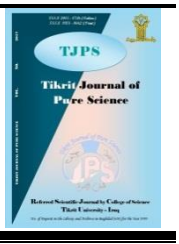
TJPS

TIKRIT JOURNAL OF PURE SCIENCE

Journal Homepage: http://main.tu-jo.com/ojs/index.php/TJPS/index



Antimicrobial activity of *Loranthus europaeus* L. and *Lawsonia inermis* L. extracts against clinical Methicillin-resistant *Staphylococcus aureus* isolated from boil infections

Abbas yaseen Hasan[1] , Thuraya Kadhum Ismael [2]

[1]*Department of Biology , College of Science , University of Diyala , Diyala , Iraq*
[2]*Department of Biology , College of Science , University of Tikrit , Tikrit , Iraq*

ARTICLE INFO.

Article history:

-Received: 12 / 12 / 2017
-Accepted: 12 / 3 / 2018
-Available online: / / 2018

Keywords: Boil Infection, *S. aureus*, extracts, *Loranthus europaeus* L. ,*Lawsonia inermis* L.

Corresponding Author:

Name: ThurayaKadhum Ismael

E-mail:
Thuraiyakadhum@gmail.com

Tel:

Abstract

This study included to collect 106 samples boil swab from patients, males and females, at Baqubah Teaching Hospital whose ages ranged between (1-80) years old. At the period of 1st July 2016 to the end of January 2017. Aimed to identification of bacterial isolates and determine the antibiotic resistance of isolates. In addition to determine the activity of aqueous and alcohol of *Loranthus europaeus* and *Lawsonia inermis* extracts on bacterial isolates. The results showed a significant difference ($p < 0.05$) in the percentage of infected females (58.49%) compared to the infected males (41.51%). The study involved isolation and diagnosis of (41) (38.67%) of *Staphylococcus aureus*, of which, 5 (12.20%) were methicillin-resistant isolates. Bacterial isolates were identified initially depending on cultural and microscopic features and biochemical reactions test. Antibiotics sensitivity of *Staph. aureus* conducted for 14 β-lactam antibiotic groups. All of bacterial isolates (100%) were resistant to ceftriaxone, cefotaxime and cefepime, and (92.68%) to cefoxitin and penicillin G, while (100%) of isolates were sensitive to Imipenem and Meropenem. Regarding plant extracts, various concentrations of hot aqueous extracts were prepared for each one of them (12.5, 25, 50, 100 and 200) mg/ml against methicillin-resistant *Staph. aureus*. When *Loranthus europaeus's* extract was used, the diameters of inhibition zone were (14.57, 15.28,16.00, 23.71 and 25.00 mm) while (0.00, 11.85, 18.00, 21.28 and 24.14 mm) when *Lawsonia inermis's* extract was used. Like aqueous extracts, same range of concentrations were prepared for alcoholic extracts. The diameters of inhibition zone were (7.57, 8.42, 10.57,13.28, 17.28), (11.14, 17.00, 20.85, 25.42, 28.42) when alcoholic extracts of *Loranthus europaeus* and *Lawsonia inermis*, respectively were used. Comparing the activity of hot and alcoholic aqueous extracts of the tested plants, alcoholic extracts of *Lawsonia inermis* had a higher inhibitory effect against *S. aureus* isolates.

Introduction

Boils are deep inflammation of hair follicles, leading to accumulation of pus and dead tissues. Boils appear as red swollen nodules in many parts of the body caused by *Staph. Aureus* [1] and especially Methicillin - resistant *Staph. aureus* (MRSA), the most serious pathogen of the skin and soft tissue. This disease is often recurrent and may spread among family members, where some healthy people are considered as carriers of *Staph. aureus*[2]. Various tissue diseases caused by *Staph. aureus* are nosocomial infections spreading through USA, and Europe[3]. However, *Staph. aureus* now appears in the general public as well as the healthy, with 10% infection in some hospitals and nearly 65% in others[4]. Spreading of these infections and antibiotic resistance are due to the abuse and overuse of antibiotics as well as horizontal gene transfer among microbial community[5]. Resistance of *Staph. aureus* to antibiotics such as Oxacillin, Methicillin and β-lactam group antagonists is due to the production of β-lactamase enzymes. These enzymes are reducing the amount of Penicillin Binding Protein (PBP2), and

