ANTIBATERIAL ACTIVITY OF (*HERICIUM ERINACEUS)* EXTRACT ON SOME CLINICAL PATHOGENIC ISOLATES

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ABSTRACT

The purpose of this research was to find the effect of alcoholic extract of the lion's mane mushroom (*Hericium erinaceus*) against certain clinical pathological isolates isolated from Iraqi patients suffering from various diseases such as gingivitis, urinary tract infection, diarrhea and severe vomiting, and also a biopsy was taken from the stomach of people suffering from ulcers. Out of 150 males and females sample 107 samples were isolated and identified by chemical detection and VITEK 2 technique, and by PCR for *Halicobacter pylori* as follows: *Staph aureus*(56),*Salmonella typhi(*6),Pseudomonas aeruginosa(10),*Escherichia coli*(15),*Halicobacter pylori*(10),*Klebsiella pneumonia*(10),*Candida albicans*(10) samples. lion's mane mushroom powder was extracted with 80% ethyl alcohol, and active components were investigated by chemical reagents and Gc-mass. The chemical detection of Alcoholic extract revealed the existence of flavonoids, alkaloids, phenols, terpenoids, glycosides, and polysaccharides. The efficacy of the extract was tested using 4 concentrations (400, 200, 100, and 50 mg/ml) on bacterial and yeast isolate (*C.albicans*).The diameter of the inhibitory zone increased with increasing extract concentrations, according to the findings. Zone of inhibition for all isolates started at 100mg/ml except *C.albicans*(50mg/ml)and *E.coli* (200mg/ml)

Key words: chemical detection, mushroom ,sensitivity test, natural products

الفعالية المضادة للبكتيريا لمستخلص *Hericium erinaceus* على بعض العزلات الممرضة السريرية

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المستخلص

ان الغرض من هذا البحث هو إيجاد تاثير المستخلص الكحولي لفطر عرف الأسد (*Hericium erinaceus*) ضد بعض العزلات المرضية السريرية المعزولة عن مرضى عراقيين يعانون من أمراض مختلفة مثل التهاب اللثة والتهاب المسالك البولية والإسهال والقيء الشديد كما تم أخذ خزعة من معدة الأشخاص الذين يعانون من القرحة. من بين 150 من الذكور والإناث عينة تم عزل 107 عينة وتحديدها بواسطة تقنية الكشف الكيميائي Api20 وتقنية VITEK2، وعن طريق تفاعل البوليميراز المتسلسل لـ *Halicobacter pylori* على النحو التالي: (56) *Staph aureus*، (6) *Salmonella typhi*، *Pseudomonas aeruginosa* (10)، *Escherichia coli* (15)، *Halicobacter pylori* (10)، *Klebsiella pneumonia* (10)، *Candida albicans* (10)عينات. تم استخلاص مسحوق فطر عرف الأسد مع 80٪ من الكحول الإيثيلي ، وتم فحص المكونات النشطة بواسطة الكواشف الكيميائية وتقنية Gc–Mass .بين الكشف الكيميائي للمستخلص الكحولي عن وجود مركبات الفلافونويد والقلويدات والفينولات والتربينويدات والجليكوزيدات والسكريات. تم اختبار فاعلية المستخلص باستخدام 4 تراكيز (400 ، 200 ، 100 ، 50 ملغم / مل) على العزلات البكتيرية والخميرة (*C.albicans*)، ازداد قطر المنطقة المثبطة مع زيادة تركيزات المستخلص حسب النتائج. بدأت منطقة التثبيط لجميع العزلات عند 100 ملغم / مل ما عدا المبيضة البيضاء (50 ملغم / مل) والإشريكية القولونية (200 ملغم / مل).

الكلمات المفتاحيه: الكشف الكيميائي، فطر، اختبار حساسيه، منتجات طبيعيه.

