	13	4	1	0	0	100	25	16	4	100	25	17	9	48	12	25
Staph.lentus	0	0	100	1	0	0	100	1	0	0	0	0	0	0	100	1	0	0	0	0	1
Staph.xylosus	71	1	50	7	57	8	13	9	0	0	0	0	21	3	100	14	50	7	42	6	14
Staph.haemolyticusi	0	0	25	1	0	0	25	1	0	0	0	0	0	0	100	4	50	2	25	1	4
Staph.sciuri	50	1	50	1	50	1	100	2	0	0	0	0	50	1	100	2	50	1	100	2	2
Staph.caseolyticus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2

Tobramycin =TN ,AK=Amikacin,(NitroFurantion=NIT Tetracyclinn=T)NIV=Novobiocin TM=Trimethoprim Gentamycin, Chloromphenicol = C ,Imipenem=IMI

Diagnosis of fungi producing the metabolic compounds

After obtaining the fungal species producing the metabolic compounds, they were then developed in the agricultural areas Sabouraud dextrose agar Potato dextrose agar After the emergence of the colonies, the different isolates were selected in size, color and shape and were developed to obtain colonies that were pure and preserved in tubes and plates containing a PDA medium at 4 ° C until completion of the diagnosis preserved in tubes and plates containing a PDA medium at 4 ° C until completion of the diagnosis It is observed that when farms are older growth, the posterior areas are less visible because new conidoid colonies are outside the conical ring region. The isolates of the disjointed shape can observe the appearance of the ring areas at the modern growth stage only. (18)

The color of the colonies is due to the pigments found in the germs Phialospores. In addition, there are other factors that affect the color of the plant, including the amount of germs produced or formed that do not know the color of the colony is dark or light, some isolates produce secretions of pigments, The pH of the medium,dye the environment, The growth of the colony also affects its color (19).

Diagnosis the fungus *Trichoderma koningii*

Most species of Trichoderma breed rapidly grow on the industrial food environment, producing many kinds of small conidic green or white bacteria from cells called Conidi-ogenous located at the end of the many confessional counties, but identifying species is a difficult process very, Since there is considerable overlap between species characteristics, and it is difficult to establish the specific name of species only after extensive study[20].

The species of the sex based on the shape and color of the colony, and appeared colonies of fungi T.koningii when developing on the center of the SDA green color and soon become olive green color with the progress of the age of the colony, while the type of microscopic examination if found that the innate yarn is divided and the emergence of conidies and ends with spherical vesicles However, in this type, the branches appear thick and short and tend to get out of the rack in a wider angle than those in the Verticillium mushroom. In this group of isolates, the system of laying of the Quindian pregnant women is simple and less regular. The offspring are less contiguous. These qualities are apply perfectly of the fungus, which he described T.Koningii.[21].

diagnosis Fungus *Trichoderma viride*

After the diagnosis of genus fungus, the species was identified through phenotypic examination, if the colonies of the T.viride mushroom grow very rapidly if they are initially transparent and then become green. The microscopic examination shows that the conidiophores of the fungus branch out profusely in pyrimidal fission, If the short branches are close to the top, while their longer branches are in the lower part of the colony base and the branch is usually angular (Domsch et al., 1980)), pregnant women with large branches of this species carry Phidalides either individually or in groups, These condiments consist of sticky wet condid spores [22]. Conidical spores produced by T.viride fungi are large, spherical, translucent, and transparent with a diameter of 4.5-3.6 µm. The wall is usually rough (Pitt and Hocking, 1997; Rifai, 1969), that these qualities fully apply to the taxonomic characteristics of the T.viride mushroom described by[23].

Preparation of fungal extracts of fungi isolated from soil

The process of preparation of fungal extracts was developed after the development of fungal isolates in 200 ml of liquid yeast extract at 25 °C and for 72 hours in the shaking incubator at 150 cycles / min. The liquid produced is separated from the filter using filtration through the Nalgene filter unit as in[24].

The size of the leachate produced from the isolates *T koningii* and 150,100 *T.viride* milliliters respectively, and the weight of the biomass caused by toxicity was 3.67, 4.20 g, respectively, the difference in the amount of biomass in the fungal isolates is produced from different The ability of fungal isolates to grow in the center of Yeast Extract Broth.

