Antibacterial effect of Non-Steroidal Anti-Inflammatory Drugs (NSAIDs)

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Abstract :-

Effect of five concentrations of five non-steroidal anti-inflammatory drugs (NSAID) included (acetyl salicylic acid , ibuprofen , sodium diclofenate , mefenamic acid ,and piroxicam) against five bacterial species (*E. coli* , *Pseudomonas* , *Klebsiella* , *Staphylococcus* , *Bacillus)* via measuring the inhibition zone of drug . All drugs were effected on all bacterial species except (mefenamic acid , piroxicam) which inhibited only Gramm positive bacteria . Statistical analysis results showed that the most effected drug was acetyl salicylic acid and the less effective one was mefenamic acid.

Introduction

The anti-inflammatory ,analgesic ,and anti-pyretic properties of non-steroidal anti-inflammatory drugs (NSAIDs) are particularly useful in treating rheumatic and other musculoskeletal disorders .Theses drugs have been introduced on the market of the commercial potential for such compounds and testing to their utility in the treatment of pain and inflammations of varying origin (Nakka,*et al.*,2011).

The NSAIDs are among the most widely described drugs worldwide ,being the drug of the first choice in the treatment of degenerative inflammatory diseases. Arachidonic acid (AA) is subsequently converted by lipooxygenases and cyclooxygenases (COX) to eicosanoids . Inhibition of cyclooxygenases and therefore prostaglandin production is common mechanism of action of the NSAIDs .In addition AA is precursor for the production of eicosanoids ,known virulence factor ,stimulating germ tube formation ,and inflammation during infection and can be incorporated in to the phospholipids . NSAIDs are inhibitors of the cyclooxygenases (COX) isoenzymes .these drugs block the synthesis of mammalian prostaglandin by inhibiting one or both of COX isoenzymes .Eicosanoids two pathways ;the cyclooxygenase and lipooxygenase pathway .COX is the key enzyme in the synthesis of prostaglandin .Two isoforms of the cox enzymes have been characterized COX1 and COX 2 which they are identical in structure but have different substrate and inhibitor selectivity .(Rusu 2011).

Anti-inflammatory non- steroidal agents could be divided in to four main groups 1- compounds capable of producing full inhibition of both COX1&COX2with poor selectivity 2- compounds inhibits both COX1&COX2with preference towards COX2, 3- compounds that strongly inhibit COX2 and 4- compound that appeared to weak inhibitors for both COX 1&COX2(Warner &Mitchel2004, Howard &Delafoutain 2004).

Different studies investigated the antibacterial and antifungal effect of the non- steroidal anti-inflammatory drugs . NSAIDs possessed a moderate powerful effect against Gramm negative bacteria like *Salmonella* , *E. coli* ,*Helicobacter pylori* , *Klebsiella* ,and *Enterobacter* , as

well as Gramm positive bacteria such as *Staphylococcus ,Bacillus , Mycobacterium ,and Listeria monocytogenes* (Mazumdar*et al* .,2009, Al-Janabi,2009,Al-Janabi,2010, Bend *et al* .,1999 *et al*,2003).

Studies on NSAIDs also explored their effect against fungal infection such as *Candida, Cryptococcus ,Aspergillus, Penicillium ,Trichoderma*(Deva *et al*., 2000,Novert et al,2003,Rusu et al .2009,Al-Bader,2009)

The present study was designed to investigate whether the pathogenic bacher the pathogenic bacteria from different sources were susceptible to different type of non -steroidal anti-inflammatory drugs .

Materials and Methods:-

Bacterial Isolates:

Different pathogenic bacteria ,i.e. *E. coli , Klebsiella , Staphylococcus* from urinary tract infection and *Pseudomonas* from wound infection were used in present study ,while *Bacillus* was the onlyenvironmental isolate (all bacteria were obtained from bacteriology laboratory /dept. of biology /college of science.

