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Antibacterial Activity of a novel Lectin produced by bee honey *Bifidobacterium adolescentis* against Multidrug Resistant *Salmonella typhi*

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Abstract

Lectins are glycoproteins attached to carbohydrate noncovalently. Probiotic bacterial lectins have immunomodulatory effect, antibacterial and antifungal effect, supporting consortium and maintaining healthy coordination among microbes or between microbes and host. Colonization of Bifidobacterium in gastro-intestine of host are essential, the attachment of Bifidobacterium to the mucosa of host was mediated by lectins. This study aimed to isolation of *B. adolescentis* from bee honey, detection, extraction and purification of lectin produced by *B. adolescentis* and investigation of the antimicrobial activity of crude and purified *B. adolescentis* lectin. Sealed honeybee colonies were obtained for *B. adolescentis* isolation directly from honey. Honeybee colonies were provided from honeybee colonies from Hella city- Iraq during May 2017. Six isolates of *B. adolescentis* isolated were obtained by cultivation of bee honey on De Man, Rogosa and Sharpe (MRS) and were identified by API32CH. Two methods were performed for lectin screening detection: semi-quantitative detection and quantitative hemagglutination assay. The results revealed that, human erythrocytes AB^+ and O^+ and sheep erythrocytes gave hemagglutination activities with all *B. adolescentis* but it was higher against O^+ human erythrocytes. Lectin was extracted and purified and the protein content of lectin was estimated and was 0.13 mg/ ml. Thirteen isolates of *Salmonella typhi* isolated from stool samples obtained from patients admitted to Al-Escan Hospital / Baghdad-Iraq and were diagnosed by VITEK-2 system then tested for their susceptibility to antibiotics via disc diffusion method. *Salmonella typhi* appeared to be multidrug resistant. Crude and purified *B. adolescentis* lectin were subjected to antibacterial activity against *S. typhi* pathogen. The results indicated that *B. adolescentis* lectin at both concentrations, 32 and 64 µg/ml possesses significant antibacterial activity against multidrug resistant *S. typhi* as compared with control ($P<0.05$). The antibacterial activity of crude and purified lectin in concentration 64 µg/ml was higher than antibacterial activity of concentration 32 µg/ml($P<0.05$). Furthermore, the antimicrobial activity of purified lectin was significantly higher than crude lectin, $P<0.05$. In conclusions *B. adolescentis* found to be one of the components of bee honey. Bee honey *B. adolescentis* isolates produced a lectin but they varied in production amounts. Lectin hemagglutinate human blood groups AB^+ and O^+ and sheep erythrocytes but it was higher active against O^+ human erythrocytes. The crude and purified lectin had antibacterial activity against multidrug resistance *S. typhi. Bifidobacterium adolescentis* lectin at 32 and 64 µg/ml possesses significant antibacterial activity against multidrug resistant *S. typhi* as compared with control but antibacterial activity of crude and purified lectin at 64 µg/ml was higher than that of concentration 32 µg/ml. Moreover, antimicrobial activity of purified lectin was significantly higher than crude lectin.

Keywords, lectin, antibacterial activity, *B. adolescentis* ,*Salmonella typhi*, bee honey.

INTRODUCTION

Lectins are glycoproteins attached to carbohydrate noncovalently. They are molecules that have a high affinity to sugar moieties of some other compounds. Lectins are distinctive molecules able to recognize the targets on the cells and engage in several roles in biological detection involving cells, carbohydrates, and proteins. Lectins also involved in affection and attachment of viruses and bacteria to their cognate receptors [1]. The majority of lectins are widely distributed and have no enzymatic activity. They could attached to carbohydrate in soluble form or to the glycolipid or glycoprotein possessing moiety of carbohydrate. For this reason, They usually agglutinate some kind of cells like red blood cells of human or animal origin [2]. There are about 20 families of lectins have been discovered yet now and are participated in metabolism regulation and they worldwide participant in biotechnology procedures [3, 4, 5, 6]. The relationships connecting probiotic bacteria and macroorganisms are regulated mostly by the action of probiotic bacterial lectins [7]. Moreover, probiotic bacterial lectins have immunomodulatory effect, antibacterial and antifungal effect, supporting consortium and maintaining healthy coordination among microbes or between microbes and host [8]. As immunomodulatory molecule, lectins induce TNF-α production by lymphocytes of peripheral blood of humans as well as modulate the migration of macrophage [9, 10]. Lectins proved to be antifungal agent and act both as fungicidal and fungistatic toward clinical *Candida albicans* resistant to nystatin. Furthermore, lectins have anti-biofilm of both *Staphylococcus aureus* and *C. albicans* but anti-biofilm property depend largely on the isolated strain and kind of lectin [10]. Phylogenetic analysis based on partial sequences of 16S rRNA gene sequences of honey bee gut microbiota revealed presence of *Bifidobacterium* spp. hence the presence of this bacterium in bees honey [11]. Colonization of Bifidobacterium in gastro-intestine of host are essential, the attachment of Bifidobacterium to the

mucosa of host and assimilation of carbohydrate was mediated by lectins[12] . This study aimed to isolation of *B. adolescentis* from bee honey, detection, extraction and purification of lectin produced by *B. adolescentis* and investigation of the antimicrobial activity of crude and purified *B. adolescentis* lectin.

MATERIALS AND METHODS

Bacterial Isolates

Sealed honeybee colonies were bring to the laboratory for *B. adolescentis* isolation directly from honey. Honeybee colonies were provided from honeybee colonies in Hella city- Iraq during May 2017. Six isolates of *B. adolescentis* isolated were obtained by cultivation of bee honey on De Man, Rogosa and Sharpe (MRS) and identified by API32CH Biomerieux (France). Thirteen isolates of *S. typhi* isolated from stool samples obtained from patients admitted to Al-Escan Hospital / Baghdad-Iraq and were diagnosed by VITEK-2 system at the same period.

B. adolescentis lectin Production detection

Two methods were used for lectin screening detection: semi-quantitative detection and quantitative hemagglutination assay.

1-Semi-Quantitative detection

The semi-quantitative screening was done on microscopic glass slide as following: 25µl of bacterial suspension at dilution 10^9 was mixed with 25 µl of 0.02M Phosphate Buffer Saline (PBS) pH 7.2 on glass slide, then 25 µl of blood suspension for sheep and human erythrocytes types AB^+ and O^+ at a concentration of 3%, blending the mixture well by wooden chopsticks and then moved the glass slide and gently examined near the light source to note the agglutination. Agglutination within 5 minutes referred to positive result. The control was PBS with blood without bacterial suspension [13].

2- Quantitative Hemagglutination Assay

The quantitative screening was done in microtiter plate as following: a serial two-fold dilution of bacterial suspension or lectin solution 50 µl in microtiter U-plates was mixed with 50 µl 0.02M PBS pH 7.2 was mixed with the same volume of a 3% suspension of sheep and human erythrocytes in the same buffer and incubated at 37°C for 2 hours. The activity was expressed as hemagglutination units (HU). One HU was defined as the inverse of the highest dilution still capable of causing agglutination. The control was PBS with blood without bacterial suspension or lectin solution [14].

Extraction of Lectin

The bacterial isolate was grown on Colonization Factors Antigens medium (CFA) composed of the following (g/L):10g gasamino acid, 1.5g Yeast extract, 0.05g$MgSO4$ and 0.005g $MnCl_2$at 37°C for 24 hour, then the cells was harvested by centrifugation at 8000rpm for 30 min, washed twice and re-suspended in 0.02 MPBS pH 7.2. Cells were disrupted by glass beads for 50 min at 4°C using the vortex. Residual whole cells and cell membrane fragments were removed by centrifugation 8000 rpm for 20 min. The hemagglutination activity of lectin and protein concentration was measured for the resulting crude cell extract [15].

Purification of Lectin

The crude cell extract was fractionated with saturation ammonium sulfate at concentrations 30-55% and the precipitate obtained after centrifugation at 8000 rpm for 30 min was suspended in 0.02M PBS pH 7.0 and the hemagglutination activity and protein concentration were measured. The dialyzed protein was applied to a DEAE - cellulose column (2.5×20cm) previously equilibrated with the same buffer. The protein was washed with the same buffer and eluted with a salt gradient containing 0.1–0.5M NaCl [16] The hemagglutination activity for each fraction was assayed as described above. The fractions that revealed significant peak of activity were mixed together and lyophilized.

Estimation of Protein Content

The protein content of lectin was estimated by using the method of Bradford (1976)[17] and using bovine serum albumin as a standard.

Antibiotic Sensitivity Test

Salmonella typhi isolates were tested for their susceptibility to some potential antimicrobial agents including some β-lactams, aminoglycosides, tetracycline and quinolones. Conventional disk diffusion method [18] was performed according to the CLSI (2013) instructions [19]. Table 1 illustrates the antibiotic discs, disc potency and manufacturing company of antimicrobial agents used in this study.

