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Antibacterial Evaluation of *Saussurea costus* Alcoholic extract against MDR-*K. pneumoniae* isolated from Patients infected with COVID-19.

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Abstract - The emergence of resistant *Klebsiella pneumoniae* assosiated with COVID-19 demonstrate a primary challenge for the antimicrobial therapy of infectious diseases and increases the incidence of mortality and morbidity. *K. pneumoniae* isolated from COVID-19 patients sputum with ratio (100%). All *K. pneumoniae* clinical isolates had 100% resistance to ceftriaxone , piperacillin (80%) , cefepime (60%) , amikacin (40%) , and meropenem =levofloxccin (20%). Bacterial isolates gave positive result for MHT, also all isolates formed *β*- lactamase at a rate of 100% by using spectrometry β-lactamase assay. Costunolide (38.3 %), Rutin (15.33%), Pentadecanoic acid (6.54%), Oliec acid (4.77%), and Caproic acid (3.22%) considered as major compounds in ***Saussurea costus*** were identified by GC-Mass spectrometry . The β-lactamase produced by *K. pneumoniae* were inhibited by ***Saussurea costus*** with a strong statistical significance at P- value : <0.001.

Keywords - ***Saussurea costus , antibacterial activity ,*** *Klebsiella pneumoniae , Costunolide* .

1. Introduction :

Secondary infections are common with COVID-19 in respiratory tract infections and are a significant cause of morbidity and mortality that requires prompt diagnosis and antibacterial therapy [1]. Pandimic Corona virus is an outbreak of a novel detected virus that spread rapidly[2][3]. The incidence, frequency, and properties of bacterial infection in patients infected with corona virus are unknown and have been identified as a major gap in knowledge [4]. The awareness of the proportion of COVID-19 patients with acute bacterial co-infection and responsible pathogens is crucial to the management of COVID-19 patients and the responsible use of antibiotics and to minimize the adverse effects of overuse. [5] .*MDR- K. pneumoniae* is considered to be the second most prevalent Gram-negative pathogen in nosocomial settings, after *Escherichia coli.* Multidrug-resistant organisms (MDR) or

superbugs have become more severe these days [6] . The source of great economic benefit is medicinal plants. In synthesizing medicinal compounds, plant herbs are naturally talented[7].

In light of scientific progress in understanding the medicinal properties of plants, such natural antibiotic sources have received increased interest because of their low toxicity, pharmacological activities, and economic viability[8]. The extract of S. costus exhibited broad antimicrobial activity against a variety of human pathogens. Additionally, multiple studies have demonstrated anti-ulcer, anti-inflammatory, hepatoprotective, immunomodulatory, hypoglycaemic, spasmolytic, anticonvulsant, antidiarrheal, and antiviral activity in *S. costus roots*. [9].

This work aimed to determine the antibacterial of *Saussurea costus* and β- lactamase activity producing *K. pneumoniae* and identification bioactive compounds in the plant responsible for the antibacterial.

2. Materials and Methods

2.1 Collection of samples and bacterial isolates:

Sputum samples were collected from patients infected with Covid-19 that hospitalized in COVID-19 department of Al-Ramadi Teaching Hospitals , located in the West of Iraq, between December 2020 and November 2020. K. pneumoniae diagnosis and susceptibility profile assessment was carried out using the VITEK 2 (bioMérieux) method according to the recommendations of the Institut for Clinical and Laboratory Standards. [10]. Roots of *S. costus* were obtained from Ramadi , Al-anbar goverment. *Prof.* Dr. Mohammed Othman identified *S. costus* at Anbar University's Herbarium Center of Desert Studies . The roots were air dried and were grinded to uniform powder using grinder (Philips, India), and stored in air-tight bottles till further analysis. According to **Kirby-Bauer** disc diffusion method , All K. pneumoniae were screened for resistance to six antibiotics: piperacillin/tazobactam, ceftriaxone, cefepime , meropenem , levofloxacin and amikacin .

2.2 Solvents extraction

The extraction was done according to [11] . Using a maceration process, plant material was extracted. The plant's roots were compressed into small fragments, then used electrical mixer grinder to a fine powder. Mixing fine powder with 70% ethanol and 80% methanol solvents separately was used to macerate plant material.

2.3 Bioactive compounds of plant extract Analysis :

Constituent identification was carried out on the basis of retention time(RT), calculated with reference to MS library search, and comparison with MS literature results. Relative amounts of individual components were determined without correction factors based on the GC peak area. