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A comparative study of the antibacterial activity of Quercus robur (Ethanolic extract) and Zinc oxide nanoparticles on Salmonella (In vitro)

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Abstract

This study was planned to estimate the inhibitory effect of Ethanolic extract of (***Quercus robur***) and Zinc oxide nanoparticles on the growth of one genus of enterobacteriacae (***Salmonella***). *In vitro*. For this aim graduate concentrates for plant extract (50, 100, 200, 400) mg/ml which prepared and compared with Zinc oxide nanoparticles of different concentration (2,1,0.5 and 0.25) µg/ml, and examined. The result demonstrated that the studied medicinal plant has antibacterial activity against this bacteria which used. The result showed that the plant has good activity in decrease the growth of this bacteria. The results of the study also showed that the nano-ZnO has very effective antibacterial action against the studied bacteria which was Salmonella, nanoparticles concentrations lead to increasing in the inhibition zones of tested bacterial growth.

We also study the effect of three antibiotics Lomefloxacin(LOM), Ciprofloxacin (SIP) and Rifampin (RA) and the result showed, in a comparison within the tested bacteria, *Salmonella* had a significant inhibition increase in Lomefloxacin; the ciprofloxacin showed effect on tested bacteria. However, Rifampin did not show any effect on tested bacteria.

Keywords: *Quercus robur, enterobacteriacae,* extract, nanoparticles, ZnO, Lomefloxacin (LOM), Ciprofloxacin (SIP) and Rifampin (RA).

CHAPTER ONE
(GENERAL INTRODUCTION)

1.1 *Enterobacteriaceae* :

is a family of Gram-negative, and one of the most important non-spore-forming groups of bacteria. Many foodborne pathogens such as Shigella spp, *Cronobacter spp, Yersinia enterocolitica*, pathogenic *Escherichia coli* (including E. coli O157:H7), and *Salmonella* belong to this family. Opportunistic pathogens are also included especially in clinical settings (e.g.,*Citrobacter spp, Serratia* spp. and *Klebsiella spp*.). Some species of the family are also associated with food spoilage and therefore participate in significant economic losses for the agricultural and food industries. Plant and fruit diseases, for example have a strong relationship with Erwinia spp., and the more recently introduced Pectobacterium spp. and Brenneria spp. Many other members of the Enterobacteriaceae are responsible for spoilage of a variety of foods including fruit and vegetables, meats, poultry, eggs,]1[milk and dairy products, as well as fish and other seafoods.

1.2 *Salmonella*

I.Characteristic of *Salmonella*:

Salmonellae are non-fastidious as they can survive under various environmental conditions outside the living hosts. They can grow in the absence or presence of sodium chloride in concentration up to 0.4 to 4%. Most Salmonella serotypes grow at temperature range of 5 to 47°C with optimum temperature of 35 to 37°C but some can grow at temperature as low as 2 to 4°C or as high as 54°C (2), They are sensitive to high temperature and often killed at temperature of 70°C or above. The optimum pH range is between 6.5 and 7.5, but also can grow in a pH range of 4 to 9. High water activity (aw) between 0.99 and 0.94 (pure water aw=1.0) is required, yet can live at dried foods.

II. Clinical manifestation:

Four disease patterns can be distinguished in the clinical pattern of salmonellosis in human namely enteric fever, gastroenteritis, bacteremia and other complications of nontyphoidal salmonellosis as well as chronic carrier state. Enteric fever Salmonella Typhi causes typhoid fever, whereas Paratyphi A, B and C cause paratyphoid fever with symptoms which are milder and a mortality rate that is lower for the latter. Both serotypes are exclusively human pathogens. Infection classically occurs due to ingestion of food or water contaminated with human waste. In recent years, antibiotic-resistant strains have been isolated in most endemic areas, particularly Southeast Asia, India, Pakistan and Middle East (3).

Roughly 10% of patients may relapse, die or face serious complications such as typhoid encephalopathy, gastrointestinal bleeding and intestinal perforation. Deterioration is the most

common occurrence probably due to persisting organisms within reticuloendothelial system (RES). Typhoid encephalopathy, often come with shock, is associated with high mortality. Minor gastrointestinal bleeding can be fixed without blood transfusion but in 1 to 2% of cases can be fatal if a large vessel is implicated. Intestinal perforation may present with abdominal pain, rising pulse and dropping blood pressure in sick people. Hence, it is very serious in 1 to 3% of hospitalized patients (4)(5).