The inhibitory effect of fungal metabolic compounds against the most resistant bacteria Staphylococcus to antibiotics

Results showed that the inhibitory effect of T.koningii fungi increased with the increased of used concentration rate, the inhibitory diameter reach the highest range to Staph.aure bacterial in case of use the concentration of 100 mg / ml 18 mm. In the case of concentration of 150 mg / ml, the damping diameter was 24 mm while the inhibitory strength increased to 26 mm in case of use The concentration is 250 in the case of bacterial strain Staph. Saprophyticus e Concentration status of 100 mg / ml The diameter of the inhibition was 16 mm. If the concentration was 150 mg / ml, the inhibitory rate was 20 mm, while the inhibitory capacity increased to 24 in the case of use concentration 250, in the case of Staph. Epidermidis was the highest inhibition in the case of use concentration of 100 mg / ml 10 ml. In the case of use concentration of 150 mg / ml, the inhibition diameter was 12 ml, while the inhibitory capacity increased to 14 ml if concentration was used. 250 other bacterial species were inhibited as shown In the table(3).

The inhibitory effect of the metabolic compounds to fungi of T. Koningii demonstrated a high inhibitory effect against Staphylococcus bacteria. This can be attributed to the efficacy of these secondary metabolites to break peptide bonds in the prokaryotic ribosomes of proteins [25].

The metabolic compounds of fungi T.viride there effect were also based on the concentration of the extract with the highest rate of inhibition diameter of Staph.aure bacterial.

In the case of concentration use 100 mg / ml 18 mm. If the concentration of 150 mg/ml was used, the diameter of the inhibition was 24 mm while the inhibitory capacity increased to 26 mm, In the case of bacterial type of Staph. Saprophyticus in the case of the use of concentration 100 mg / ml 16 mm, while in the case of the use of 150 mg / ml concentration, the diameter of the inhibition was 16 mm while the inhibitory capacity increased to 18 mm in case of concentration 250, Either in the case of bacterial type Staph. Epidermidis was the highest diameter inhibition in the case of use concentration of 100 mg / mL 10 ml. In the case of use concentration of 150 mg / ml, the inhibitory diameter was 12 ml while the inhibitory capacity increased to 14 ml in the case of use concentration 250 and other bacterial species were inhibited as shown in table(4).

In a study on the effect of secondary metabolites of fungus *T.viride* on five types of plant pathogens, it was observed that the fungus had the ability to inhibit the efficacy of fungal pathogens. These results were attributed to the ability of *fungus T.viride* to secrete compounds with inhibition ability of pathogenic microorganisms[26].

Table (2) the inhibitory efficacy of *Trichoderma koningii* extract on Staphylococcus specis

***Trichoderma koningi*i Concentration of fungus**			Source samples	Samples	
250	150	100			
20	18	14	wound	*Staph.aureus*	1
18	14	10	Urin	*Staph. aureus*	2
16	12	10	Urin	*Staph. aureus*	3
22	18	16	wound	*Staph. aureus*	4
18	14	12	brun	*Staph. aureus*	5
14	10	8	wound	*Staph. aureus*	6
26	24	18	brun	*Staph. aureus*	7
22	18	12	brun	*Staph. aureus*	8
14	12	10	wound	*Staph. aureus*	9
18	16	14	chronic otitis media	*Staph. aureus*	10
20	16	14	pharynx infections	*Staph. aureus*	11
18	14	12	Wound	*Staph. aureus*	12
16	14	10	chronic otitis media	*Staph. aureus*	13
14	12	10	pharynx infections	*Staph. aureus*	14
16	14	10	Urin	*Staph. saprophyticus*	15
18	16	14	Urin	*Staph. saprophyticus*	16
14	12	10	brun	*Staph. saprophyticus*	17
16	14	12	Urin	*Staph. saprophyticus*	18
14	12	8	chronic otitis media	*Staph. saprophyticus*	19
24	20	16	Urin	*Staph. saprophyticus*	20
18	14	12	Urin	*Staph. saprophyticus*	21
18	14	10	Urin	*Staph. Saprophyticus*	22
16	14	12	Urin	*Staph. saprophyticus*	23
20	16	14	Urin	*Staph. saprophyticus*	24
16	12	10	wound	*Staph .haemolyticus*	25
14	10	8	wound	*Staph .haemolyticus*	26
24	20	16	Urin	*Staph .haemolyticus*	27
20	16	14	Urin	*Staph . haemolyticus*	28
14	12	10	Urin	*Staph. lentus*	29
16	14	10	chronic otitis media	*Staph. xylosus*	30
18	16	14	chronic otitis media	*Staph. xylosus*	31
18	14	10	brun	*Staph. xylosus*	32
18	16	12	Urin	*Staph. sciuri*	33
18	14	12	Urin	*Staph. caseolyticu*	34

