Biofilm,Antimicrobial& Reductive ability of *London rocket S. irio*

Abstract: *Sisymbrium irio* is a plant used in folk medicine in Asian peoples. In 79 samples collected from hospitals, 71 were infected with Gram positive bacteria. Performing API 20 samples showed 17(23.94%) were *S.pyrogens* and 54(76.06%) were *S.aureus*. In an experiment for antibiotic sensitivity, the samples showed 100% sensitivity to penicillin, cephalexin, cefotaxime, tetracycline, Amoxicillin and methicillin. However, the sensitivity was less in Vancomycin, clindamycin, Rifampin. Moreover, it was resistance to ciprofloxacin.

Test tube method used to detect ability of pathogenic *S. aureus* isolates which isolated from skin of children had impetigo for biofilm formation. The result illustrated the high percent of *S. aureus* isolates were able to produce biofilm. 47 (87%) *S. aureus* isolates produce biofilm with different degree of thickness and only 6 (13%) isolates unable to produce biofilm. The total flavonoids content was determined by spectrophotometer. The ethanol, metabolic and aqueous extract of *S. irio* as rutin the best standard substance for flavonoids. The best absorbance was methanol extract followed by water then, ethanol extracts.Reductive ability was carried out to know the effect of free radicals.The best extract was methanol followed by ethanol then, water extract.

Key words: London Rocket, Gram positive bacteria, Antibiotic sensitivity, plant extracts, reductive ability.

Introduction: The cruciferae family is a vast plant family that incorporates imperative sustenance crops, herbs,ornamentals and weeds1. Forty six genera of this family are circulated in Jordan,including the variety Sisymbrium. There are seven species having a place with this class in Jordan (Al-Eisawi 1982). A standout amongst the most vital types of this class is Sisymbrium irio, which is developed in numerous parts of the world. The plant is a rich wellspring of flavoinoids(Del Pero de Martinez and Aguinagalde 1982) and glucosinolates (Cole 1976, Griffiths, Deighton et al. 2001). It has a sharp flavor and can be utilized in plates of mixed greens. The plant is utilized in society drug as a febrifuge, an invigorating poultice, treating asthma and for contaminations of the throat and chest (Shinwari and Khan 2000). Impetigo is an endemic bacterial skin disease most normally connected with the pediatric populace. Topographically, this contamination is generally found in tropical regions around the world. Impetigo has the biggest increment in occurrence rate, when contrasted with different skin contaminations found in kids. The real trademark saw in this contamination is injuries. The essential causative living beings for impetigo incorporate Staphylococcus aureus and streptococcus pyogenes (Rørtveit, Skutlaberg et al. 2011, Patty Ghazvini* 2017) . Antimicrobial ,biofilm and reductive capacity were determined for this vital plant.

Materials and Methods:

Plant Extraction

Seeds of London Rocket *Sisymbrium irio* were acquired from the local market of Baghdad, Iraq. Seeds were washed, dried and ground to fine powder by utilizing an electric blender. 50 g of seeds powder was used in200 ml of 90% ethanol, methanol

and water independently. The jars were incubated at room temperature for 2 days with shaking at 140 rpm on an orbital shaker. The rough concentrate was separated by utilizing 0.22µ filter unit. The ethanol and methanol filtrate let dry at room temperature while fluid filtrate dried and thought by rotating evaporator. Dried rough concentrate was broken down in DMSO independently to the last grouping of 300 mg/ml (Kamel, Mohamed et al. 2017).