Gastroenteritis Nontyphoidal salmonellosis or enterocolitis is caused by at least 150 Salmonella serotypes with Salmonella Typhimurium and Salmonella Enteritidis being the most common serotypes in the United States. Infection always occurs via ingestion of water or food contaminated with animal waste rather than human waste. The emergence of multidrug-resistant S. Typhimurium DT104 has been associated with outbreaks related to beef contamination and resulted in hospitalization rates twice than that of other foodborne salmonellosis (2)(6).

Ciprofloxacin is often administered at the first sign of severe gastroenteritis whereas ceftriaxone is given to children with systemic salmonellosis. In production animals like swine, treatment is usually contraindicated but, when necessary, can be given by injection with several treatment options based on considerations such as withdrawal time. Antibiotic treatment is usually not recommended except for rare cases because it can prolong the presence of bacteria in the stool (2), (6). Bacteremia and other complications of nontyphoidal salmonellosis About 8% of the untreated cases of salmonellosis result in bacteremia. Bacteremia is a serious state in which bacteria enter the bloodstream after passing through the intestinal wall. It has been related with highly invasive serotypes like Cholearaesuis or Dublin.

III. Bacteremia:

In cases of fever of unknown origin; bacteremia caused by Salmonella should be considered. Patients who have bacteremia and other complications should be treated with antibiotics (3). Chronic carriers who potentially infect many individuals can result in prevalence of chronic carrier state Salmonellosis, especially those who work in food-related industries. Factors contributing to the chronic carrier state have not been fully explained. On average, nontyphoidal serotypes persist in the gastrointestinal tract from 6 weeks to 3 months, depending on the serotypes. Only about 0.1% of nontyphoidal Salmonella cases are shed in stool samples for periods exceeding 1 year. About 2 to 5% of untreated typhoid infections result in a chronic carrier state. Up to 10% of untreated convalescent typhoid cases will excrete S. Typhi in feces for 1 to 3 months and between 1 and 4% become chronic carriers excreting the microorganism for more than one year (3)(5).

1.3 The Plants

Have a great ability for producing new drugs of great benefit to mankind. There are .]7[many approaches to the search for new biologically active principles in higher plants Many efforts have been done to discover new antimicrobial compounds from various kinds of sources such as soil, microorganisms, animals and plants. One of such resources is folk medicine and systematic screening of them may result in the discovery of novel effective compounds. Further, scientific investigation and information of the therapeutic potential of]8[the plant material is limited.

1.4 Nanotechnology:

The idea of nanotechnology was introduced by Richard Feynman in 1959, when he gave a talk called “there is plenty of room at the bottom”. In his talk he never explicitly mentioned about nanotechnology, he proposed that it will eventually be possible to control atoms and molecules precisely. The invention of the Scanning Tunneling Microscope in 1981 and the discovery of fullerenes in 1985 are considered as the convergence of experimental advances which leads to the emergence of nanotechnology in 1980’s. nanoscience and nanotechnology are widely cited to be the defining technology for 21st century and nanotechnology is defined as the control and manipulation of matter at nano dimensions. The introduction of nanotechnology resulted in development of Nano powders that can be used for wide range of purposes. Study has been made for nano-size materials in recent years because of their exceptional character differing from those in the bulk state. One of the huge promises that nanoparticles of metal oxides hold in chemical applications is their amazing ability to chemically absorb a wide range of molecules, especially organic molecules that are concern as environmental hazards.

1.5 Zinc oxide

In latest years' zinc oxide has promoted itself as an attracting metal oxide material because of its exceptional physical and chemical properties such as high chemical and mechanical stability, wide range of radiation absorption, elevated catalysis activity, electro chemical coupling coefficient, nontoxic nature etc. In materials science, zinc oxide is counted as a semiconductor in group II-VI, with a wide energy band of 3.37 eV and high band energy of 60meV. Because of its distinct properties its extensively used in many fields such as rubber industry, pharmaceutical and cosmetic industries, textile industries, electronics and electro technology industries etc. Nanometri zinc oxide can occur in variety of structures. It can exist in one dimensional (1D), two dimensional (2D) and three dimensional (3D) structures. One dimensional structures make up the largest group including needles, helixes, nanorods, ribbons, belts, wires and combs. Zinc oxide can exist in two dimensional structures such as nanopellets, nanosheet/nanoplate.[9] Examples of three dimensional structures of Zinc oxide involve snowflakes, dandelion, flower etc. In this paper the method of synthesis, characterization, surface modification is discussed. Zinc oxide can be produced using several different methods including micro emulsion synthesis, spray drying, sol-gel method, pyrolysis, controlled precipitation, RF plasma synthesis, vapour transport method etc. controlled precipitation method was used here for the synthesis of zinc oxide nanoparticles.

1.6 Drug resistant pathogens:

The increase of drug resistant pathogens is one of the most dangerous threats to successful treatment of microbial diseases. Down the ages essential oils and other extracts of plants have induced interest as sources of natural products. They have been selected for their possible uses as alternative preparations for the treatment of many infectious World Health Organization (WHO) observed that majority of the world's]10[diseases population depends on conventional medicine for primary healthcare. Medicinal and aromatic plants which are broadly used as medicine and comprise a major source of

natural organic compounds. Mainstream medicine is increasingly responsive to the use of antimicrobial and other drugs originated from plants, as traditional antibiotics (products of microorganisms or their produced derivatives) become ineffective and as new, particularly viral, diseases remain intractable to this type of drug. (11)

1.7 The aim of study:

The study aimed to estimate the antimicrobial activity of the extract of medicinal plant (Ethanolic extract) on the growth of ((***Salmonella*),** invitro, and compare the activity of these extracts with Nano-particles have the antibacterial activity when interact with bacterial surface and then entering inside the cell causing its destruction. Sometimes, these materials may have bactericidal effects that are essential in numerous antimicrobial applications just like in food industry [12]. The use of nano-particles (ZnO) concentrations are also important in medical applications like: therapeutics, diagnostics and surgical tools [13;14]. Also with several profiles of susceptibility and resistance to(Lomefloxacin), (Ciprofloxacin) and (Rifampin).

CHAPTER TWO
Materials and methods

2.1- Plant collection and preparation:

In this study we used local medicinal plant (*Quercus robur)* (the fruit) which obtained from the local market of Al- Qadisiyah city and identified by the (national Iraqi institute for herbs), we take the fruit of this plant, then drying for ten days, after drying, we grinded it very well until it became as a powder. The Ethanolic extraction for plant was by using of Ethanol at a concentration (96%).]15[done by used Harborn method

2.2- Nanoparticles material preparation:

A white powder of nano-ZnO was prepared nanoparticles (20µg.) powder and dissolved in 10 ml of dimethyl sulfoxide(DMSO) to prepare 2 µg /ml, the tested concentrations were prepared from this solution that include (2 – 1 – 0.5 & 0.25) µg /ml for agar wells diffusion method.

2.3- Bacterial Isolates:

bacterial Isolates were identified by laboratory of Microbiology \College of Medicine at Al-Qadisiyah University.

2.4- Antibiotics

In this study we use (3) antibiotics to compare their antibacterial effect with that of the medicinal plant (Ethanolic extract) due to their broad spectrum activity and these antibiotics include: Lomefloxacin (LOM) 10 mcg, Ciprofloxacin (SIP) 5 mcg, Rifampin (RA) 5 mcg.

2.5- Serial dilutions:

For the tested medicinal plant, we had been made a serial dilution to study the effect of it in the inhibition of the growth of *examined bacteria.* at a different concentrations and choose the most effective concentration of the plant extract based on the zone of inhibition of bacterial growth that been given by each concentration , we began with a concentration

(400)mg/ml (prepared by add 10 ml from Ethanol 96% to 4 gm of the plant extract) and the second concentration is 200 mg/ml(prepared by taking 4ml from the first dilution and we add 4 ml of ethanol 96% to it ,the third dilution is 100 mg/ml (prepared by taking 2ml from the second dilution and add 2 ml of ethanol 96% to it) ,the fourth dilution is 50%(made by taking 2 ml from the third dilution and add 2 ml of ethanol 96 % to it). These serial dilution was decided depending on clinical trials.