Table 3: The inhibitory efficacy *ofTrichoderma viride* extract on bacterial isolates

Concentration of fungus *Trichoderma viride*			Source samples	Samples	
250	150	100			
18	16	12	wound	*Staph.aureus*	1
16	12	8	Urin	*Staph. aureus*	2
14	10	8	Urin	*Staph. aureus*	3
16	14	10	wound	*Staph. aureus*	4
18	16	14	brun	*Staph. aureus*	5
14	12	10	wound	*Staph. aureus*	6
24	20	18	brun	*Staph. aureus*	7
20	18	12	brun	*Staph. aureus*	8
12	10	8	wound	*Staph. aureus*	9
20	16	14	chronic otitis media	*Staph. aureus*	10
18	14	12	pharynx infections	*Staph. aureus*	11
18	14	12	Wound	*Staph. aureus*	12
14	12	10	chronic otitis media	*Staph. aureus*	13
12	10	8	pharynx infections	*Staph. aureus*	14
18	16	12	Urin	*Staph. saprophyticus*	15
12	10	8	Urin	*Staph. saprophyticus*	16
12	10	8	brun	*Staph. saprophyticus*	17
14	12	10	Urin	*Staph. saprophyticus*	18
12	10	8	chronic otitis media	*Staph. saprophyticus*	19
16	14	12	Urin	*Staph. saprophyticus*	20
14	12	10	Urin	*Staph. saprophyticus*	21
18	14	12	Urin	*Staph. Saprophyticus*	22
14	12	10	Urin	*Staph. saprophyticus*	23
16	14	12	Urin	*Staph. saprophyticus*	24
12	10	8	wound	*Staph .haemolyticus*	25
14	12	10	wound	*Staph .haemolyticus*	26
22	18	14	Urin	*Staph .haemolyticus*	27
18	14	12	Urin	*Staph . haemolyticus*	28
18	16	12	Urin	*Staph. lentus*	29
16	12	10	chronic otitis media	*Staph. xylosus*	30
18	14	12	chronic otitis media	*Staph. xylosus*	31
12	10	8	brun	*Staph. xylosus*	32
18	14	10	Urin	*Staph. sciuri*	33
16	14	10	Urin	*Staph. caseolyticu*	34

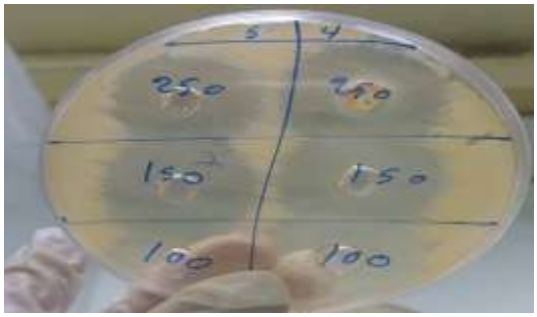

Figure (1-1) Effect of differen concentrations of *Trichoderma koningii* and *Trichoderma viride* by products on staph. auerus grown on muller-Hinton agar

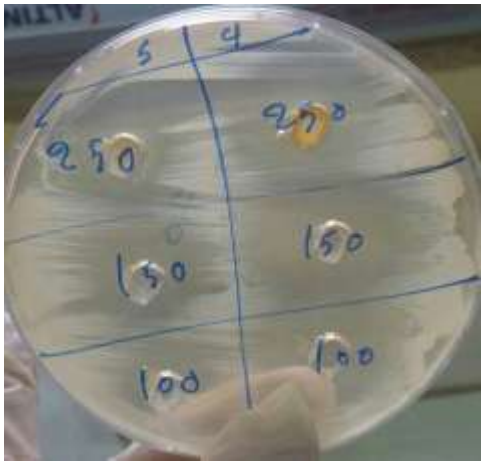

Figure (1-2) Effect of differen concentrations of *Trichoderma koningii* and *Trichoderma viride* by products on staph.saprophyticusgrow on muller-Hinton agar

References

1-Al-Jebouri MM and Mdish SA. (2013). Antibiotic Resistance Pattern of Bacteria Isolated from Patients of Urinary Tract Infections in Iraq. *O.J.U*. 3(2).

2- **Klevens RM, Morrison MA, Nadle J, Petit S, Gershman K, *et al*. (2007).** Invasive methicillin-resistant *Staphylococcus aureus* infections in the United States. *JAMA*. 298: 1763-1771.

3-Farah CS, Lynch N, Mc Collough MJ(2010) Oral fungal infection : An update for general practitioner. Aust. Dent .J (sup. 1) 48-54.

4-Parisien A, Allain B, Zhang J, Mandeville R, Lan CQ (2008) Novel alternatives to antibiotics: bacteriophages, bacterial cell wall hydrolases, and antimicrobial peptides. J Appl Microbiol 104: 1–13

5-Hussain H, Krohn K, Ahmed I, Draeger S, Schulz B, Pietro S, et al. (2012) Four new pyrenocines from an endophytic fungus, Phomopsis sp. Eur J Org Chem , 1783-1789.

6-Holt, J.; Krieg, N.R.; Sneath, P.; Staley, J. and William, S. (1994). Bergys Manual of determinative bacteriology. 9th ed. William and Wilkins publication, New York.

7-Harley, J.P. and proscott, L.M. (1996). Laboratory Exercises in Microbiology, 3rded. WCB/ Mc Graw – Hill.

8-Koglin, A.; Walsh, C.T.(2009). Structural insights into nonribosomal peptide enzymatic assembly lines. Nat. Prod. Rep. 26, 987-1000.

9-Koneman, E.W.; Allen, S.D.; Juda, W.M.; Schreckenberger, P.C. and Winn, W.C. (1997). Color Atlas and Text book of diagnostic microbiology. 5th ed. Lippincott- Raven puplishers, Philadelphia, USA

10-Rashan, I.J.; Abed, A.A. and Aziz, A.A. (1992). Further observation on the pharmacological activities of the aqueous extract of Aristolochia bottae stems. Fitoloerapial XIII; 4:350-352.

11-Morello JA, Granato PA and Mizer HE. (2003). Laboratory manual and workbook in Microbiology applications to patient care. 7th ed., McGraw-Hill company. New york, USA.

12-Heilmann, C. and Peters, G.(2000). Biology and pathogenic-city of *Staphylococcus* epidermidis- D.C: American society for Microbiology .4:442-449.

13-Linda, P.; Rosalind, M. and Julie, M. (1985) *Staphylococcus saprophyticus* as a urinary pathogen: a six year prospective survey. *British Medical Journal.* 26.(291):1157-1159.

14-Brooks GF, Carroll KC, Butel JS, Morse SA and Mietzner TA. (2014). Jawetz, Melnick, & Adelberg's Medical Microbiology. 26th ed. McGraw-Hill Companies. USA. Pp: 200- 205.

15-Mahmood LJ. (2013). Molecular Study on *Staphylococcus aureus* Causing Burn Wound Infections. M.Sc. thesis. Hawler Medical University. Erbil, Iraq.

16-Brooks, G.F., Butel, J.T., Morse, Jawetz, (2001) Melinck Adelbergs medical Microbiology. 20th ed. Lang medical books – McGraw- Hill. USA.,197-203.

17-Smith S. A., Directory of Services, Path Group, Associated Pathologists, Virginia, USA, 2009

18-Hovelius, B.; Collen, S. and Mard, P.A. (1984). Urinary tract infections in men caused by Staphylpcoccus Saprophyticus Scand. J. Infect. Dis. 6: 328 – 326

19-McMullin D. Roderick (2014) Structural characterization of secondary metabolites produced by fungi obtained from damp Canadian bulildind. A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of PhD. Ottawa-Carleton Institute of Chemistry .Carleton University.Ottawa, ON, Canada.

20-Harsukh Gajera*, Rinkal Domadiya, Sunil Patel, Mansukh Kapopara, Balubhai Golakiya(2013) Molecular mechanism of *Trichoderma* as bio-control agents against phytopathogen system – a review . Current Research in Microbiology and Biotechnology Vol. 1,

21-Watanabe , T . (2002) . Pictorial atlas of soli and seed fungi : morphologies of cultured fungi and key to species . 2nd . ISBN 0-8493-1118-7.