Extraction non -steroidal anti-inflammatory drugs (NSAIDs):-

Five type of non-steroidal anti-inflammatory drugs were used in this study including peroxicam ,ibuprofen , aspirin (salicylic acid) , ponstan (mefenamic acid),and voltarin(diclofenate sodium),all drugs were extracted from tablets or capsule according to their solubility.

Piroxycam extracted by dissolving 1gm powder in 15 ml chloroform with stirring for 1 hour ,filtered , and evaporated at room temperature in dark.(Nakka*et al.* ,2011)

Ibuprofen was extracted by powdering 600 mg of tablets after removing the red coat ,homogenized , and dissolved in 10 ml chloroform with stirring for 5 minutes ,centrifuged ,excluded of precipitate and left supernatant to dried at room temperature .(matcovic et al .,2005)

Salicylic acid was extracted by powdering 600 mg of aspirin tablets ,and dissolving in ethyl acetate with vigorous shaking for 2 minutes, filtered ,and dark dried at room temperature.(Williamson ,1989)

Mefenamic acid was extracted by dissolving 10 mg of ponstan powder in glacial acetic acid at 70 ^{o}c. with stirring ,filtered and dried atroom temperature(Othman and Awades,2008) .

Diclofenate was extracted by dissolving 5mg of voltarin powder in 5ml methanol ,filtered ,and dried at room temperature .(Jawla,and Jain,2010)

Preparation of stock solution : To obtain the stock solution (100000µg/ml) of each drug,0.1gm from each extract was dissolved in 1 ml of DMSO (Dimethyl sulfoxide).Then a serial dilution (10-10000µg/ml)from the stock solution were prepared .

Preparation of bacterial culture :-One colony from each bacterial stock culture was inoculated in 4 ml nutrient broth and incubated at 37^{o}c for 24 hours .

Antibacterial assay :- 0.1 ml from each bacterial broth was spreaded on nutrient agar plate by using sterile L-shape rod , then well made with sterile cork-borer ,after that 0.1 ml from each concentration of each NSAIDs was added to well .All plates were incubated at 37^{o}c for 24 hours ,then diameters of inhibition zone were measured for antibacterial effect determination(Al-Janabi ,2009).

All data were analyzed statistically by using ANOVA test .

Results and Discussion:-

Large amounts of NSAIDs are consuming every day all over the world for treatment of many inflammatory diseases. Obtaining data about antibacterial action of such drugs is still unclear due to variability of influencing factors.

Measurement of inhibition zone of non-steroidal anti-inflammatory drugs against bacterial spp. Revealed that aspirin was the most effective drugs among the studied NSAIDs followed by ibuprofen and voltarin while each of ponstan and piroxicam gave the lowest effect .According to studied bacteria, results showed that *Bacillus* was the most affected even at low concentration with large inhibition zone . All of the 5 NSAIDs affected the growth of Gramm positive bacteria at different concentrations .Susceptibility of Gramm negative bacteria towards NSAIDs were varied.

Table (1) Diameter of inhibition of NSAIDs exposed bacteria

Bacteria / NSAIDS		*E. coli*	*Pseudomonas*	*Klebsiella*	*Staphylococcus*	*Bacillus*
Salicylic acid	1*10^1		6			4
	1*10^2		6	6		6
	1*10^3		12	10	8	10
	1*10^4	12*	18	12	20	12
	1*10^5	22	20	40	38	22
Ibuprofen	1*10^1	4	4			
	1*10^2	10	6			6
	1*10^3	12	10	6	4	8
	1*10^4	16	12	10	12	12
	1*10^5	20	14	12	18	16
Diclofenate sodium	1*10^1					6
	1*10^2			8	8	10
	1*10^3			10	14	14
	1*10^4	12		12	22	18
	1*10^5	12		12	34	30

Mefenamic aci	1*10^1					
	1*10^2					6
	1*10^3					10
	1*10^4					14
	1*10^5				12	16
Piroxicam	1*10^1					8
	1*10^2				10	8
	1*10^3				10	8
	1*10^4				12	10
	1*10^5				18	18

* Measurement of inhibition zone (mm)

