

**Evaluation of Antimicrobial activity of flavonoides Extract from  
*Lawsonia inermis*****Alyaa Sebti Jasim****College of Veterinary Medicine- University of Basrah. Basrah .Iraq****Key wards: *Lawsonia inermis* , *Flavonoides* , *Antimicrobial activity*****Abstract:**

Investigation of antibacterial activity of flavonoides extract of *lawsonia inermis* leaves was carried out on gram positive and gram negative pathogenic bacteria , the results exhibited a variable susceptibilities of microorganism for different concentration of flavonoides extract (6.25 ,12.5 ,25 ,50 ,100 mg/ml). The activity of this extract was associated with high concentration (100 mg/ml) using plate methods flavonoides extract of *L. inermis* had the highest effect and wide diameter of growth inhibition zone agiants *Streptococcus* sp. And it has no effect of growth of *Burkholderia pseudomallei* and *Staphylococcus aureus* was noticed except at very high concentration.

**Introduction:**

The increased prevalence of antibiotic resistant bacteria emerging from the extensive use of antibiotic may render the current antimicrobial agents insufficient to control at least some bacterial infections (1). Many of today's modern drugs have their origin in traditional plant medicine as well as natural products are a significant source of synthetic traditional herbal medicine that still primary health care system (2). *lawsonia inermis* is a small shrub of henna that widely cultivated and use in many Oriental, Middle Eastern and Northern African countries (3) this dwarf shrub grows outdoor unsheltered of temperature higher than 11oC (68oF) , it needs around 5 years to mature and produced leaves (4), It grows better in arid regions than moisture regions and achieves a high of 8 to 10 feet (5). The plant belongs to family lythraceae and is best known for its coloring matter contained leaves (6). Henna was then used for the treatment of headaches, migraine, albinism, skin abrasions and ulcers, burns, small pox, leprosy boils, wounds, some mycotic infection and cancers. It was also use for the treatment of scalp and hair infections and ailment (7,8). The aim of this study was to investigate the anti microbial effect of Henna leaves flavonoides on bacterial growth in vitro.

**Materials and methods****Plant material & Extraction:**

*Lawsonia inermis* were purchase from the local market of Basrah. The leaves of henna were sun-dried and powdered. A (40)gms of powder were extracted by mixing with 200ml Ethanol 80% for 24 hrs using magnetic stirrer. The extract was filtered through wattmann No. 31 to remove particles then supernatant was mixed with 1% lead acetate , filtered with wattmann No. 31. Residue was dissolved with 25ml acetone and 30ml HCl concentrated. The extract was filtered by wattmann filter paper No.1 then left to dry by rotary evaporator to contain black solid residue about 5gm (3) which was chromatographed over silica gel (TLC) by successive elution with 7:2:1 solvent mixture of Formic acid , water and second butanol for 40 minutes and range of follow the flavonoides extract was  $RF_1 = 0.2$  and  $RF_2 = 0.3$  that showed in figure (3) another ways we investigate about the flavonoides which produce brown residue when mixed with ethanolic KOH. These amount of flavonoides were diluted by normality<sub>1</sub> X volume<sub>1</sub> = normality<sub>2</sub> X volume<sub>2</sub> to prepare the diluted concentration (6.25, 12.5, 25, 50, 100 mg/ml)

### Sensitivity Test:

Seven types of pathogenic bacteria were previously isolated and identified by other workers from department of biology collage of science university of Basrah were used. To study the antimicrobial activity of flavonoides extracts of *lawsonia inermis*, muller – Hinton agar medium was used for bacterial growth  $10^5$  cell/ml. Discs were cut from filter paper No.1 and impregnated with the extract that carefully placed into the cultured plates and allowed to stand for a few minutes then plates were incubates at 37°C for 24 – 48 hrs. The method of well contain extract were used and the inhibition zones were measured by scales compared with the control (9).

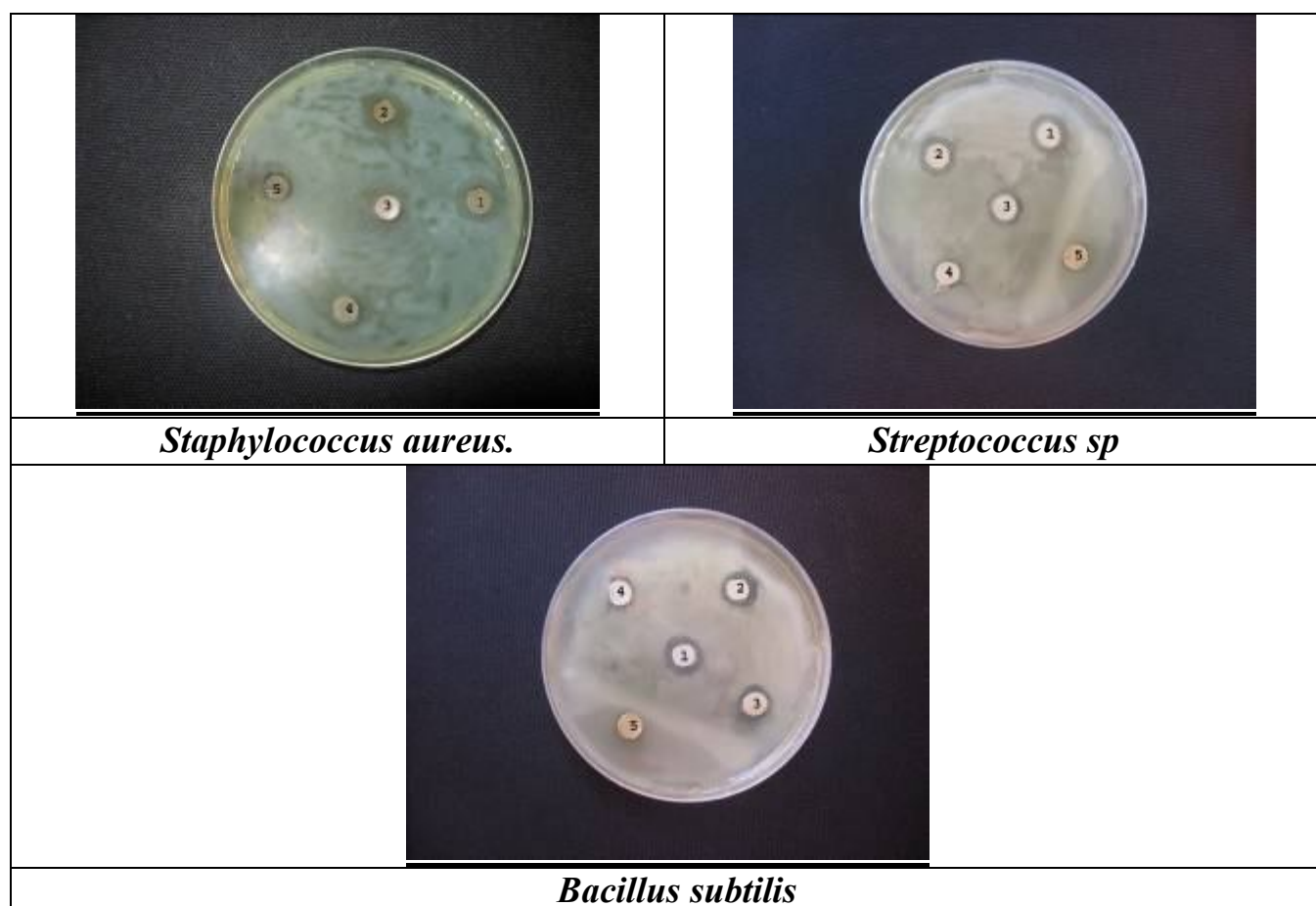
### Results:

The results of the study are summarized in Table 1. Showed the diameter of inhibition zone induced by flavonoides extract of *lawsonia inermis* leaves on the growth of bacteria. We studied the minimum inhibition concentration (MIC) of these bacteria, the (MIC) was less than 6.25 mg/ml for *Staphylococcus aureus* , *Streptococcus sp.* and *Bacillus subtilis* and less than 12.5 mg/ml for *Pseudomonas aeruginosa* and *Klebsiella pneumonia* , less than 6.25 mg/ml for *Escherichia coli* and *Burkholderia pseudomallei*. The inhibition zone induced by extract also illustrated by photographs (Fig. 1,2) . Antimicrobial activity was recorded when the zone of inhibition is greater than 6mm (10). The flavonoides extract was strongly inhibit the growth of many types of bacteria above the concentration 50mg/ml. the growth of *streptococcus* is inhibited with less than 6.25mg/ml. But as the concentration increased antibacterial activity was obvious. In contrast, the growth of *staphylococcus aureus* and *Burkholderia pseudomallei* was less inhibited even with high concentration.

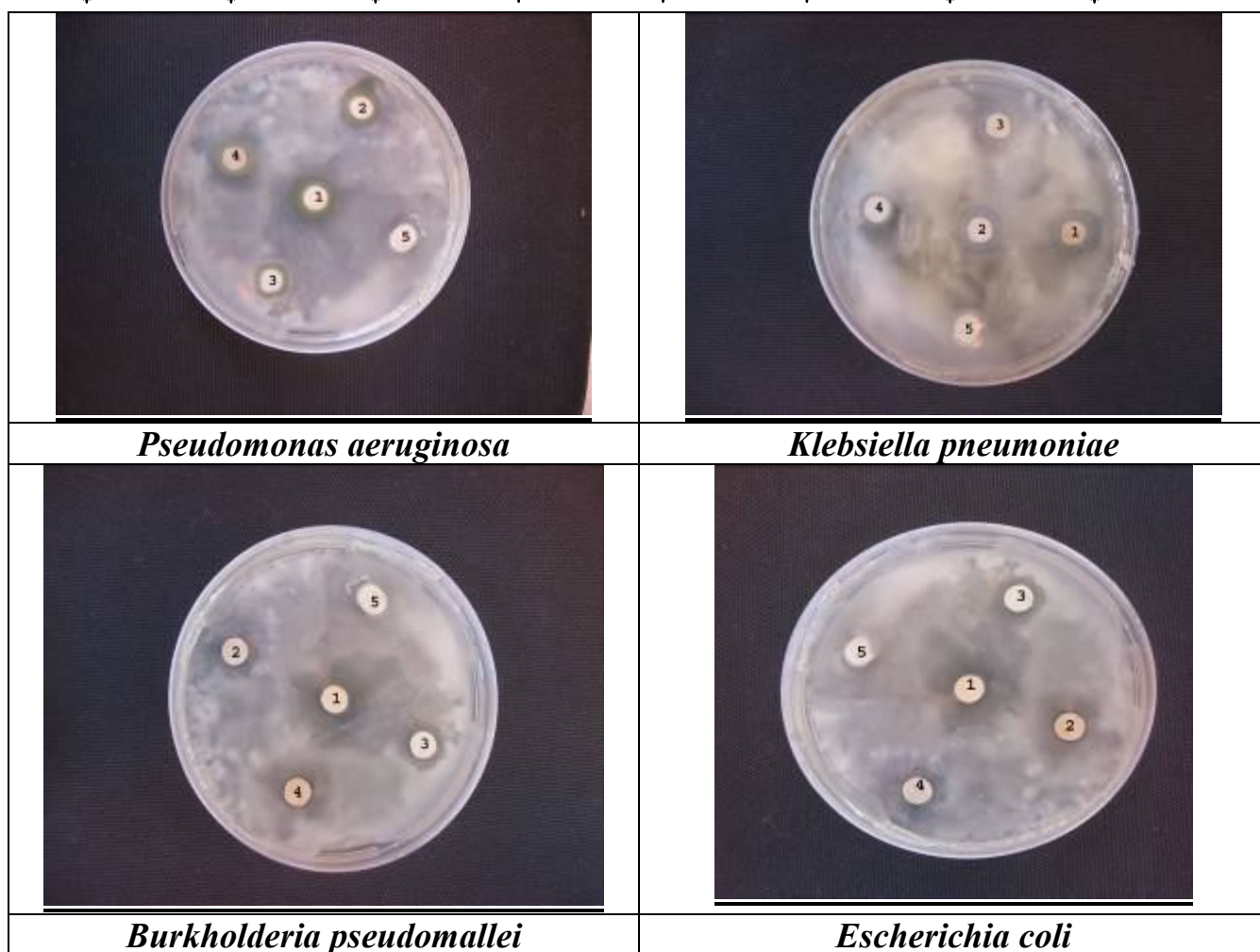
**Table 1: The diameter of the inhabitation zones induced by flavonoides extract of henna on tested bacteria**

Microorganism	Concentration of flavonoides Extraction(mg/ml)				
Gram Positive	6.25	12.5	25	50	100
	DIZ(mm)	DIZ(mm)	DIZ(mm)	DIZ(mm)	DIZ(mm)
<i>Staphylococcus aureus</i>	4	6	8	8	15
<i>Streptococcus sp.</i>	8	18	24	25	30
<i>Bacillus subtilis</i>	9	11	13	15	18
Gram Negative					
<i>Pseudomonas aeruginosa</i>	0	3	4	4	-
<i>Klebsiella pneumoniae</i>	0	0	0	8	-
<i>Escherichia coli</i>	8	9	4	15	15
<i>Burkholderia pseudomallei</i>	3	3	4	10	-