22-Domsch, K. H. ; Gams, W. and Anderson, T. H (1980). Comependium of Soil Fungi. Vol. 1, Academic Press, London, U.K. 859 pp.

23-Almassi F, Ghisalberti EL, Narbey MJ, Sivasithamparam K. (1991) New antibiotics from strains of *Trichoderma harzianum*. J Nat Prod 1991; 54: 396-402.

24- Kalpana Devi, M., Rasheedha Banu, A., Gnanaprabhal, G.R., Pradeep, B.V. and Palaniswamy, M. (2008). Purification characterization of alkaline protease enzyme from native isolate *Aspergillus niger* and its compatibility with commercial detergents. Indian J. of Sci. and Tech; 2008: Vol.1, No 7.

25-Hameed, Nazeeh Maher. (2010). In Vitro Evaluation of the Activity of *Aspergillus niger* Extract on the Viability of *Staphylococcus aureus* and Protoscolices of Hydatid cysts.

26-Carrasco, L., M. Barbacid and D. Vizques (1973), The *Trichoderma* group of antibiotics , Inhibitors peptide bond formation by Eukaryotic Ribosomes. *Biochimica et Biophysica Acta*, 312 , 368-376.

Tikrit Journal of Pure Science 22 (11) 2017 *ISSN: 1813 – 1662 (Print)*
E-ISSN: 2415 – 1726 (On Line)

تحديد الفعالية التثبيطية للمستخلصات الاحيائية تجاه بعض انواع بكتريا Staphylococcous المتعددة المقاومة للمضادات الحيوية والمعزولة من مصادر سرسرية مختلفة

رحاب سلمان كردي[1] ، محمد نظير معروف[2]

[1]قسم علوم الحياة ، كلية العلوم ، جامعة تكريت ، تكريت ، العراق

[2]قسم علوم الحياة ، كلية التربية ، جامعة تكريت ، تكريت ، العراق

الملخص

تضمنت الدراسة جمع 225 عينة من المرضى الراقدين في مستشفى تكريت العام خلال فترة تراوحت من9/1الى 12/15 سنة 2016.تضمنت العينات مسحات من الحروق والجروح وعينات الادرار والأذن الوسطى والبلعوم .تم تنمية العينات على اكار المانتول والذي يعتبر وسط انتقائي وتفريقي للمكورات العنقودية وأظهرت النتائج عزل100 عزلة (44.4%) د. ثم اعتمادا على الخصائص المظهرية والاختبارات الكيموحيوية تم تشخيصها على مستوى النوع اذا تم تشخيص انواع بكتريا *Staph. aureus* ,*Staph.Saprophyti s Staph. haemolyticusi* , *Staph. Xylosus Staph. Lentus caseolyticus* , *Sciuri Staph. haemolyticusi* .*Staph* .وتم فيما بعد اختبار انتاجها لعدد من الانزيمات مثل انتاجها لإنزيمات اختزال النترات والهيمولاسين وDNase حيث اظهرت العزلات القدرة على انتاج هذة الانزيمات.

اختبرت قابلية العزلات البكترية على مقاومة 10 مضادا حيويا وجد ان البكتريا اظهرت مقاومة عالية لمضاد PenicillinG (94), Tobramycin (40%), Amikacin (39%), NitroFurantion (38%) Tetracyclin (36%), Novobiocin (24%), Trimethoprim (23%), Gentamycin (13%), Chloromphenicol (10%), Imipenem (3%).,كذلك تم تشخيص نوعين من الفطريات وهي *Trichoderma koningii* و*Trichoderma viride*,المنتجه للمركبات الايضية واختبار فعاليتها التثبيطية بعد استخلاصها تجاه انواع بكتريا Staphyloccus , بينت الفعالية البايلوجية لكل من التراكيز 100,150,250ملغم/مل فعالية تثبيطية ضد انواع بكتريا Staphyloccus,كما تبين ايضا انه كلما ازداد تركيز المستخلص ازدات الفعالية التثبيطية .كما بينت النتائج ان بكتريا Staph.aurea هي الاكثر تأثرا بالمركبات الفطرية اذا كانت اعلى نسبة تثبيط عند التركيز 250ملغم /مل 26ملم في حالة المستخلص *Trichoderma koningii* في حين بلغت نسبة التثبيط عند نفس التركيز 24مل في حالة المستخلص *Trichoderma viride*.