enhancing bacterial resistance to antibiotics. This binding protein, which is encoded by *mecA* gene, has a low affinity for the β-lactam antagonists, lead to resistance for all β -lactam antagonists[6]. These bacteria cause killing and rapid diseases such as necrotizing pneumonia and necrotizing fasciitis[7]. Biologists refereed to the activity of medical herbs as effective antimicrobial agents and as alternative treatment for antibiotics. The side effects of these antibiotics on human health also encouraged many people to use alternative medical herbs as effective therapeutic substances[8]. The interest of companies to manufacture pharmaceuticals and medical drugs from raw plants has increased because of the importance of plant extracts as safe substance compared to antibiotics, in addition to their availability, accessibility at cheapest price, stability, and bioactivity[9]. *Lowsonia inermis* plant extracts were used as traditional treatment among Arabs for different skin infections[10] The leaves, flowers, seeds, roots and bark were used as ancient traditional medicine for various diseases, such as arthritis, headache, ulcer, leprosy, heart disease, and diabetes [11]. It is also used as an antifungal, antiviral and antiparasital agent[12]. *Loranthus europaeus* is used as treatment for eczema, foot ulcer, granulomatous tumor, and tuberculosis[13]. It has also been used since ancient times to treat various dermatologic infections including genital warts, psoriasis, basal cell carcinoma, Baghdad boil or sore, as well as boils and abscesses [14].This study aimed to evaluate of the antimicrobial effect of *Loranthus europaeus* and *Lawsonia inermis* extracts against clinical isolates of Methicillin resistant *Staph. aureus* isolated from boil infection.

Materials and Methods

A total of (106) swabs were collected from boil infection patients who visited the consultative clinic of Baqubah teaching hospital in Diyala governorate for the period from the first of July 2016 to the end of January 2017. The ages of patients ranged from (1-80) years old for both sexes. The samples were cultured on the blood agar and incubated at 37 °C for 24 hrs. The pure single colonies were then transferred and cultured on the Mannitol Salt Agar and incubated at 37 ° C for 24-48 hrs. The appearance of yellow golden medium is an indication of Mannitol fermentation by these bacteria[15].Initially, *Staph. aureus* were identified depending on the cultural, microscopic, and biochemical tests[16]. using API Staph System. [17], and Vitek 2 device, which consists of 64 biochemical tests.

Antibiotic susceptibility testing

The disk diffusion method was done using Mueller Hinton agar medium. colonies (3-5) with the same morphologic features growing on the blood agar were transferred by sterile-inoculating loop to a tube containing 5 ml of physiological saline solution. Turbidity of bacterial growth was compared with standard McFarland No 0.5, which gives an approximate number of cells (1.5×10^8 cell/ml). Aliquots, 100µl of bacterial suspension was transferred by the micropipette, and spread by swab on overall the surface of the Muller-Hinton Agar. Then, the inoculated agar plates were left to dry at room temperature for (10-15) min. The antibiotic discs listed in table (1) were then applied into the Muller-Hinton Agar using sterile forceps. The agar plates incubated at 37 °C for 24 hours. The diameter of the inhibition zones around the antibiotic discs was measured and compared with the standard chart to decide wither bacteria are sensitive or resistant. The isolates of MRSA were identified by measuring the sensitivity of the bacteria to the Oxacillin[18].

Table (1) List of Antibiotics used in Study

Antibiotic type	Symbol of antibiotic	Concentration µg /ml
Amoxicillin / Clavulonic acid	AMC	20/10
Ampicillin /sub lactam	SAM	10/10 µg
Augmentin/ Sublactum	AMS	30/15
Cefachlor	CFC	30
Cefazolin	CZ	30
Cefoxitin	FOX	30
Ceftriaxone	CRO	30
Cefotaxime	CTX	30
Cefepime	FEP	30
Imipenem	IPM	10
Meropenem	MEM	10
Oxacillin	OX	1
Penicillin G	P	10 IU
Pipracillin/tazobactam	TPZ	100/10
Amoxicillin/ Clavulonic acid	AMC	20/10

Preparation of Hot Aqueous Extract

According to Chanda and Parekh method[19], 50 g of dry plant was weighed and dissolved in 500 ml of boiled distilled water. After cooling to 60°C and placed in a 1000 ml flask, the solution was placed on the hot plate-Magnetic stirrer for 2 hrs. Following that, the mixture was filtered using medical gauze, then filtrated by Whatman No.1 filter papers. The supernatant was transferred into centrifuge tubes at a speed of 3000 cycles/min for 10 min. After that, the supernatant was evaporated by rotary evaporator under low pressure at (40-50°) C. The remainder extract was dried using dishes with a large surface area in the oven at of 40°C until the water was evaporated completely. The powder of aqueous extract was placed in tightly sealed glass tubes. The tubes were labeled and kept in the refrigerator at 4°C until use.

Preparation of alcoholic extract

According to Jameela method [20], 50 g of dry plant was weighed and dissolved in 500 ml of ethanol 70% and put in a 1000 ml tightly-closed flask and kept in opaque place for 7 days. The mixture was filtered using gauze, and complete the extraction according the previous aqueous extract.

Effect of *Loranthus europaeus* and *Lawsonia inermis* on bacterial growth

Well diffusion assay was used according to Al-Okaili method[21]. Bacterial suspension was prepared from (3-5) colonies with the same morphologic features

growing on the blood agar. These colonies transferred by standard wire loop into a tube containing 5 ml of physiological saline solution. Turbidity of bacterial growth was compared to the standard McFarland No 0.5, which gives an approximate number of cells (1.5×10^8 cell/ml).Aliquots 100 µl of bacterial suspension was transferred by the micropipette, and spread by swab overall agar plate. The inoculated agar plates were left to dry at room temperature for 5 min. Wells with 6 mm in diameter were made on agar.

After that, 20 µl of the extract were added to each well at pre-determined concentrations (12.5, 25, 50, 100, and 200 mg/ml) and left for 1 h to ensure proper distribution. Distilled water and phosphate buffer saline were added as control for boiled aqueous and alcoholic extract, respectively. Following that, agar plates were incubated at 37°C for 24 hrs. The activity of each plant extract was determined by measuring the diameter of inhibition zone in millimeters by a standard scale.

Result and Discussion

The data of current study showed that the number of females infected with boils 62(58.49%) was more than male 44(41.51%) as shown in (table 2). These results were in agreement with Shallcross[22]..The variation in infection number and percentage between the sexes may be due to the quantity and quality of the normal flora of skin in different sexes[23]. Furthermore, different methods of sample collection in addition to the physiological condition of humans especially depression, secretion of female hormones may play a role in decreasing the number of males infection[24].

Table (2) Distribution of patients with boils by sex

Gender	Number	Percentage %
Male	44	41.51%
Female	62	58.49%
Total	106	100%

Forty one isolates (38.67%) of *S. aureus* were identified from a total of (106) samples. *S. aureus* were the dominant bacterial isolates. This findings were in line with the study of Ibler and Kromann[2]. In our study, 5 (12.20%) isolates were methicillin-resistant *Staph. aureus* and 36 (87.80%) identified as methicillin-sensitive isolates (table 3). The results of our study are in agreement with study performed in Egypt by Abdel-Maksoud *et al*., [25], in which methicillin-resistant *S. aureus* was (11.5%) of 343 samples.

A mass of pus is accumulated in hair follicles after *S. aureus* infection which usually occurs after puberty. This mass develops and opens either spontaneously or as a result of external pressure [26].

The reason for the dominance of *Staph. aureus* infection is due to its ability to develop antibiotic-resistant and production of β-lactamase enzymes. Moreover, *S. aureus* rapidly multiply and possess many virulence factors such as production of enzymes: catalase, coagulase, deoxyribonuclease, lipase, hemolysin, leucocidin, PVL, enterotoxin and hyaluronidase. Capsule and surface proteins play an important role in the invasion of host tissues and the spread of bacteria. The β-Lactamase-produced enzymes contribute to bacterial resistance to many antibiotics[27].

Table (3) Classification of *Staph. aureus* according to its resistance to Methicillin

Bacteria	Number	%Percentage
Staph. aureus	36	%87.80
Methicillin resistant *Staph. aureus*	5	%12.20
Total	41	%100

In the current study, the sensitivity of (41) isolates of *S. aureus* were examined to 14 commonly used β-lactum antibiotics. All *S. aureus* isolates were 100% resistance to ceftriaxone, cefotaxime and cefepime, and 92.68% of isolates were resistant cefoxitin and penicillin G.