Detection the ability of bacteria for biofilm formation (Test tube method)(Kristensen and Christensen 1982)**.**

This method included inoculation 5 ml of (Trypton soya broth) with particular isolates and incubated for 48 hours at 37 ±°C, after that, the contents of the tubes were removed carefully and added the crystal violet stain (1%) to each tube for 15 minutes then rinsed the tubes and let tubes to dry at room temperature (20-25)°C. The result was read by notice the formation of biofilm as a layer at the internal wall of tubes by naked eye and comprise with the negative control (tube contains Tsb medium without inoculation), thickness and color of layer consider a parameter of bacterial ability for .biofilm formation

Antibacterial activity by agar well diffusion method

The agar plate surface is inoculated by spreading100 µL of the microbial inoculum over the entire agar surface. Then, a hole with a diameter of 6 to 8 mm is punched aseptically with a sterile cork borer, and a volume (100 µL) of the extract solution at desired concentration is introduced into the well. Then, agar plates are incubated overnight at 37°C. The antibacterial activity determined by measurement of inhibition zone (Balouiri, Sadiki et al. 2016).

Determination of Total Flavonoids

Total flavonoids content was determined by spectrophotometer. The ethanol, metabolic and aqueous extract of *S. irio* as rutin (flavonoids standard) equivalent by aluminum chloride colorimetric method as described by (Sakanaka, Tachibana et al. 2005). Then, the absorbance was measured at 450 nm with a spectrometer. A similar procedure was applied to six concentrations (2.5, 5, 10, 20, 40 and 80 µg), and from which a standard curve was prepared (Figure 1). The total flavonoids content was determined using a curve-fitting equation of the standard curve.

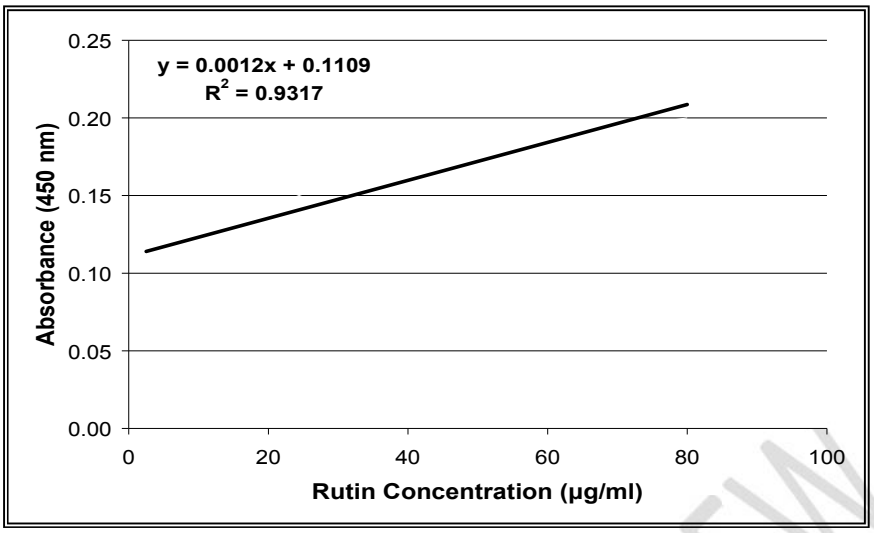

Figure 1: Standard curve for determination of rutin concentration.

Assessment of Anti-oxidant Activity *in vitro*

As Anti-oxidant activity of the *S. irio* extracts was assessed through previously describe by (Fu, Chen et al. 2010)**.**

Results:

Isolation and identification bacteria from Specimens

Seventy-nine samples obtained from school children by swabbing for detecting the presence or absence of skin infection (Impetigo) at Al-Yarmook teaching hospital in Baghdad during the period (from Oct. -2017 to Mar. - 2018).

All the isolates were identified by using cultural, morphological and biochemical tests(Cruckshank, Dagaid et al. 1975). Results showed that 71 out of 79 samples gave a positive culture while 8 samples were negative as shown in the table (1).

Table(1) percentage of positive and negative culture of specimens.

Culture	No.of Isolates	Percentage %

1-	Positive	71	89.88
2-	Negative	8	10.12

Impetigo is the most common bacterial infection in children. This acute, highly contagious infection of the superficial layers of the epidermis is primarily caused by *Staphylococcus aureus* or *Streptococcus pyogenes*(Rørtveit, Skutlaberg et al. 2011).