Aspirin gave the effect on all studied bacteria at high concentration and the diameter of inhibition zone decreased with increasing of dilution .Table (1) figure(1)

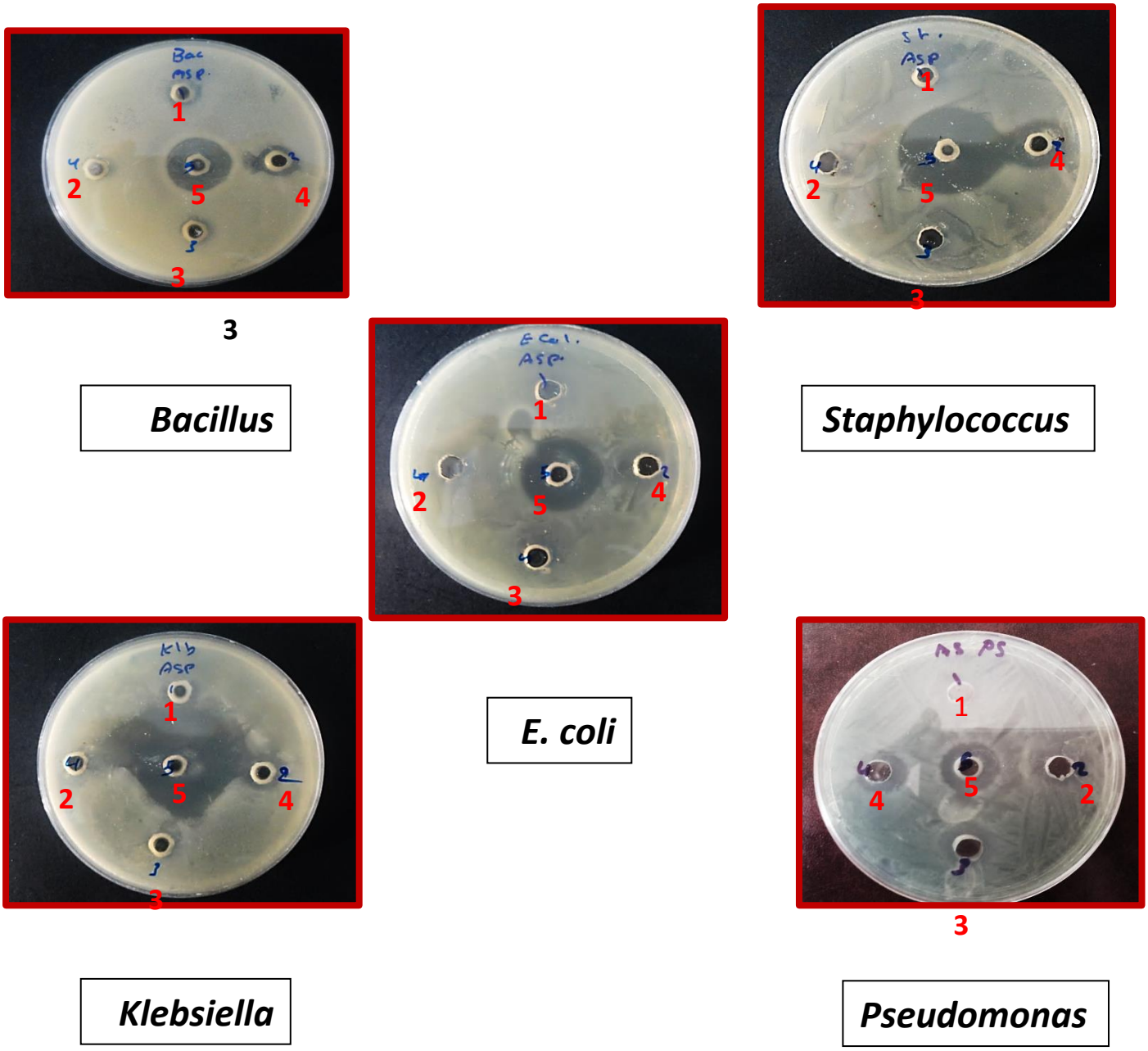

Figure (1)Antibacterial effect of aspirin (salicylic acid) . measurement of diameter of inhibition zone of each concentration 1=1*10^1,2=1*10^2,3=1*10^3,4=1*10^4,5=1*10^5 in mm.