Our findings are in agreement with the results of Al-Timeemi [28]. The percentage of bacterial resistance was moderate toward some antibiotics, such as Ampicillin/Sulbactam and Augmentin (consists of two antibiotics Amoxicillin and Clavulanic acid). Amoxicillin inhibit cell wall synthesis, while clavulanic acid encounter with penicillinase production. On the other hand, the isolates showed low resistance to Augmentin/Sulbactam, cefachlor, cefazolin, and oxacillin (85.54%, 36.58%, 26.83%, and 12.19%), respectively. All *Staph. aureus* were sensitive to Carbapenems, which include Meropenem and Imipenem. The broad spectrum of these antibiotics associated with their effectivity against many skin and soft tissue pathogens[29]. the Gram-positive and Gram-negative bacteria [30]. table (4), β-lactam antibiotics inhibit bacterial cell wall synthesis by interfering with peptidoglycan synthesis. Resistance to β-lactam antibiotics is possibly due to production of β-lactamase which break the β-lactam ring or reduce the affinity of antibiotic binding with the bacterial penicillin-binding proteins. Moreover, the binding site could be modified by adenylation, phosphorylation and acetylation enzymes, or a chromosomal mutation. all of these reasons lead to loss of the antibiotic affinity to bind with the target protein and reduction of bacterial cell permeability to antibiotics [31].

Table(4) Susceptibility of *Staph. aureus* to β -lactam antibiotics

Antibiotic	Number of isolations	Sensitivity %	Number of isolates	Resistance %
Ampicillin/Sulbactam	13	31.71	28	68.29
Amoxicillin/Clavulanic acid	16	39.03	25	60.97
Augmentin/Sulbactam	17	41.46	24	58.54
Cefachlor	26	63.42	15	36.58
Ceftriaxone	0.00	0.00	41	100.00
Cefotaxime	0.00	0.00	41	100.00
Cefoxitin	3	7.32	38	92.68
Cefazolin	30	73.17	11	26.83
Cefepime	0.00	0.00	41	100.00
Imipenem	41	100.00	0.00	0.00
Meropenem	41	100.00	0.00	0.00
Oxacillin	36	87.81	5	12.19
Piperacillin/Tazobactam	24	58.53	17	41.46
Penicillin G	3	7.32	38	92.68

In this study, the inhibitory effect of hot and alcoholic extracts of *Loranthus europaeus* and *Lawsonia inermis* was determined against *Staph. aureus*. These extracts showed variable inhibitory effect against the tested bacterial isolates. Table (4) showed that the hot aqueous extract of the *Loranthus europaeus* was significantly more effective at the highest concentration (200 mg/ml) followed by the concentrations (100, 50, and 25 mg/ml) with which, the inhibition zones were(123.7, 16.00 and 15.28 mm), respectively. While, the lowest effect was at the concentration 12.5 mg/ml with which, the diameter of inhibition zone was 14.57 mm. There was no significant difference in bacterial growth inhibition between the concentrations 12.5 and 25 mg/ml at ($p<0.05$).

Table (5) Effect of aqueous extract of *Loranthus europeaus* on *Staph. aureus*

Concentration . mg/ml	Inhibition zone diameter Mean± St. Error
0.00	0.00 ± 0
12.5	14.5 ± 0.99
25	15.28 ± 0.99
50	16.00 ± 0.98
100	23.71 ± 0.78
200	25.00 ± 0.81

In addition, the results showed that the alcoholic extract of *Loranthus europaeus* had an inhibitory effect on the methicillin-resistant *S. aureus* bacteria, which was 17.28 mm at the concentration of 200 mg/ml, followed by the concentrations (100, 50, 25 mg/ml) with which, the diameter of inhibition zones were (13.28 , 10.57, 8.42 mm), as shown in table (5). Also, there was no significant difference in *Staph. aureus* inhibition between the concentrations (12.5, 25.50 mg/ml), while the differences were significant ($p <0.05$) among other concentrations.

Table (6) Effect of Alcoholic extract of *Loranthus europeans on Staph. aureus*

Concentration (mg/ml)	Inhibition Zone(mm) (Mean± St. Error)
0.00	± 0.00 0.00
12.5	7.57± 0.53
25	8.42± 0.78
50	10.57± 0.97
100	13.28± 0.75
200	17.28 ± 0.98

There was a significant difference between the hot aqueous and alcoholic extracts at ($P <0.05$).

Our study was on contrary to the findings of Salem (2017) [32]. who indicated that the inhibitory concentration of hot *Loranthus europaeus*'s extract against *Staph. aureus* was at a concentration of 40 mg with diameter of inhibition zone of (14 mm), while it was (17 mm) were produced when alcoholic extract of the same plant used at the same concentration. Antimicrobial activity could be attributed to the active substances of boiled extract of *Loranthus europaeus* such as tannins, terpenoids, flavonoids and alkaloids phyto-constituents, which are considered as antibacterial compounds. However, Uzochukwu Osadebe [33]., and EL-Sharquie [34], reported that the alcoholic extract contains polysaccharides and aldehydes, which accelerate wound healing and have antimicrobial activity, preventing secondary bacterial infections. The study of Al-Rubaee [35]. also showed that the wounds of laboratory rabbits infected with *S. aureus* were healed when treated with *Loranthus europaeus* extract. Tannins and Flavonoides, existed in *Loranthus europaeus*, deposit in the bacterial cell membrane, and inhibit the action of metabolic enzymes leading to bacterial death [36].