Table 1: Antibiotic discs used for *S. typhi* susceptibility test

Id	Antbiotic Discs	Code	Disc Potency (µg/disc)	Manufacturing Company
1.	Amoxicillin-clavulanic acid	AUG	20/10	Mast
2.	Ampicillin-sulbactam	SAM	10/10	Oxoid
3.	Cefazolin	CZ	30	Mast
4.	Cefepime	CPM	30	Mast
5.	Cefotaxime	CTX	30	Mast
6.	Cefoxitin	FOX	30	Mast
7.	Ceftazidim	CAZ	30	Mast
8.	Ciprofloxacin	CIP	5	Mast
9.	Gentamicin	GM	10	Mast
10.	Nalidixic acid	NA	30	Mast
11.	Temocillin	TEM	30	Mast
12.	Tetracycline	TE	30	Bioanalyse
13.	Tobramycin	TN	10	Mast

Antibiotic susceptibility test was done to *S. typhi* isolates used in this study according to Kirby and Bauer (1966).

Antibacterial Activity of crude and purified lectin

The crude and purified lectin produced by *B. adolescentis* B 5, the potential producer of lectin as tested by semi-quantitative detection and quantitative hemagglutination, were subjected to antibacterial activity against *S. typhi* pathogen. The concentrations of crude and purified lectin were 32 and 64 µg/ml. Agar well diffusion method was used to detect antibacterial activity of lectin according to Batdorj *et al.*(2006)[20].

Statistical Analysis
Results were analyzed as the mean ± standard deviation (SD). The intergroup variation was assessed by one way analysis of variance (ANOVA) P<0.05 using sigma state statistical software.

RESULTS AND DISCUSSION
Lectin Production
1-Semi-Quantitative Analysis of *B. adolescentis* lectin production
The results revealed that human erythrocytes AB^+ and O^+ and sheep erythrocytes gave hemagglutination activities with all *B. adolescentis* but sheep erythrocytes gave lower hemagglutination activities. The isolate *B. adolescentis* B 5 showed higher hemagglutination level (+ + + + +) with O^+ blood group (table 2). Blood group O^+ was found the best among the other blood groups, then AB^+ blood group.

2- Quantitative Analysis for lectin production from *B. adolescentis*
The hemagglutination activities in microtiter plate were revealed with all *B. adolescentis* isolates and *B. adolescentis* B 5 showed higher hemagglutination value against O^+ blood group then AB^+ blood group in the second rank, while sheep blood showed lower values (table 2) therefore; these results demonstrated that hemagglutination activity was not blood-type specific.

Purification of Lectin
The purification procedure of lectin production from *B. adolescentis* B5 included two steps [21] started with ammonium sulfate saturation for 50%, followed by ion exchange chromatography by DEAE-cellulose column. An ammonium sulfate fractionation revealed that 50% saturation led to increase the specific activity to 296.65 U/mg (table 3). Ammonium sulfate was chosen as precipitating agent in this study due to its high solubility in water and produces high ionic strength. Increase in ionic strength decreases the protein solubility. Desalting was done by using the dialysis with phosphate buffer saline and the dialysis was performed in the cold condition to prevent possible denaturation of the protein. This step led to remove ammonium sulfate from the sample. After dialysis step, the sample was applied to DEAE -cellulose column. A gradient concentrations of NaCl from 0.1 to 0.5M were used in elution process and led to appear four peaks of proteins and only one peak showed hemagglutination activity (figure 1) in this step 13% the yield of lectin and 6.066 a purification fold. The protein content of purified lectin was estimated and was 0.13 mg/ ml.

Table 2.Hemaaglutination activity of *B. adolescentis* lectin by microscopic glass slide and microtiter plate methods

Isolate No.	Degree of Hemagglutination			Titer of Hemagglutination		
	Sheep	Human AB+	Human O+	Sheep	Human AB+	Human O+
B1	+	+	++++	8	32	64
B2	+	++	+++	8	16	64
B3	++	++	+++	2	16	32
B4	+	+	++	4	8	32
B5	+++	++++	+++++	32	64	128
B6	++	+++	++++	16	64	64