Salicylate and related compounds such aspirin are known to have a variety of effect on microorganisms (Price *et al* .,2000) These effects include changes in membrane potentials and virulence factors production, reduction in extracellular polysaccharide production (Price *et al.* 2000; Wang *et al.* 2003)

Aspirin possessed a broad spectrum antimicrobial activity against *E. coli* and *Pseudomonas aeruginosa* (Al-Bakri, *et al* .,2009).

Another study showed that when aspirin administered to mice undergoing treatment of tuberculosis infection (*Mycobacterium tuberculosis*) to determine if these non-steroidal anti-inflammatory drugs enhance pyrazinamide activity *in vivo*(Byrne *et al* .,2002).

Diclofenate sodium revealed a significant effect against Gramm positive bacteria. *Pseudomonas* not affected even at high concentration .Table (1) figure (2)

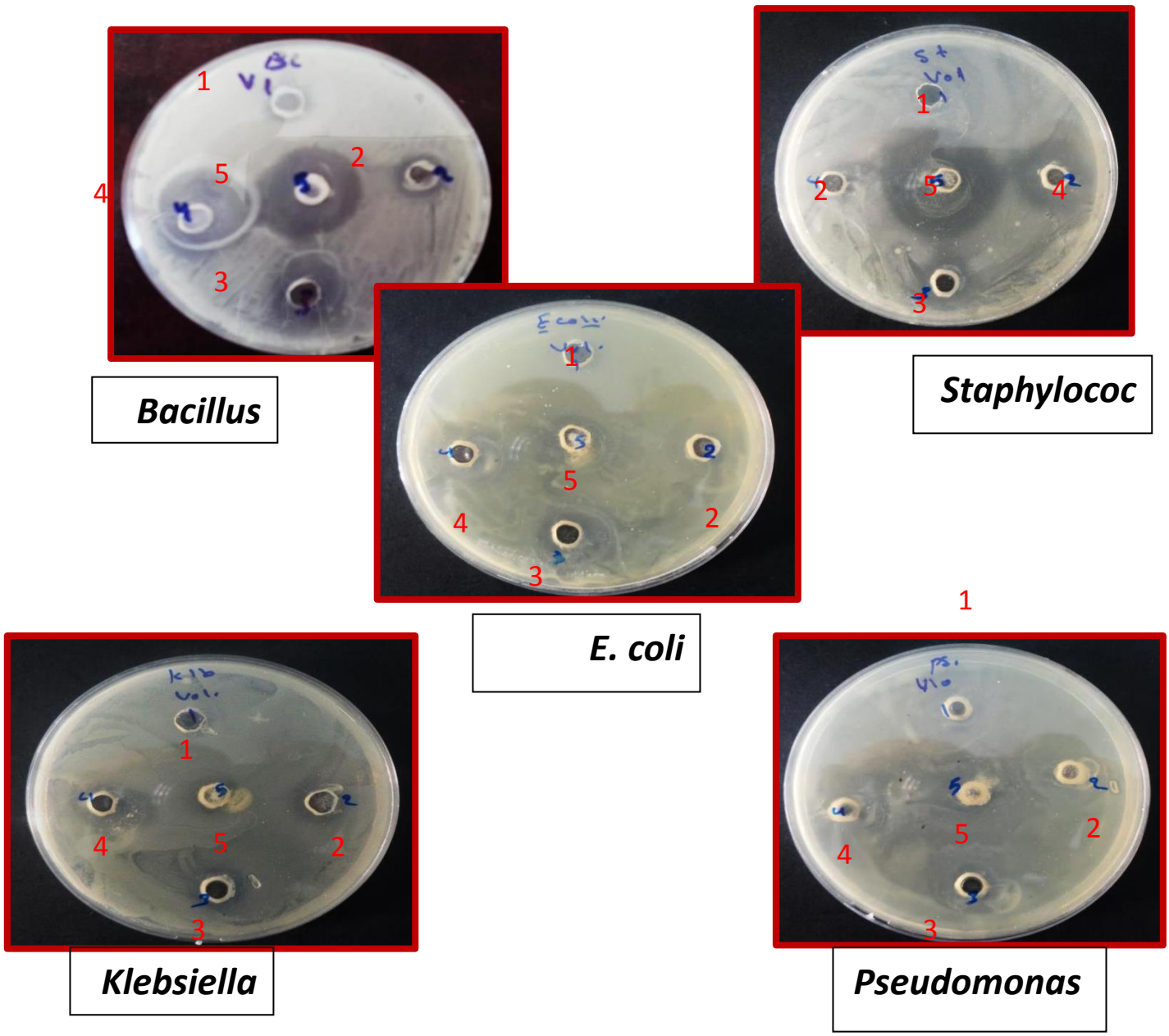

Figure (2)Antibacterial effect of voltarin (diclofenate sodium) . measurement of diameter of inhibition zone of each concentration 1=1*10^1,2=1*10^2,3=1*10^3,4=1*10^4,5=1*10^5 in mm.

A time kill study of diclofenate come in part from its ability to inhibit the DNA synthesis of *E. coli* and *Listeria monocytogenes*(Mazmudar*et al* .,2009) .Dutta et al .,(2007) had demonstrated that diclofenate had the ability to protect animal from lethality of *Salmonella* .

Ibuprofen has been inhibited the growth of both Gramm negative and Gramm positive bacteria , as illustrated in table (1)and Figure (3)

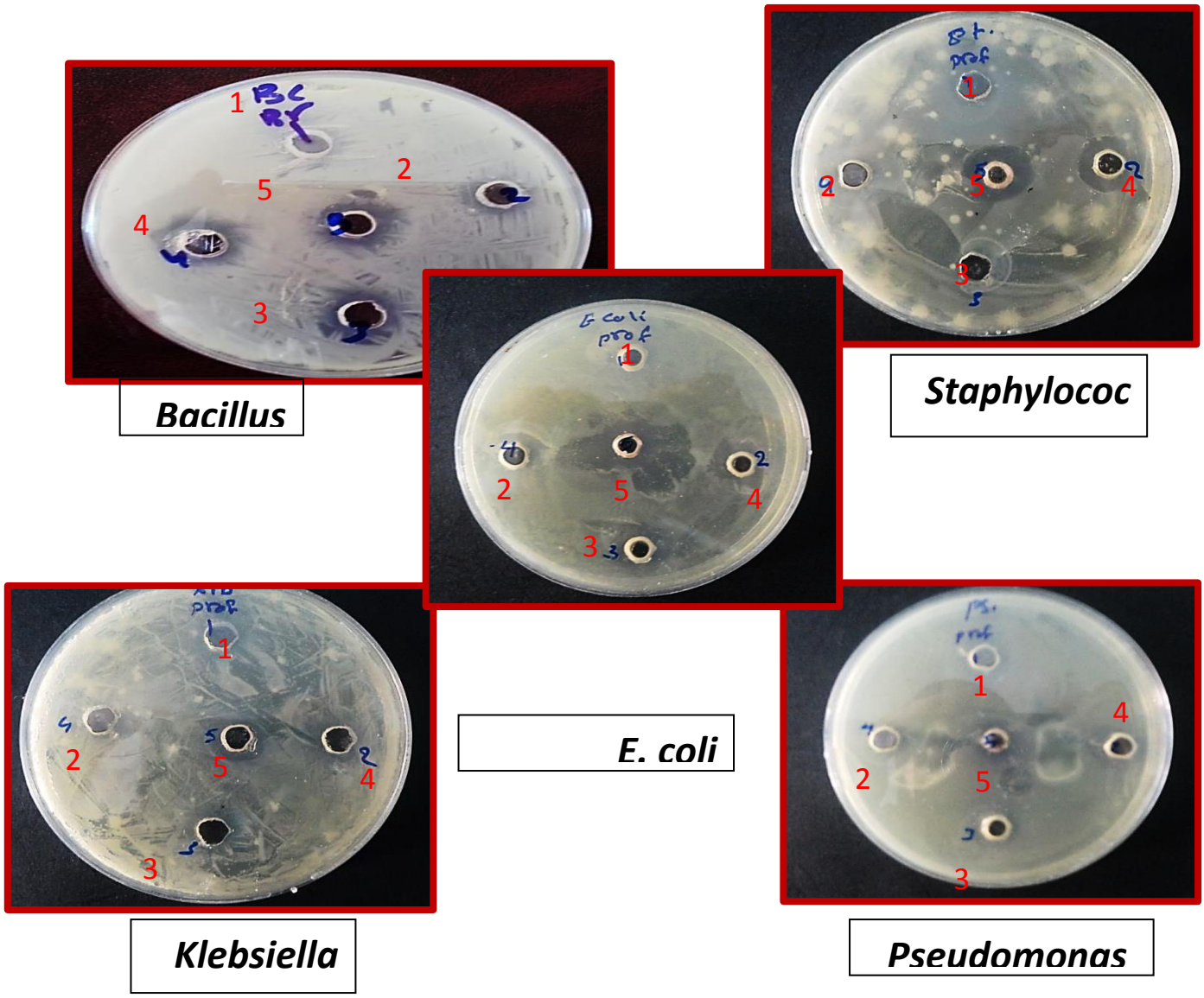

Figure (3)Antibacterial effect of ibuprofen . measurement of diameter of inhibition zone of each concentration 1=1*10^1,2=1*10^2,3=1*10^3,4=1*10^4,5=1*10^5 in

Ibuprofen belongs to propionic family of NSAIDs which consist of a phenol group in its structure . The antibacterial effect of ibuprofen due to the formation of free radicles and its hydrophobicity of the individual compounds ,which by turn damage DNA and enzymes (Hensch *et al.* .,2000; Boyd *et al.* ,2001) .

The less effected compounds of studied NSAIDs were both of

mefenamicacid and piroxicam which inhibited the growth of Gramm positive bacteria ,but gave no effect on Gramm negative bacteria .