2.6-Senstivity test study:

After formulation of all the **Serial dilutions of** Ethanolic extract and activation of the pathogenic bacteria by the nutrient broth, the Mueller Hinton Agar (HIMEDIA -India) was made by (dissolve 38 gm from the agar powder in 1000 ml of distilled water in a flask and shaking it well to dissolve the agar and then we start heating it by a Benzen burner in attempt to complete dissolving of all the agar powder and after that the agar was sterilized by by means of of autoclave at 15 IP for 15 minutes) . After preparation of Mueller Hinton agar, we transferred it in to Petri plates and after solidification of the agar we made 4 wells (5 mm diameter) in each one of the Petri plates except those of the antibiotics discs, Petri plates having Mueller Hinton agar were used in this study {(4 plates for the medicinal plant extract study and (3 Petri plates for antibiotics: and 2 control plate}. This study was done by taking swap from the test tube that contain the bacterial suspension and inoculated it on the Petri plates that contain the Mueller Hinton agar, and then we added 0.1 ml of each concentration of plant extract on its own plates (in well), and we put on the chosen antibiotics discs in its plates. two control plate (the 1st one contain the plant extract only, and the 2nd one which contain the antibiotic discs only),After complete applying of all the medicinal plant extract concentrations and the Antibiotics we incubated all the Petri plates at 37^{o}c for 24 hours .The sensitivity of microorganisms towards the plant extract was screened by following the agar well –diffusion method .The tested concentrations were prepared from this solution that include 4 – 6 – 8 & 10 mg/ml for agar diffusion, had been prepared for the determination of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC).The zone of inhibition (diameter in mm) in .]16[triplicates was measured and the mean value (μ) was tabulated

CHAPTER THREE
Result and discussion

In this study, *Quercus robur* and Nanoparticles materials (ZnO), show antibacterial activity against ***Salmonella***, the genus of Enterobacteriacae which chosen as studied bacteria.

The results of the study demonstrated that the Ethanolic extract of Oak give antibacterial activity against the studied bacteria which was: Salmonella as follow for the studied concentrations (400,200,100,50) mg\ml appeared as a zones of inhibitions for the studied concentrations as follow (18.8, 18.2, 15.6, 14.1) mm respectively: (table 1) (Fig.1).

In (Table1) that deals with the inhibition zones relationships between the tested Enterobacteriaceae bacteria (*Salmonella*) and the different ethanolic extract concentration s (400,200,100 and 50) mg/ml, the results were detailed as follow:

Table (1): The inhibition zones of Ethanolic extract of *Quercus robur*) on *Salmonella*

	Concentration (mg/ml)			
Salmonella	**400**	**200**	**100**	**50**
inhibition zones(mm)	**18.8**	**18.2**	**15.6**	**14.1**

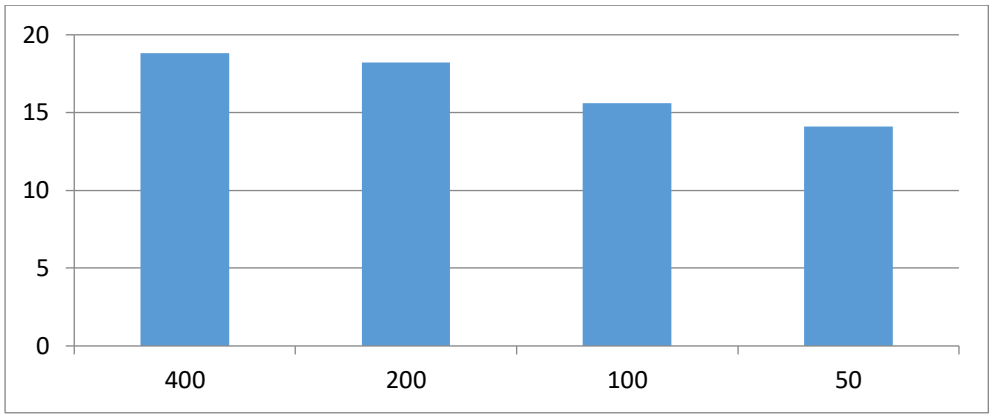


Figure (1): The inhibition zones of Ethanolic extract of *Quercus robur*) on *Salmonella*

The influence of the selected plant (Oak)as antimicrobial was recognized in a laboratory experiment in which the pure plant (Oak) contain in its structure :Tannins ,Polyphenols and , Therefore, their manner of antimicrobial action]17[Quercetin (available commercially) and inactivate]18[may be related to their capability to complex with polysaccharide microbial adhesins, enzymes, cell envelope transport proteins, etc. also The antimicrobial importance of this specific activity has not been explored. So, Tannins in plants inhibit insect reviewed]21[. Scalbert]20[and interrupt digestive actions in ruminal animals]19[growth the antimicrobial properties of tannins in 1991. He listed several studies may reach around 33 studies which had recorded the inhibitory activities of tannins up to that point. According to all these studies, tannins can produce toxic to filamentous fungi, yeasts, and bacteria. Condensed tannins have been established to bind cell walls of ruminal bacteria, stopping .]22[growth and protease activity

The results of the study showed that the nano-ZnO antibacterial activity against the studied bacteria which was: Salmonella as follow for the studied concentrations (2,1,0.5,0.25) µg/ml appeared as a zones of inhibitions for the studied concentrations (Table2) (Fig.2).