This work showed that the hot aqueous extract of *Lawsonia inermis* effectively inhibited the growth of methicillin-resistant *S. aureus* producing inhibition zone (24.14mm) at 200 mg /mL followed by (21.28, 18.00, 11.85 mm) when (100, 50, 25 mg/ml) respectively, were used. while no effect at the concentration of 12.5 mg/ml was noticed, table (6).

Table (7) Inhibitory effect of aqueous extract of *Lawsonia inermis* against *Staph. aureus*

Concentration . mg/ml	Inhibition zone diameter Mean ± St. Error
0.00	0.00 ± 0.00
12.5	0.00 ± 0.00
25	11.85 ± 0.88
50	18.00 ± 0.81
100	21.28 ± 0.95
200	24.14 ± 0.89

In addition, the alcoholic extract of *Lawsonia inermis* had an inhibitory effect on *Staph. aureus* with inhibition zone of 28.42 mm at 200 mg/ml. With the following concentrations (100, 50, 25 mg/ml), the inhibition zones were (25.42, 20.85, 17.00 mm), respectively. In addition, the diameter of inhibition zone was 11.14 mm at the concentration of 12.5 mg/ml (table 7).

Table (8) Inhibitory effect of alcoholic extract of *Lawsonia inermis* on *S. aureus*

Concentration . mg/ml	Inhibition zone diameter Mean± St. Error
0.00	± 0.00 0.00
12.5	11.14± 0.89
25	17.00± 0.81
50	20.85± 0.95
100	25.42± 0.88
200	28.42± 0.98

Comparing the results of inhibitory effect of aqueous and alcoholic extract of *Lawsonia inermis*, it was found that there was a significant difference (P<0.05). This may be due to the effect of alcohol in the extraction of active substances[37] Al-Hamooshi at Mosul University reported that the alcoholic extract of *Lawsonia inermis* had a higher inhibition activity than its aqueous extract .

Alcohol is a good solvent of active substances such as phenols, an effective substances which form a complex with extracellular substances of the cell wall disrupt the cell membrane permeability of the bacteria. In addition, alcohol acts on protein denaturation agent in the bacterial cell and suppress the action of enzymes that responsible for metabolic reactions[38].

The antimicrobial effect of alcoholic extract of *Lawsonia inermis's* leaves against *Staph. aureus* was due to its compositions: comarins, toxic oxidizing compounds, which are highly pass through the cell wall of bacteria[39]. In addition, it contains lowsone, mannite, flavonoid and tannin compounds which inhibit the action of transporting enzymes and proteins of the cell membrane[40].

Jahan [47] reported that *Lawsonia inermis* extract, prepared from various parts of the plant and with different solutions, showed antimicrobial activity. Sudharameshwari *et al*[41] mentioned that the activity of *Lawsonia inermis* is due to the presence of active substances such as alkaloids, phenols, anthocyanin, xanthoprotein, carboxylic acid, cumarins and sterols.

In the present study, we found that the boiled aqueous extract has higher inhibitory effect than the alcoholic extract of *Loranthus europaeus*. However, the alcoholic extract of *Lawsonia inermis* has higher inhibition ability than its aqueous extract against MRSA. When comparing between the most effective inhibitor among the two extracts, there was a preference in affectivity of the alcoholic extract of *Lawsonia inermis*. It is also observed that the active ingredients of medical plants are affected by their quantity and quality[42].

When using ethanol in *Lawsonia inermis* extract, its anti-microbial activity is increased. Ethanol may increase the concentration of the active substance compared to its aqueous extract in water. In addition, the chemical components of the culture medium may oppose or reduce the effectiveness of the extract against bacteria[43].

Reference

1- El-Gilany, Abdel-Hady, and Fathy, H. (2009). Risk factors of recurrent furunculosis. Dermatology Online Journal 15 (1): 16 .

2-Ibler, K. S. and Kromann ,B .C.(2014) . Recurrent furunculosis – challenges and management: a review.J. Clinical, Cosmetic and Investigational Dermatology . (7): 59–64.

