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Biofilm, Antimicrobial & Reductive Ability of London Rocket *S. irio*

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Authors' contributions

This work was carried out in collaboration among all authors. Authors RNAC and RMA done the analytical work. Author NKA carried out experimental design and revised the manuscript. Author SAB done typing of the article and some statistical interpretation. All authors read and approved the final manuscript.

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Short Research Article

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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 detectability of pathogenic *S. aureus* isolates which isolated from the skin of children had impetigo for biofilm formation. The result illustrated the high per cent 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.

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1. 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 [1]. 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 [2] and glucosinolates [3,4]. It has a sharp flavour 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 [5]. 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* [6,7]. Antimicrobial, biofilm and reductive capacity were determined for this vital plant.

2. MATERIALS AND METHODS

2.1 Plant Extraction

Seeds of London Rocket *Sisymbrium irio* were acquired from the local market of Baghdad, Iraq. Seeds were washed, dried and ground to a fine powder by utilizing an electric blender. 50 g of seeds powder was used in 200 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 0.22 µ filter unit. The ethanol and methanol filtrate let dry at room temperature while fluid filtrate dried and thought by the rotating evaporator. The dried rough concentrate was broken down in DMSO independently to the last grouping of 300 mg/ml [8].

Detection of the ability of bacteria for biofilm formation (Test tube method) [9].

This method included inoculation 5 ml of (Tryptone 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 the naked eye and comprise with the negative control (tube contains TSB medium without inoculation), thickness and colour of layer consider a parameter of bacterial ability for biofilm formation.

2.2 Antibacterial Activity by Agar Well Diffusion Methods

The agar plate surface is inoculated by spreading 100 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 [10].

2.3 Determination of Total Flavonoids

Total flavonoids content was determined by spectrophotometer. The ethanol, metabolic and aqueous extract of *S. mario* as rutin (flavonoids standard) equivalent by aluminium chloride colourimetric method as described by Sakanaka et al. [11]. 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 (Fig. 1). The total flavonoids content was determined using a curve-fitting equation of the standard curve.

2.4 Assessment of Anti-oxidant Activity *in vitro*

Anti-oxidant activity of the *S. irio* extracts was assessed through As previously described by Fu et al. [12].

3. RESULTS

3.1 Isolation and Identification of Bacteria from Specimens

Seventy-nine samples obtained from school children by swabbing for detecting the presence

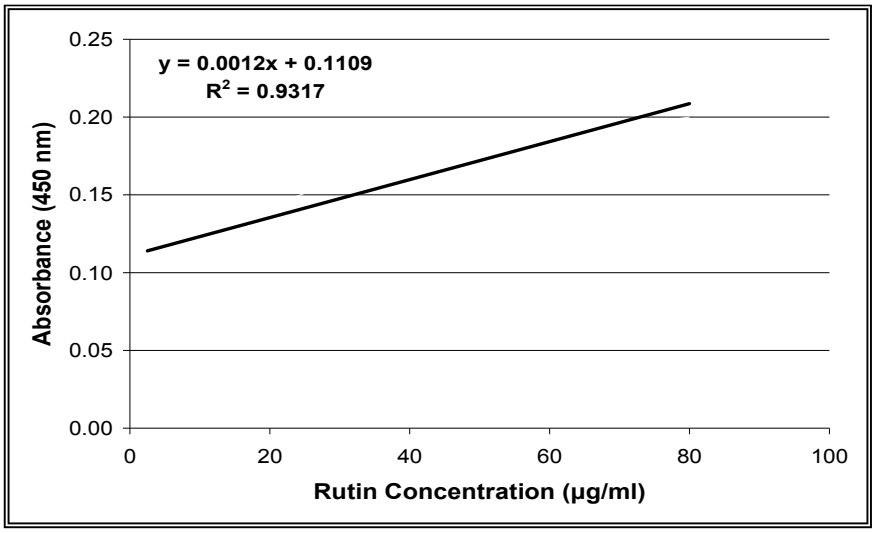

Fig. 1. Standard curve for determination of rutin concentration

Table 1. Percentage of the positive and negative culture of specimens

Culture		No. of isolates	Percentage %
1-	Positive	71	89.88
2-	Negative	8	10.12

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 [13]. Results showed that 71 out of 79 samples gave a positive culture while 8 samples were negative as shown in the Table 1.

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* [7].

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 [14] 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.

Our results agreed with [15] 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 et al. [16] 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 [17].

3.2 Antibiotics Sensitivity

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

Table 2. Percentage of bacterial isolates

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

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%

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%, Gentamycine 100% 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 resistant bacteria therapeutic problems that could be explained by the influence of excessive inappropriate antibiotic used, antibiotic resistance among pathogenic bacteria that cause infections [18].

Certain types of bacteria are inherently resistant to the effect of the particular antibiotic, this is called innate or intrinsic resistance, while the resistance of other bacteria to antibiotic types considered as acquired resistance which may result through spontaneous mutation or the acquisition of new genetic information [19,20].

Biofilm has an active role in bacterial pathogenicity because of bacteria embedded in a matrix of host proteins and microbial slime, which provided a home for the 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 Fig. 2. The best absorbance was on Methanol extract followed by water extract then, ethanol.

Assessment of reductive ability: In 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. Fig. 3.