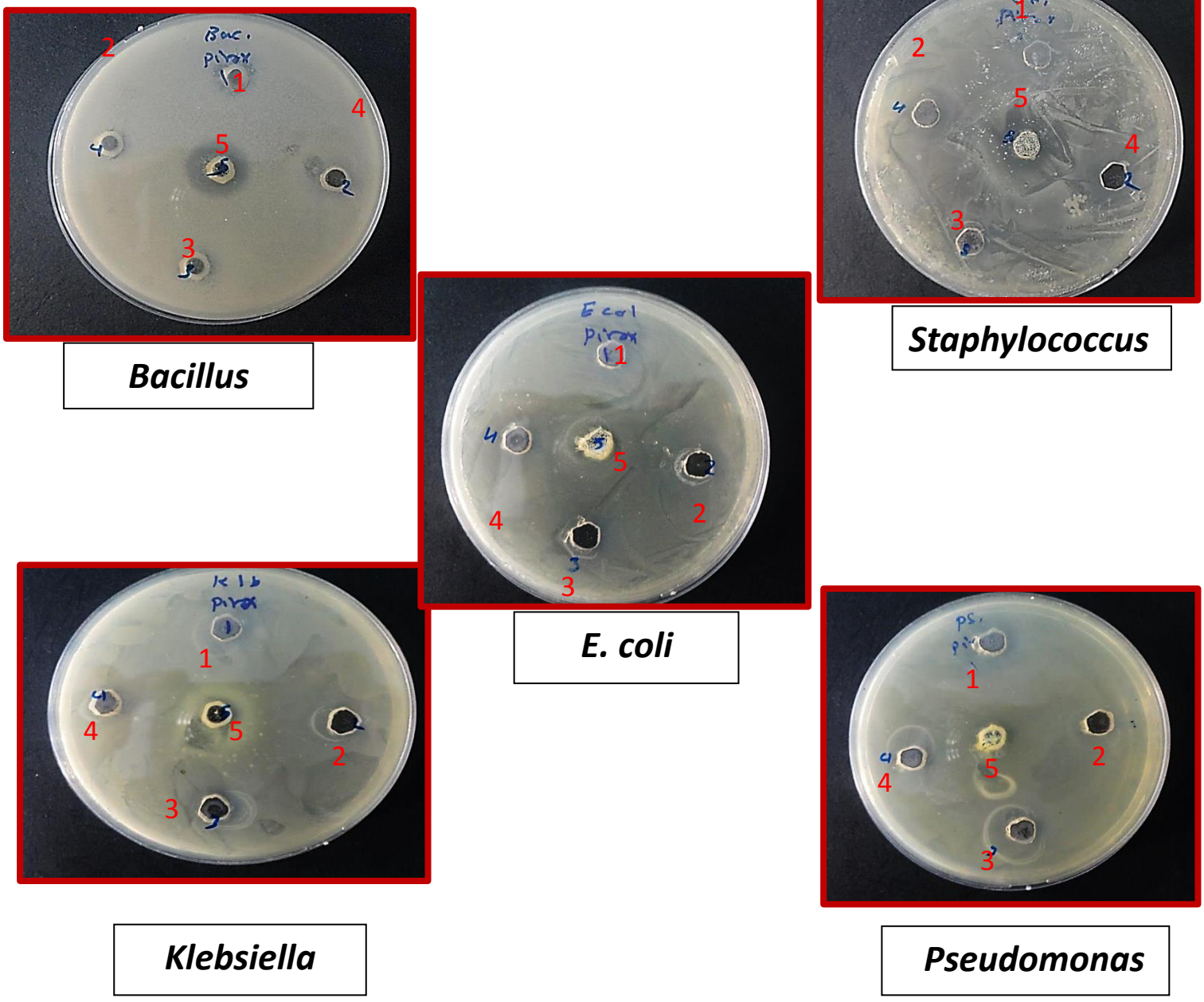

Figure (4)Antibacterial effect of piroxicam measurement of diameter of inhibition zone of each concentration 1=1*10^1,2=1*10^2,3=1*10^3,4=1*10^4,5=1*10^5 in mm.

(Nakka*et al* .,2011) found out thatsulfonate esters derived from piroxicamhave a potent activity against gram positive and gram negative bacteria, without testing of crude drug.

Kruszewska*et al*.(2006) found no activity of mefenamic acid on growth of *E. coli*which compatible to the present study while the isolated E. coli in the study of Al-Janabi (2009)showed susceptibility to mefenamicacid,butit enhanced the growth of some tested strains, especially *Staphylococcus aureus*, *Bacillus subtilis*and *Enterobacteraerogenes*in broth culture.