3-Moxnes, J.F.; de Blasio, B.F.; Leegaard, T.M. and Moen, A.E.(2013). Methicillin-resistant *Staphylococcus aureus* (MRSA) is increasing in Norway: a time series analysis of reported MRSA and methicillin-sensitive *S. aureus* cases 1997-2010 .J.PLoS One. 8(8): 70499

4- Hafeez, R.; Chughtai, S. A. and Aslam, M. (2004). Prevalence and Antimicrobial Susceptibility Methicillin Resistant *Staphylococcus aureus* (MRSA). Int. J. of Pathology. 2(1):10-15.

5- Jensen, S. O.; Lyon, B. R. (2009). Genetics of antimicrobial resistance in. *Staphylococcus aureus* .J. Future Microbiol. 4 (5): 565- 582.

6-Chan, C. L.; Gillbert , A.; Basuino, L. Hamitton, K. and Chatterjee, S. S.(2016). PBP4 mediates high-level resistance to new generation cephalosporins in *Staphylococcus aureus*. J. Antimicrob. Agents Chemother. ACC. 00358 -16 :1-24.

7-**Boyle-Vavra,** S.and Daum, R. S .(2007). Community-acquired methicillin-resistant *Staphylococcus aureus*: the role of Panton-Valentine leucocidin. J. Lab. Invest. 87 (1): 3–9.

8-El-Mahmood, M. and Amedh, J. M. (2007). In vitro antibacterial activity of Parkiabiglobosa (Jacq) root bark extract against some microorganisms associated with urinary tract infections. Afri . J. Biotech. 6(11):1272-1275.

9-Hosseinzadeh, S.; Jafarikukhdan, A.; Hosseini, A. and Armand, R. (2015). The Application of Medicinal Plants in Traditional and Modern Medicine: A Review of Thymus vulgaris. Int. J. of Clinical Medicine. 6: 635-642.

10-AL-Jubory,S.Y.(2010). Extraction and Identification of some active compounds from *Alhagimaurorum*, *Lawsonia inermis* and *Rhuscoriari* Plants and Evaluating some of their Biological effects .Ph.D.Thesis . college of science KufaUni .

11-Akter, A.; Neela, F. A.; Khan, M. S. I.; Islam, M. S. and Alam, M. F. (2010). Screening of Ethanol, Petroleum Ether and Chloroform Extracts of Medicinal Plants, *Lawsonia inermis* L. and *Mimosa pudica* L. for Antibacterial Activity. Indian J. of Pharmaceutical Sciences . 388-392.

12-Bairagi, G. B.; Kabra , A. O. and Mandade, R. J. (2011). Anthelmintic Activity of *Citrus medica* L. leaves in Indian Adult Earth worm. Int. J. of Pharm. Tech. Research. 3(2): 664-667

13-Osadebe, P.o.; Okide ,G.B. and Akabogu, I.C. (2004). Study on anti-diabetic activity of crude methanolic extracts of *loranthusmicranthus*(linn) sourced from five different host trees. Journal of Ethnopharmacology. 95:133-138

14- Habif, T. P. (2016). Sexually Transmitted Viral Infection. In: Clinical Dermatology, Chapter 11, 6th Edition, Mosby Inc. Toronto. 418-447.

15--Brooks, G. I.; Butel, J. S. and Morse, S. A. (2010). Jawetz, Melnick and Adelberg's Medical Microbiology .22nd ed. McGraw – Hill Inc. New York.

16-Macfaddin, J. F. (2000). Biochemical test for identification of Medical bacteria 3rded. The williams and wilkins. Baltimor . USA.

17-Kloos,W. E. and Schleifer, K. H. (1975). Isolation and Characterization of *Staphylococcus* from human skin .II Description of four new species :*S. Aureus, S. capitis, S. hominis and S. simulans*. J. Bacteriol. 25:62-79 .

18- CLSI. (2008). Performance Standaras for Antimicrobial Susceptibility Testing, 15th Informational Suoppolement, M100-S15. Wayne, PA: clincal and Laboratory Standards Institutes.

19-Parekh, J. and Chanada, S. (2007) .In vitro antimicrobial activity and analysis of some Indian medicinal plant . Turk. J. Bio. 13:53-58.