4. DISCUSSION

The medicinal plant extracts containing a high percentage of active compounds such as polyphenols, flavonoids and glucosinolates. The impact of natural extracts as antioxidant showed its high ability to scavenging the free radicals in the laboratory, which may have antibacterial, antioxidant and anticancer properties [21]. Plants are responsible for their therapeutic effect against cancer, diabetes, tissue inflammatory and cardiovascular diseases. It was found that high total phenols content increase antioxidant activity and there is a linear correlation between phenolic content and antioxidant activity [22]. London Rocket extract contains significant subordinate metabolite such as flavonoids, alkaloids ,tannins, phenols, saponins, ascorbic acid and essential oil especially erucic acids were concentrate in high concentration which responsible for the antibacterial activity [23]. Glucosinolates were institute to have several biological activities including anticarcinogenic, antifungal, antibacterial plus their antioxidant accomplishment [24]. The main glucosinolate in

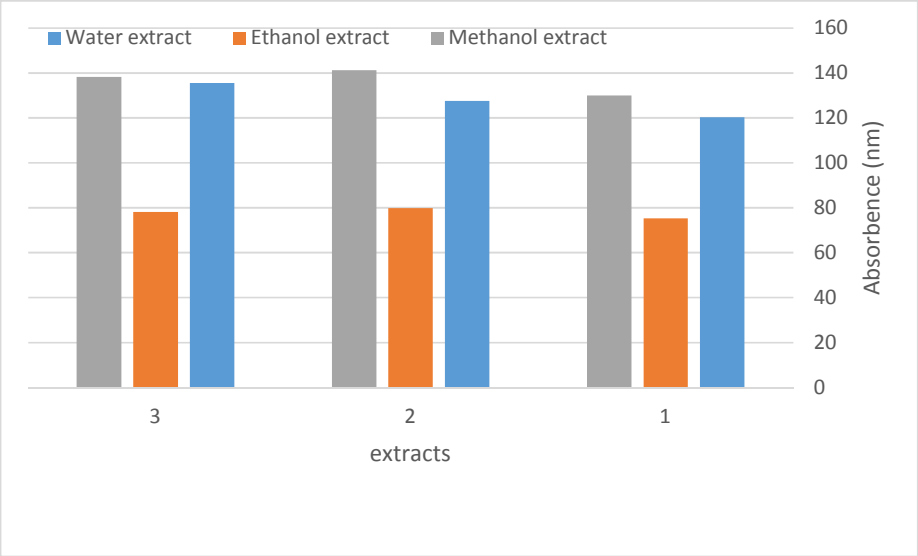

Fig. 2. Total flavonoids for water, methanol and ethanol extracts

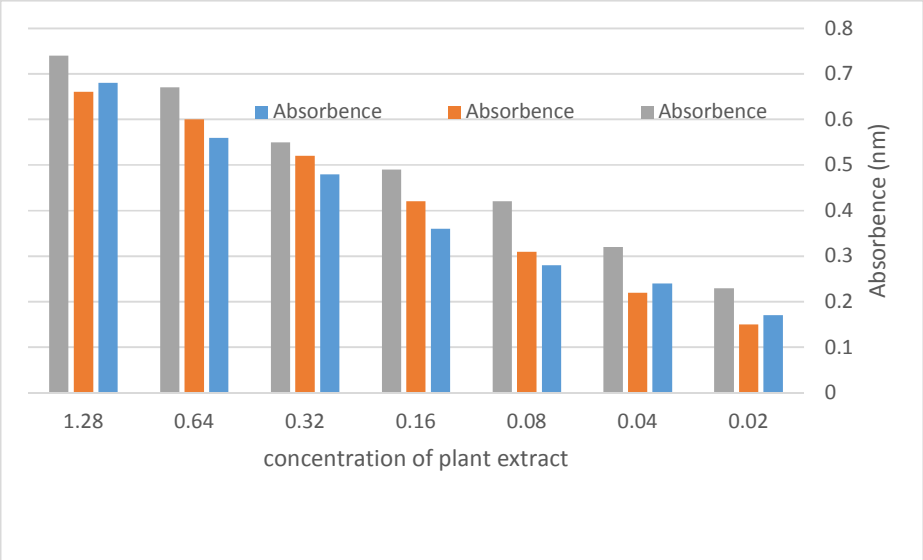

Fig. 3. Absorbance of different concentrations of water, methanol, and ethanol extracts

seeds is Erucin, which is hypothetically capable of protecting cells against oxidative stress via three instruments: (i (initiation of phase II enzymes, (ii) rummaging hydrogen peroxide and alkyl hydroperoxides accumulated in cells and peripheral blood and (iii) acting as a precursor of sulforaphane, a potent inducers detoxifying electrophiles and increase cellular antioxidant fortifications [25]. Also, the extract of the London Rocket has promising pharmacological efficacies and ensures the presence of bioactive components responsible for beneficial effects.

5. CONCLUSION

Our findings support its use in traditional medicine as antimicrobial bioagent and highlight the potential of this food plant for its possible clinical use [26]. The flavonoids and phenolic compounds in the plant have been reported to exert multiple biological effects including antioxidant, free radical scavenging abilities, anti-inflammatory, anticarcinogenic etc. [21], by the ability to electron donation capacity which reflecting the reducing power of bioactive

compounds was associated with the antioxidant activity [27].

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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