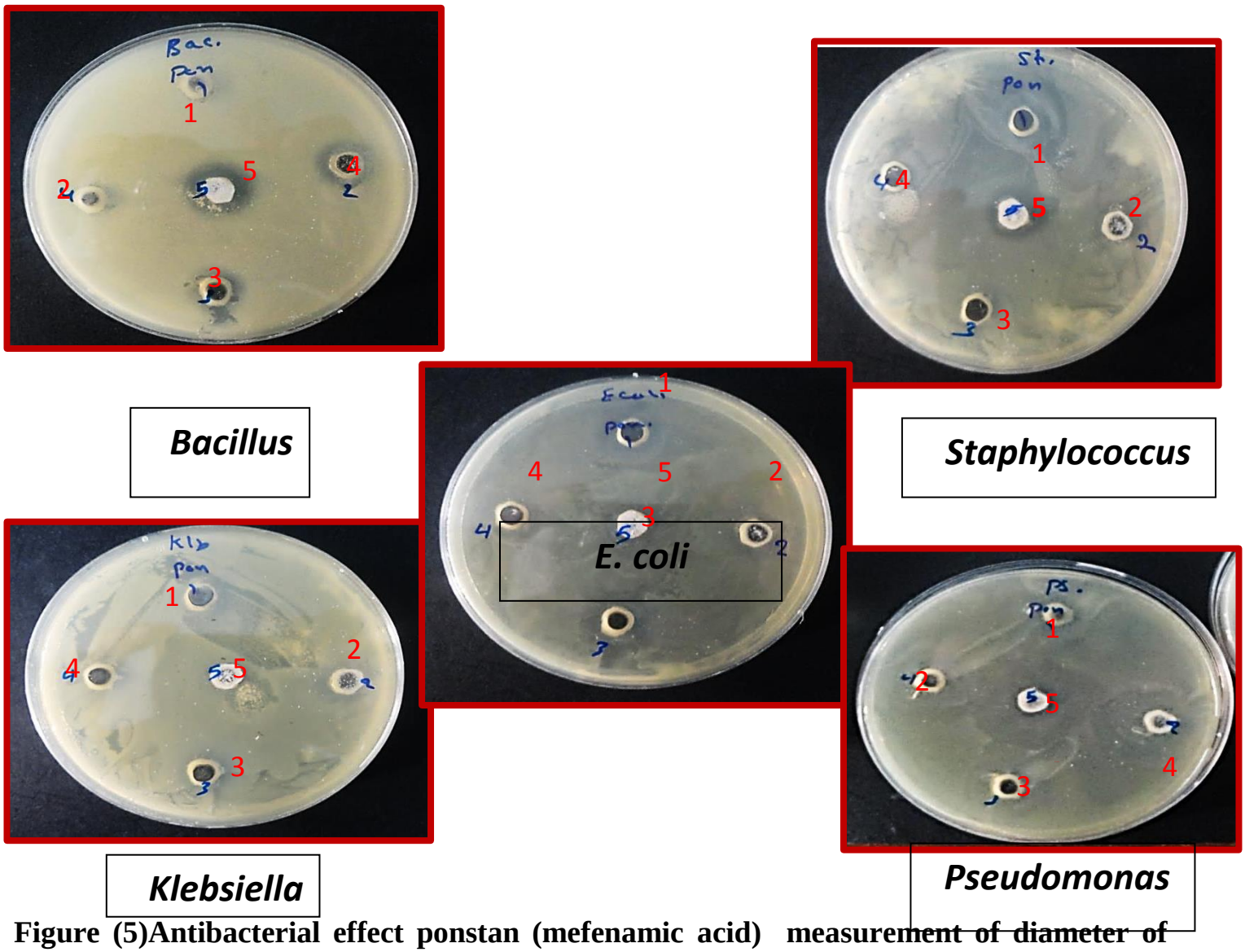

Figure (5)Antibacterial effect ponstan (mefenamic acid) measurement of diameter of inhibition zone of each concentration 1=1*10^1,2=1*10^2,3=1*10^3,4=1*10^4,5=1*10^5 in mm.

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الفعالية ضد الجرثومية للادوية المضادة للالتهابات غير الستيرويدية

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الخلاصة :-

درس تاثيرخمسة انواع من العقاقير غير الستيرويدية المضادة للالتهابات وهي (acetyl salicylic acid, ibuprofen , sodium diclofenate , mefenamic acid , piroxicam) على خمسة انواع مختلفة من الجراثيم (*E. coli* , *Pseudomonas* , *Klebsiella* , *Staphylococcus* , *Bacillus*) وبواقع خمس تراكيز لكل عقار ، حيث درس تاثير هذه العقاقير من خلال قياس قطر التثبيط . اثرت جميع الادوية المستخدمة على كافة الجراثيم المدروسة عدا(mefenamic acid , piroxicam)التي اثرت على الجراثيم الموجبة لصبغة كرام فقط.اظهرت نتائج التحليل الاحصائي ان اكثر العقاقير تاثيرا هو acetyl salicylic acid واقلها تاثيرا كان mefenamic acid