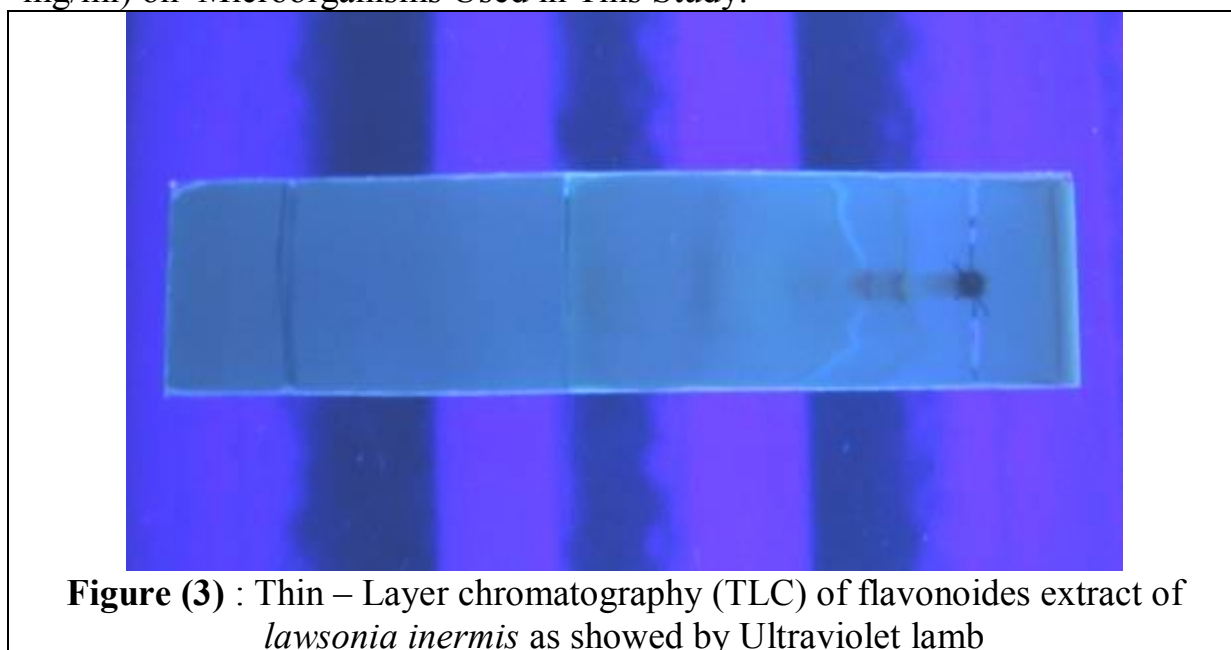
**DIZ = Diameter of inhabitation zone measured in millimeter.**



**Figure 1:** Inhibition Zones Induced by Different Concentrations of flavonoides Extract of henna leaves : (1=6.25mg/ml,2=12.5 mg/m,3=25 mg/ml,4=50 mg/ml and 5=100 mg/ml) on Microorganisms Used in This Study.



**Figure 2:** Inhibition Zones Induced by Different Concentrations of flavonoides Extract: (1=6.25mg/ml,2=12.5 mg/m,3=25 mg/ml,4=50 mg/ml and 5=100 mg/ml) on Microorganisms Used in This Study.



#### Discussion:

ISSN - 1994 - 697X

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Present study exhibited medicinal importance of the *lawsonia inermis* leaves through antimicrobial activity of flavonoides extraction. The microbes showed variable susceptibility for different concentration of flavonoides extract. The common plants contain flavonoides which is effected against bacteria (10). The role of hydroxy groups (OH<sup>-</sup>) on the flavane skeleton as well as low toxic nature and rather lipophylic properties thought to be toxic to microorganism as well as site (S) and number of hydroxyl groups on the flavonoides are responsible to their relative toxicity to microorganism that made flavonoides possessing anti – allergic , anti – inflammatory , antiviral and antibacterial activity. (11) In this study we observed that the studied gram positive pathogenic bacteria were high susceptible more than gram negative pathogenic bacteria in spite of appearance some exceptional , it may be due to the membrane of gram positive pathogenic bacteria which composed from peptidoglycan-mucopolysaccharides and phospholipids this will provide suitable for possibility to interaction and acts as bactericidal or bacteriostatic agents and give rise to affect as destructive whether on membrane or on building unit of protein structure or nucleic acid synthesis inside the bacterial cell but the cell membrane of gram negative bacteria composed of two membrane separated by periplasmic space. The cell membrane of these bacteria contains 90-95% lipids these contains were not provided suitable medium to reaction with extracts. These confirm with (11, 12). *Lawsonia inermis* possess antibacterial activity qualities, which surely enhance their application among other uses, as alternative to antibiotics for effective treatment of bacterial and fungal infections (13).

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### تأثير الفعالية المضادة للميكروبات لمستخلص الفلافونيد من أوراق الحنة

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#### الخلاصة:

درست الفعالية المضادة للميكروبات للمستخلص الفلافونيدي لأوراق الحنة وقد أختيرت بعض أنواع البكتيريا المرضية الموجبة والسالبة لصبغة كرام. بينت الدراسة ان هذه الميكروبات لها حساسية مختلفة اتجاه تراكيز المستخلص (6.25 ، 12.5 ، 25 ، 50 ، 100 ملغم/مل) وحسب نوع الميكروب والتركيز المستخدم وقد لوحظ ان التأثير الاكبر للمستخلص كان على جرثومة *Streptococcus sp.* أما النوعين *Burkholderia pseudomallei* و *Staphylococcus aureus* فلم تتأثر ألا عند استخدام التراكيز العالية جداً (100 ملغم/مل).