Received:13/8/2021, Accepted:7/11/2021

INTRODUCTION

Mushrooms are regarded as nutrient-dense meals that also serve as a source of physiologically useful drugs. The edible fungus *H.erinaceus*, Hedgehog Mushroom, also known as Lion's Mane Mushroom, has a long history of usage in traditional Chinese medicine. (12)

Mushrooms are thought to be healthful due to t heir low calorie, salt, fat, and cholesterol conte nt. As a result, they create an alliance a crucial component of a population's diet afflicted with atherosclerosis(4) *H. erinaceus* is a fungus that can be eaten,has been demonstrated to improve the effects of a number of biological processes that are commonly utilized to treat chronic superficial gastritis(15), has established itself as a strong contender in the promotion of brain and nerve health-related activities. (22). The fungus *H. erinaceus* is well-known for its therapeutic properties,with several bioactive components that have been turned into food supplements and alternative treatments.(5) Medicinal plants have long been known for their antioxidant properties, and they have long been regarded as a rich source of antibacterial agents(8). Mushroom fruit bodies, mycelia, and bioactive pure components have been shown to exhibit antibiotic, anticarcinogenic, antidiabetic, antifatigue, antihypertensive, antihyperlipodemic, antisenescence, cardioprotective, hepatoprotective, nephroprotective, and antisenescence properties.(5) Various pathogenic organisms have developed resistance to many commercially available antibiotics . As a result, several studies have been conducted to improve and focus on the pharmacological properties of plants and their parts as alternative sources for many synthetic therapeutic medications.(10) Due to its great prevalence in global populations, frequent recurrence, and rapid evolution of drug-resistant strains, *Helicobacter pylori* infection is one of the most serious public health concerns. numerous experiences have proven that *H. pylori* was inhibited by the c ethanol extracts of the fungus *H. erinaceus.* (16) Several studies discovered that extracts from mushrooms, as well as extracted pure mushroom chemicals, had antibacterial properties against foodborne pathogens like *E. coli* and *Salmonella*,(13) Methicillin-resistant MRSA, or *Staphylococcus aureus*, is one of the most common organisms in nosocomial infections. MRSA infections are a serious problem for hospitalized patients because hospital-acquired MRSA strains are resistant to a variety of medications and transfer from patient to patient via hospital personnel' transiently contaminated hands. Anti-MRSA action was discovered in extracts of the fruiting bodies and mycelia of *H. erinaceus*(11) Natural cures are currently experiencing a rebirth of interest in many regions of the world. Natural goods or traditional medicines (or alternative medicines), according to a vast number of researchers, are a promising source of new treatments Natural goods are chemical compounds or chemical found in nature that are created by living organisms as well as possessing pharmacological or biological qualities . In the creation and design of pharmaceutical medications, natural components are frequently utilized A variety of natural ingredients can be used to treat life-threatening disorders .The prophylactic and therapeutic capabilities of a variety of natural things against life-threatening diseases have been well-documented..(12). The bioactive polysaccharide has been identified in these research. Other bioactive chemicals with low molecular weight have also been discovered.(1) Proteins, polysaccharides, lipopolysaccharides, and glycoproteins are among the mushroom metabolites that have been identified as potent immunity-boosting compounds Mushrooms also make and store a number of low-molecular-weight secondary metabolites, such as phenols, polyketides, and terpenes, which are useful medicines. Unlike many existing chemotherapeutic anticancer drugs, polysaccharides from mushrooms have recently been found to have significant medical qualities and no hazardous side effects in a number of trials.(23).

MATERIALS AND METHODS

Mushroom collection

the powder of *H. erinaceus* fruiting bodies were provided from DXN pharmaceutical firm (Malaysia), Baghdad, Iraq, The alcoholic extract of *H.erinaceus* was made by

combining 10 grams of mushroom powder with 80 percent ethanol and extracting for 24 hrs at room temperature. Centrifugation (2,500 g, 5 min, room temperature) was used to collect the supernatant then alcohol was vaporized and the precipitate was kept in refrigerator until use(9).

Extraction of mushroom

The powder (ten grams) was mixed with 80% ethanol and agitated by magnetic stirrer. The extract was filtered with a vacuum through filter paper(whatman No.41), and placed in the oven at 30º C degrees for 24 hours. The extract was kept at –20°C until it was needed.(18)

Detection of active compounds

Detection of alkaloids :Dragendorff test: In 0.2 mL HCl, 60 mg Bismuth sub-nitrate were dissolved (solution A). In 1 mL Distell water, 600 mg potassium iodide (solution B)is present. An orange to brown tint indicates the presence of alkaloids when the solution [A + B] is combined and added to the extract. (1)

Detection of poly saccharides : 1 mL extract was mixed with 2 mL Benedict reagent in a liquate, which was then placed in a boiling bath for 5 minutes and allowed to cool. The presence of polysaccharides was shown by the crimson deposit. (21)