Bacterial isolates	No. of Isolates	Percentage %
Sterptococcus pyogens	17	23.94
Staphylococcus aureus	54	76.06
Total	71	100

The microscopical examination showed that all 71 (100 %) isolates were classified as Gram-positive bacteria. This result agreed with the result reported by (Templer and Brito 2009) who recorded that bacterial skin infections are a common problem encountered in clinical practice and most skin bacterial infections are caused by gram-positive bacteria, including *S. aureus*, group and, *S. viridans*.

After performing biochemical tests and API 20 for bacterial isolates, results showed that 17(23.94%) isolates were identified as *Streptococcus pyrogens* and 54(76.06 %) isolates identified as *Staphylococcus aureus* as shown in the table (2)

Table (2) percentage of bacterial isolates

Our results agreed with (Pereira 2014) who recorded impetigo is a common cutaneous infection that is especially prevalent in children. Historically, impetigo is caused by Gram-positive cocci and the most frequently isolated pathogen is *S. aureus.*

(Wu, Wang et al. 2010) who reported impetigo infections were common among Chinese children but his findings showed that *S. aureus* wasn't the main causative agent.

S. aureus is of special concern because of its ability to cause a number of life-threatening conditions and its widening resistance to currently available antimicrobial drugs which produces virulence factors, including various exotoxins and adhesions, which are associated with a variety of symptoms caused by its infections(Aung, Urushibara et al. 2011).

Antibiotics Sensitivity

Antibiotics on *S. aureus* isolates were tested by using standard disk diffusion method and results were obtained compared with the NCCLs .Results illustrated in tables (3)

It had been noticed, from the table (table 3) a range of resistance of *S. aureus* isolates which gave very high resistance percentage to (penicillin (100%), cephalexin 100%, Cefotaxime 100%, Gentamycine100% and Nalidixic acid 100%) and gave varied resistance percentage to Amoxicillin +Clavunic acid (100%),Vancomycin (93.2%), Rifampin (29.7%) and Clindamycin (11.4%). while gave no resistance to ciprofloxacin. The prevalence of antibiotic resistance bacteria therapeutic problems that could be explained by the influence of excessive inappropriate antibiotic used, antibiotic resistance among pathogenic bacteria that cause infections(D'Costa, King et al. 2011).

Table (3) The percentage of antibiotics resistance of *S. aureus* isolates.

Antibiotics		**Resistance**	
		No.	**Percentage (%)**
Penicillin	P	**44**	100%
Cephalexin	CL	44	100%
Cefotaxime	CTX	44	100%

Vancomycin	VA	41	93.2 %
Clindamycin	CD	5	11.4%
Tetracycline	TE	44	100%
Amoxicillin +Clavunic acid	AMC	44	100%
Methicillin	M	48	100 %
Ciprofloxacin	CIP	0	0%
Rifampin	RA	13	29.7%

Certain types of bacteria are inherently resistant to the effect of particular antibiotic, this is called innate or intrinsic resistance, while resistance of other bacteria to antibiotic types considered as acquired resistance which may result through spontaneous mutation or the acquisition of new genetic information(Giedraitienė, Vitkauskienė et al. 2011, Munita and Arias 2016)

Biofilm has an active role in bacterial pathogenesity because bacteria embedded in a matrix of host proteins and microbial slime, which provided a home for organism and promote increased drug resistance thus antibiotic less effective in biofilm cells than in planktonic cells.

Assessment of total flavonoid : three types of extracts were used for total flavonoids. Water, Ethanol, and Methanol extracts all gave positive results as in figure 1. The best absorbance was on Methanol extract followed by water extract then, ethanol.