20-Jameela, M.; Mohideen, A.; Sunitha, K. and Narayanan, M. (2011). Antimicrobial Activities of there Medicinal plant Extracts against fish pathogens. Int. J. of Biological Technology. 2 (2): 57 – 60

21-Al-Akili, Adnan Hanoun Abbas. (2002). Study of the effect of acetic acid and some plant extracts in the growth of bacterial burns injuries. Master Thesis, Faculty of Science, University of Mustansiriya

22-Shallcross, L. J.; Hayward, A.C.; Johnson, A.M. and Petersen, I. (2015). Incidence and recurrence of boils and abscesses within the first year. British J. of General Practice.e 65(639):e668-76 .

23-Al-Zoubi, M.; Al-Tayyar, S. I.; Ali, H. E.; Al Jabali, A. and Khudairat S. (2015). Antimicrobial susceptibility pattern of *Staphylococcus aureus*isolated from clinical specimens in Northern area of Jordan . Iran. J.Micro. 7 (5): 265-272.

24-Joshua, A.; Zeichner, M. D.; BIllary, E.; Baldwin, MD.; Cfran, E. Cook, M.D.; Dlawrence, F.; Eichenfield, M.D.; Dsheila, S.; Fallon, M.D.; Edavid, A.; Rodriguez, M.D.(2017). Emerging Issues in Adult Female Acne. J.of clinical and aesthetic dermatology January .10(1).

25-Abdel-Maksoud, M.; El-Shokry, M.; Ismail, G.; Hafez, S.; El-Kholy, A.; Attia, E and Talaat1, M. (2016). Methicillin - Resistant *Staphylococcus aureus* Recovered from Healthcare- and Community-Associated Infections in Egypt. Int. J. of Bacteriology.(1):1-5.

26-Demos, M.; McLeod, M. P. and Nouri, K. (2012). Recurrent furunculosis: a review of the literature. British Association of Dermatologists .(167):725–732.

27-Qiu, J.; Wang,D.; Zhang, Y.; Dong, J.; Wang, J. and Niu, X.(2013). Molcular modeling reveals the novel inhibition mechanism and binding mode of three natural compounds to staphylococcal alpha-hemolysin. J. *Plos one*. 8(11) : 80- 97.

28-Tamimi, Ahmed IssaJaafar. (2012). Genetic study of Staphylococcus sp. resistant to vancomycin. Master Thesis / College of Education for Pure Sciences - Diyala University.

29-Douglas, N. F. (2016). Meropenem in the treatment of complicated skin and soft tissue infections. Therapeutics and Clinical Risk Management. 2(4):401-415.

30-Al-Marjani, Mohammed Faraj. (2011). Antibiotics . Bacterial Resistance to Antibiotics. Dar Dijla, First Edition (1): 255. Amman

31-Levinson, W. (2014). Review of medical microbiology and Immunology .McGraw – Hill. New York. 13th ed. P.8.

32- Salem, Dina Kamal (2017). Studying the inhibitory effect of ginger extract and gluten against the growth of some bacterial and pathogenic fungi. Tikrit Journal of Pure Sciences. 22 (5): 55-63

33-Osadebe, P.O.and Uzochukwu, I.C. (2007). Comparative evaluation of antidiabetic activities of Flavonoids extract and crudemethanol extract of *Loranthusmicranthus*parasitic on *Kola acuminate.* J. Pharmaceut. Appl. Sci. 4: 2-7.

34-EL- Sharquie, K.; Noaimi, A. A.; Saleh, B. M.; Sharara, Z. A.; Al-Salam, W. S. (2017). Topical 40% Loranthuseuropaeus Ointment as an Alternative Medicine in the Treatment of Acute Cutaneous Leishmaniasis versus Topical 25% Podophyllin Solution. J. of Cosmetics, Dermatological Sciences and Applications. 7: 148-163.

35-Al-Rubaee,A.Methaq; Jawad, H. Ala Alden and Al-Diab, M. Jasim. (2006). Effects Of *Loranthus Europaeus* Seeds On Pyogenic Inflammation. J. Missan Researches. 2(4).

36-Mills ,E. J.; Jacques, D.; Dan, P. and Gideon K.,(2006). Herbal Medicines in pregnancy and lactation. An Evidence – Based Approach. Taylar and Francis group. London and New York .