Detection of flavonoids

Alkaline reagent test: a vivid yellow color is formed in the presence of flavonoids when a drops of 3 percent ferric chloride solution are added to the extract solution. (7) sodium hydroxide solution is mixed with a small volume of extract and left.(17)

Detection of poly phenolic compound: A brown deposition will appear when a few drops of 3 percent ferric chloride solution are added to the extract . (7)

Detection of tannins tests: The extract was spiked with a few drops of a 1 percent Lead acetate solution. The presence of tannins was detected by the formation of a gelatinous or white precipitate.(3)

Detection of terpens:

Two milliliters of chloroform and one milliliter of mushroom extract were combined with four milliliters of acetic acid anhydride and one milliliter of concentrated sulfuric acid (H2SO4). The appearance of pink tint suggested the existence of terpens(20)

Detection of saponins: A white precipitate appeared when 5 mL of mushroom extract was combined with 3 mL of mercuric chloride (1%) solution, indicating the presence of saponins.(22)

Gas chromatography-mass spectroscopic analysis: The mushroom powder was extracted with ethanol and examined with a GC-MS analyzer (GC Clarius 500 Perkin Elmer) In the Ministry of Science and Technology (Ibn Al-Bitar Department). A 30 0.25mm 1mdf Elite-1 (100 percent Dimethyl poly siloxane) column was used to gather the data.The carrier gas in the split mode was helium (99.999 percent) at a flow rate of 1ml/min (10:1). With the injector temperature set to 250oC, an aliquot of 21g of the sample's ethanol solution Injections were made into the column. The Temperature in the GC oven was started at 110°C and held for 2 minutes before being increased to 200°C at a rate of 10°C/min without holding. It was permitted to hold at 280°C for 9 minutes at a rate of 5°C/min. The injector and detector were both heated to 250°C and 280°C, respectively. The ion source was kept at a constant temperature of 200°C. To get the mass spectrum of compounds in samples, electron ionization at 70 eV was utilized, and the detector was operated in scan mode from 45 to 450amu (atomic mass units). A scan period of 0.5 seconds was maintained, with pieces ranging from 45 to 450 Da. The film lasted 36 minutes in total.(19)

Bacterial sample collection

Oral swabs were collected from people suffering from gum infections and diabetes, as well as swabs from people suffering from ulcers. Samples were collected by sterile swabs containing a nutrient medium, and then the samples were cultured on different media to identify the types of bacteria urinary tract infections and severe diarrhea. A biopsy was taken from the stomach and duodenum for people suffering from stomach

Identification of bacteria

The isolates were identified depending on chemical detection and VITEK 2 technique, and PCR for *H.pylori*

Disc diffusion susceptibility method

Microbial inoculum cultures for 24 hours a standardized inoculum (0.5 McFarland Standard) ,different type of bacteria were

cultured on the Muller Hinton agar. With the use of a sterile forceps, No. 1 Whatman filter paper discs (diameter: 6 mm) were placed on the media, and mushroom extract concentration 400g were dissolved in 5ml of DIMSO to make serial two-fold dilution of the extract(400,200,100,50)mg ,were applied to the discs in amounts of 10ml . Media plates were incubated at 37°C for 18 to 24 hours in an incubator. The chemicals seeped into the agar material from the discs. , inhibiting bacteria (if sensitive) from growing in the zone of inhibition around the disc. Each treatment's zones of inhibition were calculated the next day Bacteria were found to stop growing at the lowest quantities, (26).

RESULTS AND DISCUSSION

Chemical detection of active compounds in *Hericium erinaceus* : According to the chemical detection of *H.erinaceuse* ethanolic extract, it was appeared that the main active chemical constitutes (secondary metabolites), as shows in Table (1),All chemical detection positive result except saponins(negative result) this is because saponins are abundant in roots ,stems and leaves(14)these results are consistent with Previous studies showed that the chemical constituents of *H. erinaceus* include terpenoids, phenolics, steroids, pyranones, fatty acids, and alkaloids; thereinto (25)

Table 1. Chemical detection of some active Compounds in *Hericium erinaceus* extract

Active compound	REAGENT	RESULT
Tannins	Lead Acetate !%solution	++ve
Polysaccharides	Benedict reagent	+ ve
Alkaloids	Dragandroff reagent	+ve
Glycosides	Ked's reagent	+ve
Terpens	Four ml of acetic acid anhydride and 1 ml of concentrated sulfuric acid (H2SO4) added to 2 ml of chloroform	+ve
Saponins	mercuric chloride (1%) solution,	-ve
Flavonoids	Alkaline reagent(NaOH)	+ve
Polyphenoles	ferric chloride 3%solution	+ve