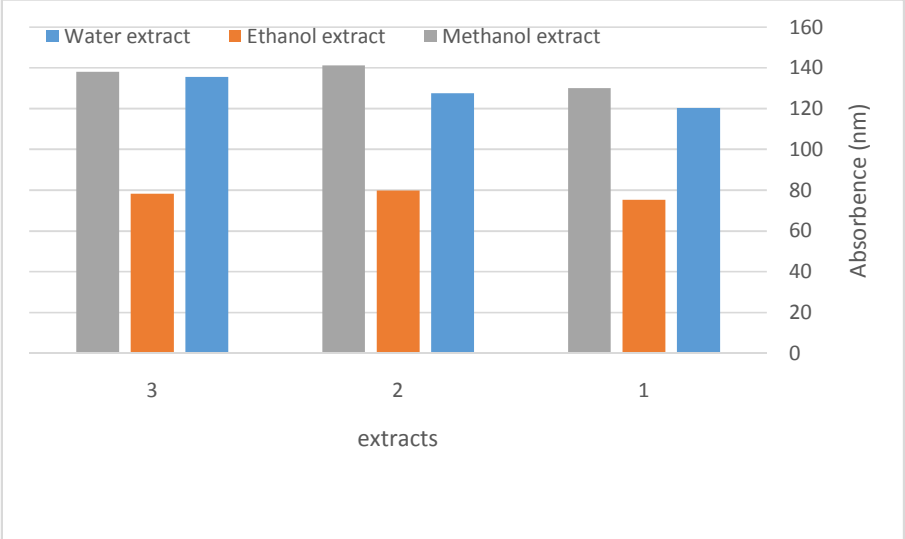

Figure 1: Total flavonoids for water, methanol and ethanol extracts

Assessment of reductive ability: In a seven concentrations of three different plant extracts the absorbance were parallel with the concentration. However, the methanol extract was the highest, ethanol extract followed then, the water extract was the last. Figure 2

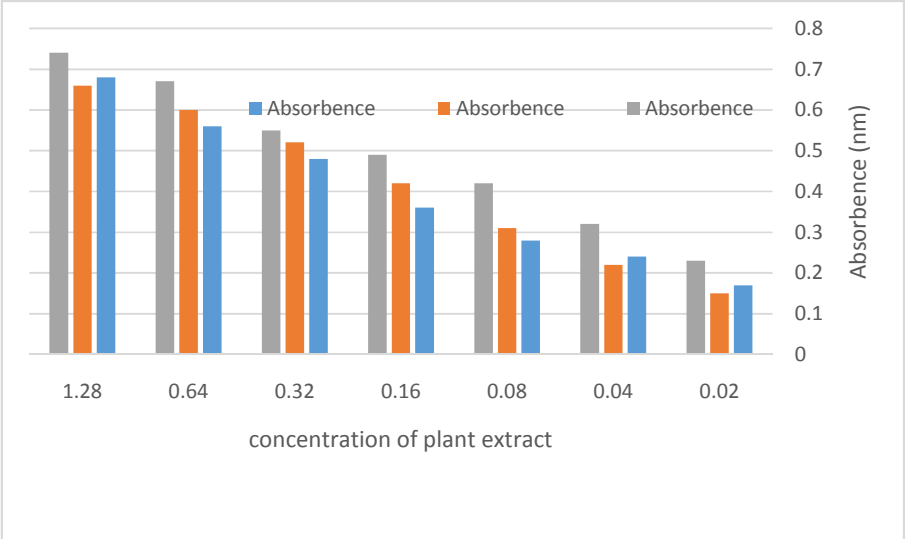

Figure 2: Absorbance of Different concentrations of water, methanol, and ethanol extracts

References :

Al-Eisawi, D. M. (1982). "List of Jordan vascular plants." Amman **152**: 79-182.

Aung, M. S., N. Urushibara, M. Kawaguchiya, T. S. Aung, S. Mya, T. San, K. M. Nwe and N. Kobayashi (2011). "Virulence factors and genetic characteristics of methicillin-resistant and-susceptible Staphylococcus aureus isolates in Myanmar." Microbial Drug Resistance **17**(4): 525-535.