In (Table2) that deals with the inhibition zones relationships between the tested Enterobacteriaceae bacteria (*Salmonella*) and the different Nano-ZnO concentrations (2,1,0.5 and 0.25) µg/ml, the results were detailed as follow:

Table (2): The inhibition zones of Nano-ZnO on *Salmonella*

	Concentration (µg/ml)			
Salmonella	**2**	**1**	**0.5**	**0.25**
inhibition zones (mm)	**26.6**	**22.8**	**19.9**	**17.5**

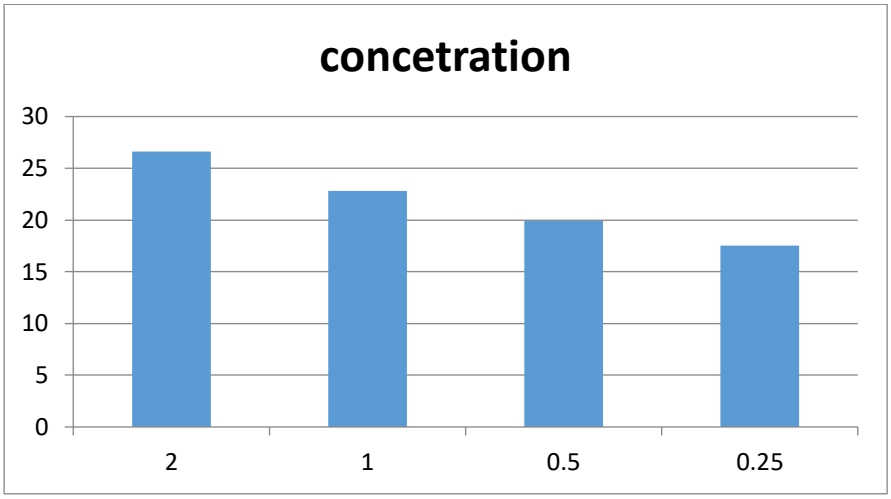


Figure (2): The inhibition zones of Nano-ZnO on *Salmonella*

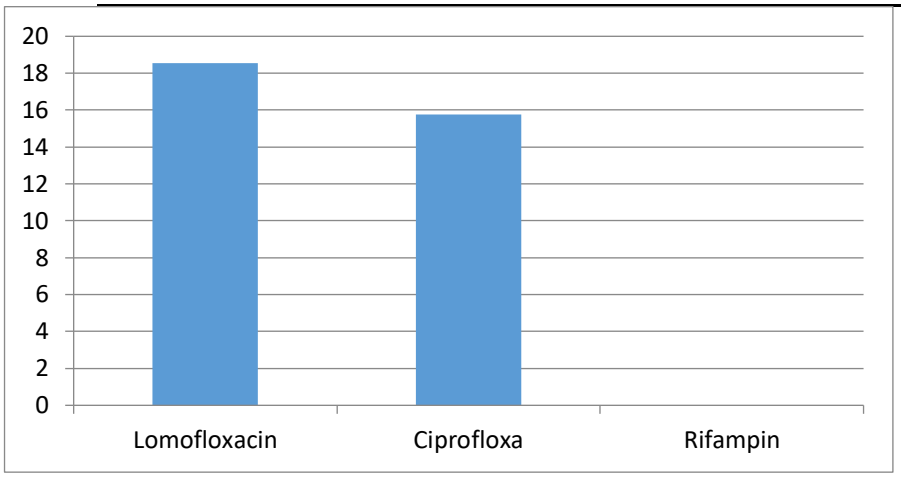
we have found that the increasing in both nanoparticles concentrations leads to increasing in the inhibition zones of all tested bacterial growth and this results are in conformity with the results of other researches [23]. Our results have clarified the antibacterial activity of nano-ZnO suspensions against (*Salmonella*). We use nano-ZnO suspensions with different concentrations (2,1,0.5 and 0.25) µg/ml, the data show all tested bacteria are totally inhibited. This is occurred because these nano-particles have the ability to penetrate the bacteria cell walls and produce damage to its cytoplasm which leads to bacteria inhibition [24]. Nano-particles ZnO have the capability to release free radical like: hydrogen peroxide (H2),OH- (hydroxyl radicals), and O(peroxide) into the medium after the exterior of the dead bacteria is entirely covered by nano-particles to avoid any bacterial action, so it shows high bactericidal efficacy [25].

Also, The antibiotics which used in this study gave antibacterial activity against the tested bacteria with the following zones of inhibitions: Lomefloxacin(18.55) mm,SIP (15.75) mm, Rifampin (0) mm . (Table3)

In (Table3) that deal with the inhibition zones relationships between the tested bacteria ((*Salmonella*) and three types of antibiotics were Lomefloxacin, ciprofloxacin and Rifampin, the results were described as follow:

Table (3): The inhibition zones of tested antibiotics discs on *Salmonella*

nella	**tion zone (mm)**		
	floxacin	**floxacin**	**ıpin**
tion zones (mm)			



Figur (3): The inhibition zones of tested antibiotics discs on *Salmonella*

The good solubility of the active constituents of this plant in organic solvent may have an . These observations can be rationalized due to]26[influence on show its antibacterial action the polarity of the compounds being obtained by each solvent, and in addition to their intrinsic bioactivity, by their capability to dissolve or disperse in the different media used in the assay. The growth media also appear to have a significant role in the determination of the , reported that Muller-Hinton agar seems to be the best medium to]27[antibacterial activity explain the antibacterial activity and the same was used in the current study.

The ethanolic extract of Oak has zone of inhibition ranged from (18.8 -14.1) mm, were ZnO ranged from (26.6 -17.5) mm.

CHAPTER FOUR
CONCLUSION

Our results have shown that the two nanoparticles(ZnO) and Ethanolic extract have antibacterial activity but nano- ZnO is better than Ethanolic extract of studied medicinal plant that have antibacterial activity against ***Salmonella*** which chosen for this study, *Invitro*. We found in this study high differences ($P<0.05$) between the effect of the studied concentrations for the tested nano-ZnO compared with medicinal plant extract and also between the different genera of bacteria that used in this study.

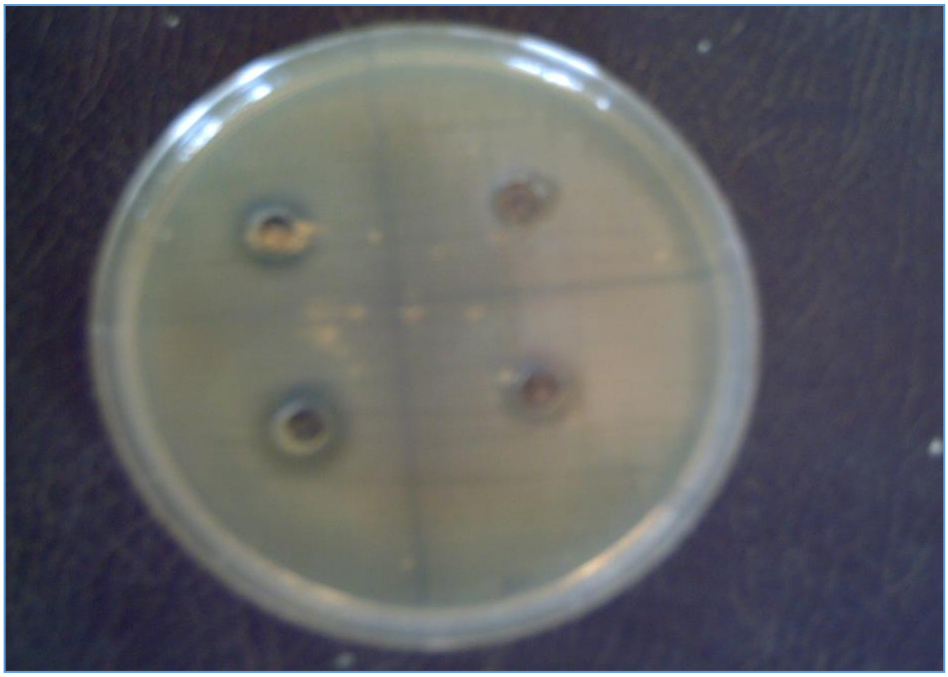


Figure (7): Inhibition zones of *Salmonella* growth on Mueller-Hinton agar produced by Ethanolic extract of *Quercus robur* the peripheral four wells contained extract concentrations (50, 100, 200, 400 mg/ml)

CHAPTER FIVE
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