GC-MS detection

Alcoholic Extract of *Hericium erinaceus* was subjected to GC – MS study for Compounds that are active are identified, In the alcoholic extract of *H.erinaceus* 9 compounds have been identified as shows in Table 2 based on mass compound interpretation (GC- MS) The research was carried out with the help of the data repository at the National Institute of Standards and Technology (NIST) . The spectra of unknown components were compared to those of recognized components in the NIST library .*H.erinaceus* identification was determined using the active principle, Molecular Weight (MW), Concentration (percentage) Peak Area, Retention Time (RT), and Molecular Formula (MF) (PA).Some of these chemicals have already been identified by previous research.

As for the GC analysis of *H. erinaceus*(figure 1): Propanamide, N-acetyl- Oxirane, (butoxymethyl)- Succindialdehyde (39.23%)**,** Oxirane, (butoxymethyl)- Oxalic acid, ethyl propyl ester Acetonitrile, hydroxyl (23.81%), 4-Pentenoic acid ethyl ester, 4-Pentenal, Methoxyacetonitrile (11.17) With other compounds present in trace amounts, These results are in agreement with the results of the study Mushroom components analysis using GC-MS a total of 88 compounds were identified (27)

Bacterial sensitivity test against antibiotics

The bacterial isolates under study were subjected to antimicrobial sensitivity tests, which are routinely used to treat infections caused by pathogenic bacteria, The most resistant isolates were selected and placed in the Table 3.

Table 2. GC- MS analysis of *H.erinaceus* extract

Pk#	RT	Area%	Library/ID	Ref#	CAS#	Qual
1	4.612	3.77	C:\GCMS\firmware\NIST11.L			
			Glycolaldehyde dimer			
Glyoxal				9224	023147-58-2	4
			Methyl Alcohol	2	6.598	
3.37	C:\GCMS\firmware\NIST11.L			210	000107-22-2	4
			Glyoxal	29	000067-56-1	2
			Oxirane, (ethoxymethyl)-			
Glycolaldehyde dimer				210	000107-22-2	4
				4273	004016-11-9	4
				9224	023147-58-2	2
3	8.628	4.42	C:\GCMS\firmware\NIST11.L			
Glyceraldehyde			Oxirane,			
(ethoxymethyl			Acetic acid,	2255	000056-82-6	9
ethoxyhydroxy-, ethyl			ester	4273	004016-11-9	9
				23000	049653-17-0	9
4	14.607	2.26	C:\GCMS\firmware\NIST11.L			
Oxirane, (ethoxymethyl)						
Oxirane, (butoxymethyl)-			Oxirane,	4273	004016-11-9	10
(butoxymethyl)-				13428	002426-08-6	42
				13435	002426-08-6	42
5	14.983	1.99	C:\GCMS\firmware\NIST11.L			
Oxirane, (ethoxymethyl) Oxirane,						
(butoxymethyl)-				4273	004016-11-9	12
			Oxirane, (butoxymethyl)-	13436	002426-08-6	40
				13428	002426-08-6	40
6	17.482	9.97	C:\GCMS\firmware\NIST11.L			
			1-Butanol, 2-nitro-			
Oxirane, (ethoxymethyl)-				9041	000609-31-4	9
Hydroperoxide, heptyl				4273	004016-11-9	28
				14429	000764-81-8	9
7	18.851	23.81				
C:\GCMS\firmware\NIST11.L						
Oxirane, (butoxymethyl)-				13435	002426-08-6	33
Oxalic acid, ethyl propyl ester				31096	1000309-25-3	7
Acetonitrile, hydroxy-				191	000107-16-4	9
8	21.197	39.23	C:\GCMS\firmware\NIST11.L			
Propanamide, N-acetyl-						
Oxirane, (butoxymethyl)-				7748	019264-34-7	12
Succindialdehyde				13436	002426-08-6	20
				1664	000638-37-9	17
9	22.108	11.17				
C:\GCMS\firmware\NIST11.L						
4-Pentenoic acid ethyl ester				12234	001968-40-7	38
4-Pentenal				1377	002100-17-6	49
Methoxyacetonitrile				595	001738-36-9	22