Table 3: Steps of *B. adolescentis* lectin purification

Purification Steps	Volume (ml)	Protein Con. (mg/ml)	Hem.Activity (U/ml)	Specific Activity(U/mg)	Total Activity(HU)	Purification Fold	Yield (%)
Crude Extract	100	3.37	389	114.836	38900	1	100
Ammonium sulphate saturation	40	2.69	798	296.65	31920	2.583	40
DEAE cellulose	13	1.49	1028	696.644	13364	6.066	13

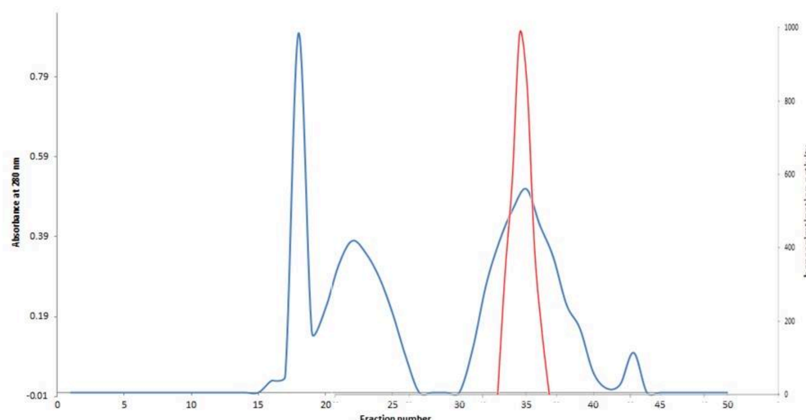

Figure 1: Ion Exchange Chromatography on DEAE -Cellulose Column for Purification of *B. adolescentis* lectin. (red)Lectin activity, (blue) Absorbance at 280nm

Antibiotic Sensitivity Test

Tested *Salmonella typhi* isolates appeared to be resistant to 4-9 antimicrobial agents out of 13 making them as multi-drug resistant isolates. Most of them are resistant to more than half of the test antimicrobial agents. Out of 13 *Salmonella typhi* isolates 8(62%) are resistant to cefazolin, 7(54%) to cefoxitin, 11(85%) to ciprofloxacin, 8(62%) to nalidixic acid and7(54%) to tobramycin (table 4). Multidrug resistance phenotype is predominant in many bacterial species of both Gram positive and Gram negative [22, 23, 24] and carry resistance genes [25]. The need of antimicrobial alternatives is necessary. Lectin produced by *B. adolescentis* suggested to be antimicrobial alternatives. Crude and purified *B. adolescentis* lectin were tested for their anti- *S. typhi* activity in this study.

Table 4: Antibiotic susceptibility results of *Salmonella typhi* isolates

Antimicrobial agents	Susceptible Out of 13 isolates (%)	Resistant Out of 13 isolates (%)
Amoxicillin-clavulanic acid	9 (69)	4(31)
Ampicillin-sulbactam	7(54)	6(46)
Cefazolin	5(38)	8(62)
Cefepime	9(69)	4(31)
Cefotaxime	7(54)	6(46)
Cefoxitin	6(46)	7(54)
Ceftazidime	7(54)	6(46)
Ciprofloxacin	2(15)	11(85)
Gentamicin	9(69)	4(31)
Nalidixic acid	5(38)	8(62)
Temocillin	8(62)	5(38)
Tetracycline	7(54)	6(46)
Tobramycin	6(46)	7(54)

Antibacterial activity of crude and purified *B. adolescentis* lectin

The results indicated that *B. adolescentis* B5 lectin at both concentrations, 32 and 64 µg/ml possesses significant antibacterial activity against multidrug resistant *S. typhi* as compared with control (P<0.05). This result may be the first report to the antibacterial activity against pathogenic *S. typhi* of lectin produced by *B. adolescentis*. It was previously shown that, the lactobacillus and bifidobacterial lectins possess antifungal activities against clinical nystatin resistant *Candida albicans* strains. Lectins revealed destructive properties with respect to *C. albicans* and *Staphylococcus aureus* biofilms [10].

The antibacterial activity of crude and purified lectin in concentration 64 µg/ml was higher than antibacterial activity of concentration 32 µg/ml. This expected result when take in consideration the effect of concentration of lectin as occurs with the increasing of antibiotics concentration. When the antibiotic concentration raised the antibacterial effect will increase [26]. Furthermore, the antimicrobial activity of purified lectin was significantly higher than crude lectin, P<0.05 (table 5). The same effect was noticed when the *B. adolescentis* bacteriocin, bifidoadocin, was tested for its antibacterial activity. Purified bifidoadocin has a strong antibacterial activity as compare with crude. This may be attributed to the increase concentration of active ingredient, low contaminants and low inhibitors [25].