Balouiri, M., M. Sadiki and S. K. Ibnsouda (2016). "Methods for in vitro evaluating antimicrobial activity: A review." Journal of pharmaceutical analysis **6**(2): 71-79.

Cole, R. A. (1976). "Isothiocyanates, nitriles and thiocyanates as products of autolysis of glucosinolates in Cruciferae." Phytochemistry **15**(5): 759-762.

Cruckshank, R., J. Dagaid, B. Morimion and R. Swain (1975). Medical Microbiology Vol. 11&, Churchill Living Stone, Edinburgh, London and New York.

D'Costa, V. M., C. E. King, L. Kalan, M. Morar, W. W. Sung, C. Schwarz, D. Froese, G. Zazula, F. Calmels and R. Debruyne (2011). "Antibiotic resistance is ancient." Nature **477**(7365): 457.

Del Pero de Martinez, M. and I. Aguinagalde (1982). "Contribucion al estudio de los flavonoides en el genero Sisymbrium." Parodiana **1**: 287-299.

Fu, W., J. Chen, Y. Cai, Y. Lei, L. Chen, L. Pei, D. Zhou, X. Liang and J. Ruan (2010). "Antioxidant, free radical scavenging, anti-inflammatory and hepatoprotective potential of the extract from Parathelypteris nipponica (Franch. et Sav.) Ching." Journal of ethnopharmacology **130**(3): 521-528.

Giedraitienė, A., A. Vitkauskienė, R. Naginienė and A. Pavilonis (2011). "Antibiotic resistance mechanisms of clinically important bacteria." Medicina **47**(3): 19.

Griffiths, D. W., N. Deighton, A. N. E. Birch, B. Patrian, R. Baur and E. Städler (2001). "Identification of glucosinolates on the leaf surface of plants from the Cruciferae and other closely related species." Phytochemistry **57**(5): 693-700.

Kamel, B. M., A. Mohamed, M. El Sherbiny, K. Abed and M. Abd-Rabou (2017). "Tribological properties of graphene nanosheets as an additive in calcium grease." Journal of Dispersion Science and Technology **38**(10): 1495-1500.

Kristensen, G. H. and F. R. Christensen (1982). "Application of cryo-cut method for measurements of biofilm thickness." Water Research **16**(12): 1619-1621.

Munita, J. and C. Arias (2016). "Mechanisms of antibiotic."

Patty Ghazvini*, P. T., Kristen Woodberry, Edouard Nerette Jr, and Hermán Powery II. (2017). "Impetigo in the Pediatric Population;." J Dermatolog Clin Res 5(1): 1092-1099 **5**(1): 1092-1099.

Pereira, L. B. (2014). "Impetigo-review." Anais brasileiros de dermatologia **89**(2): 293-299.

Rørtveit, S., D. H. Skutlaberg, N. Langeland and G. Rortveit (2011). "Impetigo in a population over 8.5 years: incidence, fusidic acid resistance and molecular characteristics." Journal of antimicrobial chemotherapy **66**(6): 1360-1364.

Sakanaka, S., Y. Tachibana, N. Ishihara and L. R. Juneja (2005). "Antioxidant properties of casein calcium peptides and their effects on lipid oxidation in beef homogenates." Journal of agricultural and food chemistry **53**(2): 464-468.

Shinwari, M. I. and M. A. Khan (2000). "Folk use of medicinal herbs of Margalla hills national park, Islamabad." Journal of ethnopharmacology **69**(1): 45-56.

Templer, S. J. and M. O. Brito (2009). "Bacterial skin and soft tissue infections." Hospital physician **26**: 9-16.

Wu, D., Q. Wang, Y. Yang, W. Geng, Q. Wang, S. Yu, K. Yao, L. Yuan and X. Shen (2010). "Epidemiology and molecular characteristics of community-associated methicillin-resistant and methicillin-susceptible Staphylococcus aureus from skin/soft tissue infections in a children's hospital in Beijing, China." Diagnostic microbiology and infectious disease **67**(1): 1-8.