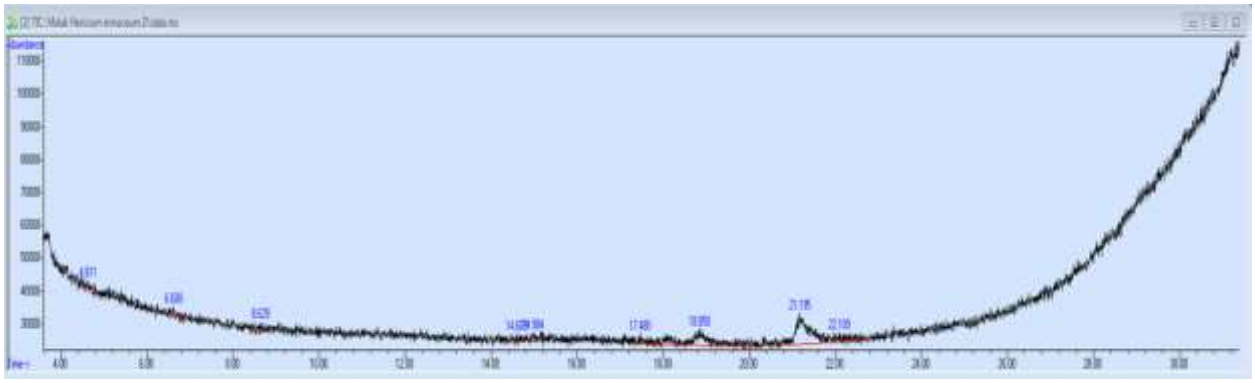

Figure 1. GC- MS analysis of *H.erinaceus* extract curve

Table 3. Bacterial isolates resistance and sensitivity against antibiotics

N	Antibiotic name	*K pneumonia*	*E coli*	S.typhi	*P. aeruginosa*	*S aureus*	*H.pylori*	*C.albicans*
1	Amoxicillin	R	R	R	R	R	R	
2	Ampicillin	R	R	R	R	R	R	
3	Ceftazidim	R	R	R	R	R	R	
4	Erythromycin	R	R	R	R	R	R	
5	Gentamycin	R	R	S	R	R	R	
6	Polymyxin	R	R	R	R	R	R	
7	Piperacillin				R	R	R	
8	Vancomycin	R	R	S	R	S	R	
9	Ciprofloxacin	R	R	S	R	R	R	
10	Cefotaxim	R	R	S	R			
11	Imipenim	S	R	R	S	R	S	
12	Trimethoprim/sulp	S	R	R	R	R	R	
13	Aztriomycin	R	S	R	R	R	R	
14	Mecillinam.	R	R	S	R	R	R	
15	Levofloxalin	R	R	R	S	R	S	
16	Nystatin							S

S:sensitive R: resistant

Determination of Inhibition zone Concentration: Table 4 shows significant differences in the value of the diameters of inhibition, the isolates that were more resistant to the antibiotic were selected in the sensitivity test (Table 3)where we notice an increase in the diameter of inhibition zone with the increase in concentration. All isolates were resistant to concentration 50mg/ml except *C.albicans* (Figure 2)which was sensitive and recorded inhibition zone 10mm. Sensitivity of all other isolates started at 100mg/ml while *E.coli* (figure 3) showed resistance for 50 and 100mg/ml. another on hand other isolates appeared sensitive at 100mg/ml and continued increasing at 400mg/ml and recorded 24,20,19,16,12,11,10 mm of inhibition zone for *S.typhi* (figure .4),*C.albicans*,*E.coli*,*S.aureus*(figure.5)*P.aeruginosa*(figure.6),*H.pylori*(figure.7),*K.pneumonia*(figure.8). By comparing the results of the extract sensitivity test with the results of the antibiotic sensitivity test, we conclude that the extract is more effective against pathogenic isolates than the antibiotics.(24)

Table 4. Zone of Inhibition for *H.erinaceus* extract concentrations against isolates