37-Hamoushi, Rawa Mahmoud and Mahmoud, Abdul Razzaq Khader. (2008). The inhibitory effect of leaf leaves of *Lowson*
ia inermis on Staph aureus species isolated from different skin infections. Journal of Science Rafidain. 19 (3): 19-1
38-Salami, Nebras Yahya Abdullah (2000). Study of the effect of *Myrtuscommunis* L. and garlic *Allium sativum* in Pseudomonas aeruginosa bacteria outside and in vivo. Master Thesis, Faculty of Science, University of Kufa.
39--Henry, J.B. (2001). Clinical diagnosis and Management by laboratory methods. 20th ed. V.3, pp 1088, 1106, 1123, Saunders Company.
40-Jahan, F; Rubina, L,; Kumar, V and Junaid , M. (2011). Evaluation of antimicrobial activity of plant extracts on antibioticsusceptible and resistant *Staphylococcus aureus*strains. J. Chem. Pharm. Res. 3(4): 777-789.
41-Sudharameshwari, K. and Radhika, J. (2007). Antibacterial screening of Aegle Marmelos, *Lawsonia inermis* and *Albizzialibbeck*. Afr. J. Trad. CAM . 4 (2): 199 – 204.
42-Varel,I. and Vincent, H. (2002). Carvacrol and thymol reduce swine waste odor and pathogenes: stability of oils. Curr. Microbial. 44: 38-43
43-Tabbene, O.; Slimene, I.B.; Djebali, K.; Mangoni, M.L.; Urdaci, M.C. and Limam, F. (2009). Optimization of medium composition for the production of antimicrobial activity by *Bacillus subtilis*, J. B38 Biotechnol. 25(5): 1267-1274.

الفاعلية الضد مايكروبية لمستخلصات نباتي حب الدبق والحناء على بكتريا المكورات العنقودية الذهبية المقاومة للميثيسيلين والمعزولة من أخماج الدمامل

عباس ياسين حسن[1] ، ثريا كاظم إسماعيل[2]

[1]قسم علوم الحياة ، كلية العلوم ، جامعة ديالى ، ديالى ، العراق

[2]قسم علوم الحياة ، كلية العلوم ، جامعة تكريت ، تكريت ، العراق

الملخص

تضمنت هذه الدراسة جمع (106) عينة من مسحات المرضى المصابون بالدمل ومن كلا الجنسين في مستشفى بعقوبة التعليمي حيث كانت أعمارهم تتراوح بين (1-80) سنة للفترة من بداية شهر تموز 2016 حتى نهاية شهر كانون الثاني 2017. الهدف من الدراسة هو الكشف عن العزلات البكتيرية وتحديد مقاومتها للمضادات الحياتية. بالاضافة الى تحديد الفاعلية للمستخلصات المائية والكحولية لنباتي حب الدبق والحناء على العزلات البكتيرية. بينت النتائج أن هناك فروقاً معنوية ($P > 0.05$) في نسبة الاناث المصابة (58.49%) مقارنة بنسبة الذكور المصابين (41.51%). تضمنت الدراسة عزل وتشخيص (41) (38.67%) من بكتريا المكورات العنقودية الذهبية، كان من ضمنها (5) (12.20%) مقاومة للميثيسيلين. تم التعرف على العزلات البكتيرية إعتمادا على الصفات التشخيصية والزرعية والفحوصات الكيميائية. تم قياس حساسية (14) مضاد حياتي من مجموعة البيتالاكتام. جميع العزلات البكتيرية كانت 100% مقاومة ل ceftriaxone, cefotaxime and cefepime, و 92.68% لمضاد cefoxitin و penicillin G ، بينما كانت العزلات 100% حساسة ل Imipenem و Meropenem. فيما يخص المستخلصات النباتية فقد تم تحضير المستخلصات المائية الحارة بتراكيز مختلفة (12.5, 25, 50, 100 and 200) mg/ml ضد المكورات العنقودية الذهبية المقاومة للميثيسيلين عند إستخدام نبات حب الدبق كانت أقطار التثبيط (14.57, 15.28,16.00, 23.71, 25.00 mm) بينما (0.00, 11.85, 18.00, 21.28 and 24.14 mm) عند إستخدام مستخلصات نبات الحناء. تم تحضير المستخلصات الكحولية بنفس التراكيز للمستخلصات النباتية وكانت أقطار التثبيط (7.57, 8.42, 10.57,13.28, 17.28) ، (11.14, 17.00, 20.85, 25.42, 28.42) عند استخدام المستخلصات الكحولية لنبات حب الدبق والحناء على التوالي.

بمقارنة فاعلية المستخلصات المائية الحارة والكحولية للنباتات التي تم إختبارها فقد كان للمستخلصات الكحولية لنبات الحناء أعلى تأثير تثبيطي ضد بكتريا المكورات العنقودية الذهبية.