Table 4: Detection of multi-drug resistant *clinical strain (RCS)* by disc diffusion method

Antibiotics	*Staphylococcus epidermis*	*Escherichia coli*	*Staphylococcus aureus*	*Pseudomonas aeruginosa* s	*Klebcilla pneumonia*
Vancomycin 30µg	I	R	R	R	R
Amikacin 30µg	R	S	R	R	I
Cefaclor 30µg	S	R	R	R	R
Amoxicillin/ Clavulinic 30µg	I	R	R	R	R
Meropenem 30µg	S	S	R	S	S
Levofloxacin 30µg	S	I	S	S	I
Cefotrocin 30µg	R	R	R	R	R
Erythromycin 30µg	I	R	R	R	R
Gentamycin 10µg	R	R	I	R	R
Cefepime 30µg	S	R	R	I	I
Ciprofloxacin 5µg	R	R	R	S	I

Table (4) showed that highest resistance percentages were achieved by Cefotrocin. Meropenem showed the lowest resistance. Three strains were sensitive to Levoloxacin and intermediate to *E.coli* and *Klebcilla pneumonia*. Many reports are available on the antiviral, antibacterial, antifungal, anthelmintic, antimolluscal and anti-inflammatory properties of plants [16]. Some of these observations have helped in identifying the active principle responsible for such activities and in the developing drugs for the therapeutic use in human beings.

Table 5: Antibacterial activity of the licorice *Glycyrrhiza glabra* L. *against clinical strain (RCS)*

Extracts	***Staphylococcus epidermis***	***Micrococcus lutes***	***Staphylococcus aureus*(CRS)**	***Klebcilla pneumonia*(CRS)**	***Escherichia coli*(CRS)**	***Pseudomonas aeruginosas* (CRS)**	***Bacillus subtlus,***	***Staphylococcus epidermis*(CRS)**	***Staphylococcus aureu***
Methanol	35	20	23	28	-	26	23	22	25
Water	-	-	-	-	-	-	-	28	-
Ethanol	-	16	20	-	-	15	-	20	25

[17] Stated that *G. glabra* root extract showed antibacterial activity against S. aureus. Also [18] stated that the extracts of *Glycyrrhiza glabra* L. showed activity against S. aureus and can be used as raw materials for phytotherapy. Table (5) showed the different level of activity with different type of solvents, methanol, water and ethanol; however methanol one was the best accordance with [19] who confirmed that plants differ significantly in their activity against tested organisms using different solvents.

In addition most likely the water extract of tested plant failed to inhibit the growth of investigated microorganisms. Consistently, [20] demonstrated that ethanolic extracts were found to be more effective than aqueous extracts. Also in present study, the extract showed response on both G+ve and G-ve bacteria. The demonstration of activities against both G+ve and G-ve bacteria is an indication that plant can be a source of bioactive substances that could be broad spectrum of activity [21].Since plants produce a variety of compounds with antimicrobial properties, it is expected that plant compounds showing target sites other than those currently used by antibiotics and will be active against drug –resistant microbial pathogens [22].

3.3 Protein analysis of bacterial cells after and before treatment with glycyrrhizin

Two kinds of bacteria were chosen for example of G-ve bacteria (*Klebcilla pneumonia*) and example of G+ve (*Staphylococcus epidermis*) to determination the effect of SDLE on both bacterial protein profiles.

A. Klebcilla pneumonia.

The protein profiles of untreated bacteria (control) differed from those of treated bacteria with glycyrrhizin for 24 h at 37ºC. Several protein bands were observed for untreated *K.penumonia* {Fig. 5, lane 1(control) and lane 2(treated)} .There was a single band for about 25 KDa on control cells and disappeared in treated cells.

The protein bands for treated bacteria in band 70KDa was very sharp and more density than it found in treated cells. In general it is clearly that glycyrrhizin decreased the numbers of protein bands after the bacterial strains were treated with glycyrrhizin. This suggests that glycyrrhizin may have caused the death of the bacteria through the destruction of cellular proteins. These results are in agreement with the findings of [23] who observed the disappearance of protein bands after exposing E. coli to an anolyte solution with an ORP of 1000 mV. Oxidizing compounds present in anolyte probably because destruction of proteins by breaking down the covalent bonds in proteins. The 10-2 anolyte dilution destroyed proteins of *P. aeruginosa* but in E. coli it resulted in appearance of even more protein bands when compared to the protein profile of the untreated bacteria.

B. Staphylococcus epidermis.

The results obtained for *S.epidermis* differed from those of *K.penumonia*. Similar to the observations made for *S.epidermis*, there were fewer protein bands from *S.epidermis* cells treated with glycyrrhizin than those of untreated cells {Fig. 5, lane 3(control) and lane 4(treated)} However, reduced the numbers of viable cells and also resulted in more protein bands.

The extra protein bands were of both low and larger molecular weight than the native proteins present in untreated bacteria. Low molecular weight protein bands probably resulted from fragmentation the proteins into smaller peptides [24] and [25]. Presence of dilute anolyte caused an unfavorable environmental condition which induced a stress response in bacteria. The level of stress response varies with the type of organism and the type of environment [26]. During exposure to stressful conditions, bacteria synthesize and replace damaged proteins from mainly recycled amino acids, and this may give rise to the altered profiles from the native proteins [27]. Also, stress initiates the activation of a variety of defense genes which encode for the scavengers of reactive radical and other stress related proteins [28] and [27].This condition may account for the presence of the additional, large molecular weight protein bands as evidenced.

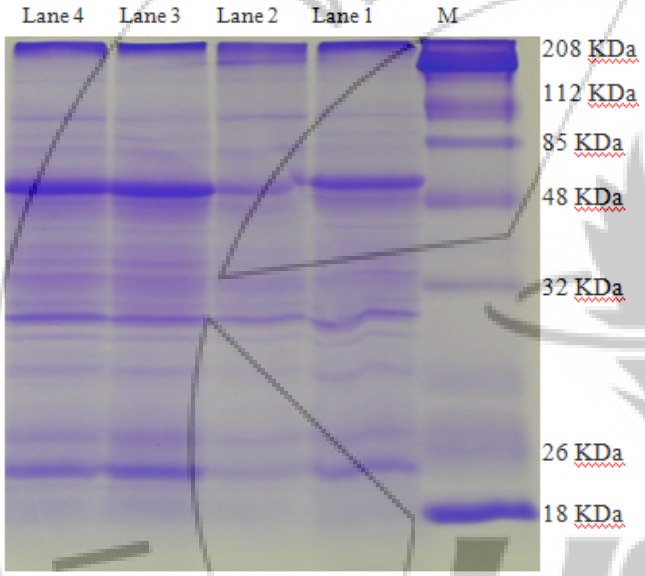

Figure 5: SDS-PAGE whole protein profiles from bacteria treated and untreated with glycyrrhizin. Lane 2 and lane 4 treated cells of *K. pneumonia* and *S. epidermis*, respectively, Lane 1 and Lane 3 untreated cells of *K. pneumonia* and *S. epidermis*, respectively, M: represent the molecular weight marker.

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