Isolate	Concentration (mg/ml)				LSD value
	400	200	100	50	
S.typhi	**24**	**21**	**17**	**0**	**5.22 ***
C.albicans	**20**	**16**	**12**	**10**	**4.51 ***
H.pylori	**11**	**10**	**8**	**0**	**3.28 ***
S.aureus	**16**	**13**	**10**	**0**	**4.06 ***
K.pneumonia	**10**	**14**	**13**	**0**	**3.15 ***
E. Coli	**19**	**10**	**0**	**0**	**3.87 ***
P.aeruginosa	**12**	**11**	**10**	**0**	**3.04 ***
LSD value	**4.27 ***	**4.09 ***	**3.66 ***	**3.17 ***	**---**

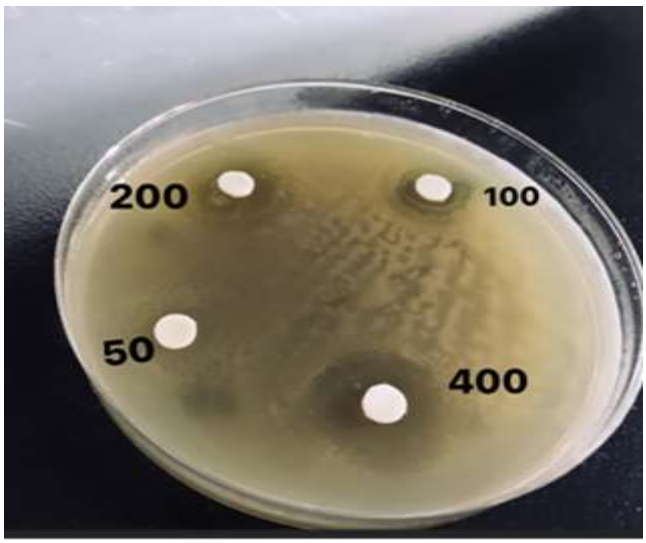