Table 5:Antibacterial Activity of crude and purified *B. adolescentis* lectin against multidrug resistance *S. typhi in vitro*

Isolates	Inhibition Zone Diameter (mm) Mean ± S.D				
	Crude Lectin		Purified lectin		Control
	32µg/ml	64µg/ml	32 µg/ml	64µg/ml	
S. typhi	7.92 ±1.54^{p1}	14.71 ±2.07^{p1}	13.15 ±1.54$^{p1\ p2}$	19.99 ±2.03$^{p1\ p2}$	0±0

P1: Probability compared to control p<0.05

P2:Probability compared to crude at the same concentration p<0.05

CONCLUSIONS

Bifidobacterium adolescentis found to be one of the components of bee honey. Bee honey *B. adolescentis* isolates produced a lectin but they varied in production amounts. *Bifidobacterium* lectin hemagglutinate human blood groups AB$^+$ and O$^+$ and sheep erythrocytes but it was higher active against O$^+$ human erythrocytes. The crude and purified lectin had antibacterial activity against multidrug resistance *S. typhi. Bifidobacterium adolescentis* lectin at 32 and 64 µg/ml possesses significant antibacterial activity against multidrug resistant *S. typhi* as compared with control but antibacterial activity of crude and purified lectin at 64 µg/ml was higher than antibacterial activity of concentration 32 µg/ml. Moreover, antimicrobial activity of purified lectin was significantly higher than crude lectin.

REFERENCES

1- Brudner, M Karpel, M. Lear,C. Chen, L. Yantosca, L.M. Scully, C. Sarraju, A. *et al* (2013). Bradley S. Schneider, ed. "Lectin-Dependent Enhancement of Ebola Virus Infection via Soluble and Transmembrane C-type Lectin Receptors". *PLoS ONE*. 8 (4 specific e code=e60838): e60838.

2- Lakhtin, V. M., Lakhtin, M. V., Pospelova, V. V., and Shenderov, B. A. (2006). Lactobacilli and bifidobacteria lectins as possible signal molecules regulating intra-and inter-population bacteria–bacteria and host–bacteria relationships. Part I. Methods of bacterial lectin isolation, physico-chemical characterization and some biological activity investigation. *Microb Ecol Health Dis*, *18*(1), 55-60.

3- Lakhtin VM, Lakhtin MV, Pospelova VV, Shenderov BA (2007) Lectins of Lactobacilli and Bifidobacteria. II. Probiotic Lectins of Lactobacilli and Bifidobacteria as Possible Signal Molecules Regulating Inter- and Intrapopulation Relationships Between Bacteria and Between Bacteria and the Host. *Microb Ecol Health Dis* 19: 153 - 157.

4- Lakhtin VM, Afanasiev SS, Aleshkin VA, Nesvizskyi UV, Lakhtin MV, Shubin VV, Cherepanova YV, Pospelova VV (2009) [Classification of lectins as universal regulator molecules of biological systems (in Russian)]. Vestnik Rossiiskoy Academii Meditsinskih Nauk No 3: 36-43. ISSN 0869-6047.

5- Lakhtin MV, Lakhtin VM, Aleshkin VA, Afanasiev SS, Aleshkin AV (2010) [Lectins and Enzymes in Biology and Medicine (in Russian)]. Moscow: "Dynasty" Publishing House, 496 pp. ISBN 978-5-98125-076-7.

6- Lakhtin V, Lakhtin M, Aleshkin V (2011) Lectins of Living Organisms. The Overview. *Anaerobe* 17: 452 - 455.

7- Lakhtin M, Lakhtin V, Aleshkin V, Afanasiev S (2011) Lectins of Beneficial Microbes: System Organization, Functioning and Functional Superfamily. *Beneficial Microbes* 2:155 – 165.

8- Lakhtin, M., Lakhtin, V., Bajrakova, A., Aleshkin, A., Afanasiev, S., and Aleshkin, V. (2012). Lectin Systems Imitating Probiotics: Potential and Prospects for Biotechnology and Medical Microbiology. In *Probiotics*. InTech.