Figure 2.*Candida albicans*

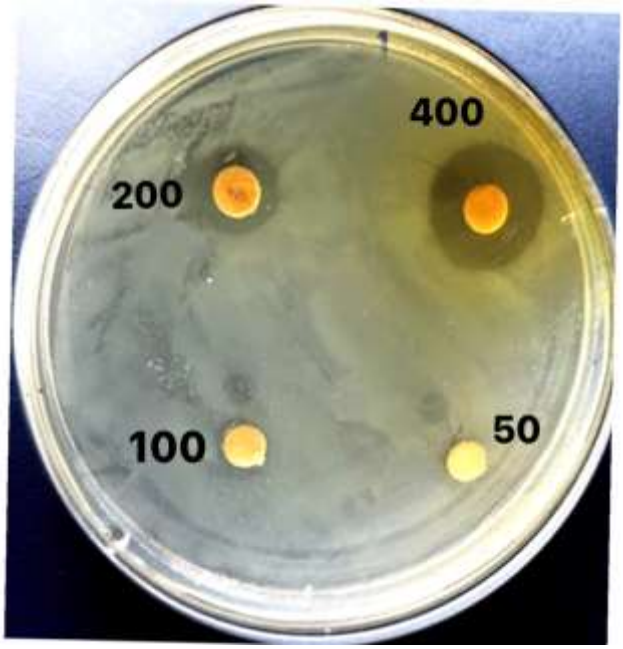

Figure 3.*Escherichia coli*

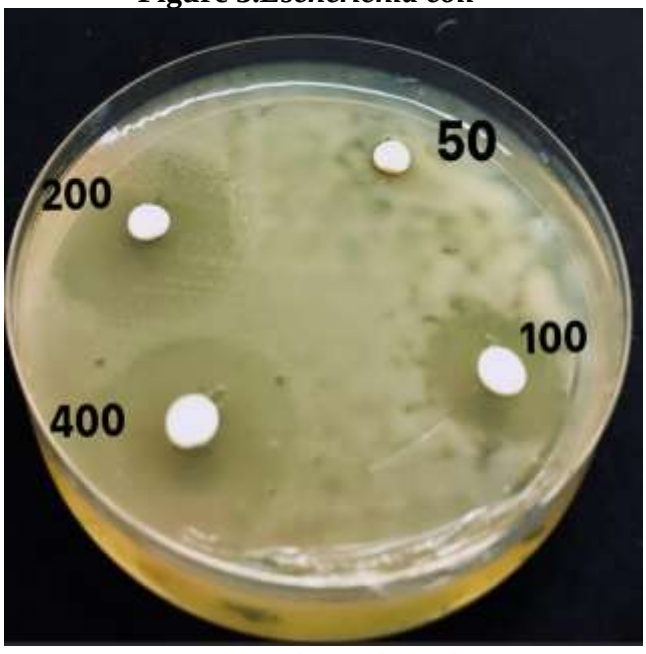

Figure 4.*Salmonela typhi*

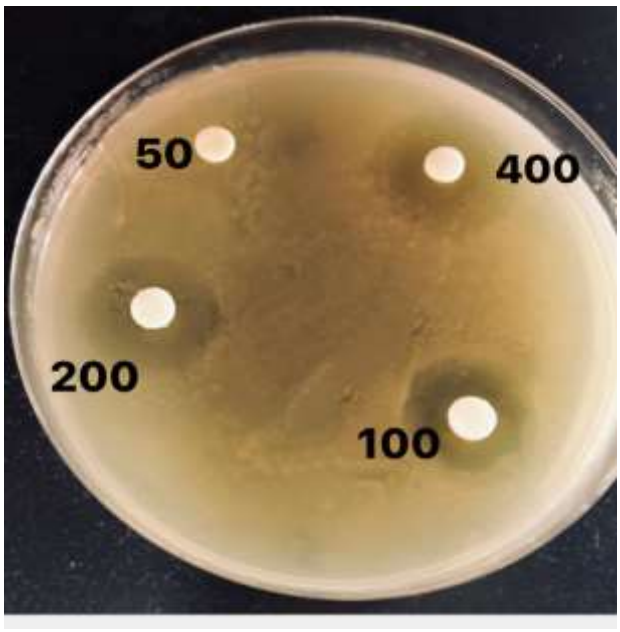

Figure 5.*Staphylococcus aureas*

Figure 6.*Pseudomanas aeruginosa*

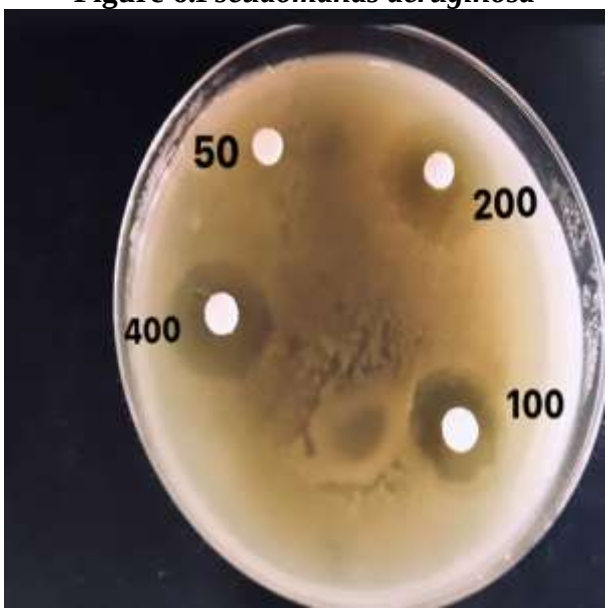

Figure 7.*Helicobacter pylori*

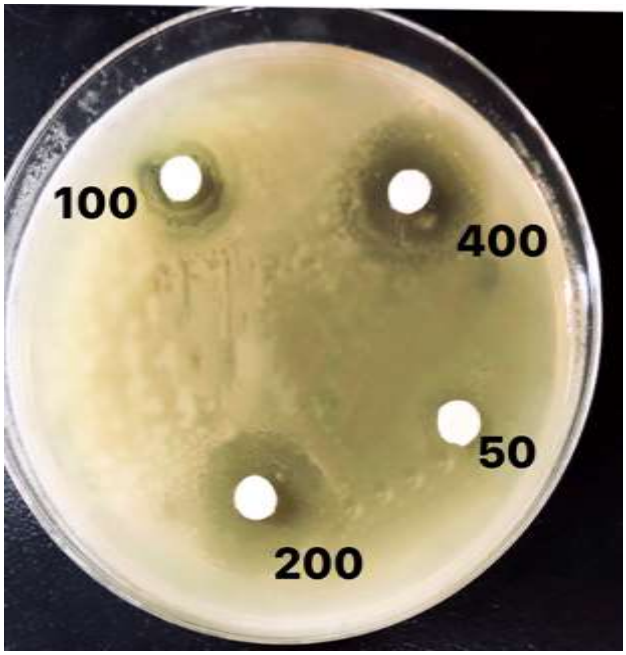

Figure 8.*Klebsiella pneumonia*

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