9- Lakhtin MV, Alyoshkin VA, Lakhtin VM, Nesvizhsky YV, Afanasyev SS & Pospelova VV (2010) The Role of Lectins from Probiotic Microorganisms in Sustaining the Macroorganism (in Russian)]. Vestnik Rossiiskoy Academii Meditsinskih Nauk 2: 3-8. ISSN 0869-6047.
10- Lakhtin M, Aleshkin V, Lakhtin V, Afanasiev S, Pozhalostina L, Pospelova V (2010) Probiotic Lactobacillus and Bifidobacterial Lectins Against *Candida albicans* and *Staphylococcus aureus* Clinical Strains: New Class of Pathogen Biofilm Destructors. *Probiotics Antimicro Prot*. 2: 186-196.
11- Babendreier, D., Joller, D., Romeis, J., Bigler, F., and Widmer, F. (2007). Bacterial community structures in honeybee intestines and their response to two insecticidal proteins. *FEMS microbiol ecology*, *59*(3): 600-610.
12- Keita, A. Nishiyam, N.Y. Sugiyama, M. Takaki, T. Urashima, T. Fukiya, S. Yokota, A. Okada,N. Takao Mukai, T. (2017). *Bifidobacterium bifidum* Extracellular Sialidase Enhances Adhesion to the Mucosal Surface and Supports Carbohydrate. mBio. 8 (5): e00928-17.
13- Eshdatand, Y. and Sharon, N. "*Escherichia coli* Surface Lectins,"in Methods in Enzymology, edited by V. Ginsburg (1982), pp. 386 – 391.
14- Mirelmanand, D. Ofek, I. "Introduction to Microbial lectins and Agglutinins,"in Microbial Lectins and Agglutinins, edited by D. Mirelman (John Willey Sons,USA,1986).
15- Ali, A. N. (2014). Cytotoxic effect of the purified lectin from locally isolate *Acinetobacter baumannii* on Hep-2 tumor cell line. *J Biolo Agricul Health.*4(26), 168-173
16- Al-Moosawi, L.H. Al-Kakei, S.N.H. Laftah, A.R. and Musafer, H.K." Purification and characterization of lectin from *Acinetobacter baumannii* isolated from urinary catheter samples and its effect on some pathogenic bacteria"4th International Conference of Genetic and Environment Proceeding. July 23-30, 2016, Cairo, Egypt, pp. 795-804.
17- Bradford, M. A (1976). Rapid and sensitive method for the quantitation of microgram quantitities of protein utilizing the principle of protein dye binding. *Annal Biochem*.72, 248-254.
18- A.W. Bauer, W.M.M. Kirby, J.C. Sherris and M. Turck. (1966) Antibiotic susceptibility testing by a standardized single disk method. *Amer I Clin Pathol*.45, 493-496.
19- CLSI., 2013. Performance standards for antimicrobial susceptibility testing; Twenty-Third informational supplement. M100-S23.33: 1.
20- B. Batdorj, M. Dalgalarrondo,Y. Choieset,J. Pedroche,F.Metro and H. Prevost. (2006). Purification and characterization of two bacteriocins produced by lactic acid bacteria isolated from Mongolian airag. *J Appl Microbiol*.101, 837-848.
21- R.K. Scopes, Protein Purification: Principles and Practice. Springer-Verlag, New York, 1994.
22- Auda, I. G. Al-Kakei, S. N. and Hamed, S. L. (2013). Occurrence of CTX-M-I Type β-lactamases Gene in Certain Gram Negative Bacteria. *Iraqi postgrad Med J*. 12(2): 306-311.
23- Punpanich, W.; Tantichattanon, W.; Wongwatcharapaiboon, S. and Treeratweeraphong,V.(2008) In vitro susceptibility pattern of cephalosporin-resistant Gramnegative bacteria. *J Med Assoc Thai*. 91:21-27.
24- Raman G, Avendano E, Berger S, Menon V. Appropriate initial antibiotic therapy in hospitalized patients with gram-negative infections: systematic review and meta-analysis. BMC Infect Dis. 2015;15:395.
25- Mahdi, L. H.; Auda, I G.; Ali, I. M. Al-saadia, L.G. and Zwain, L.A. (2018). Antibacterial activity of a novel characterized and purified bacteriocin extracted from *Bifidobacterium adolescentis*. *Rev Med Microbiol*. 29(2):73-80.
26- Auda, I.G. Expression of *mexAB-OprM* Operon of Septicemic *Pseudomonas aeruginosa* in Relation to Antibiotic Resistance. College of Medicine AL- Nahrain University. Ph.D. thesis. 2011.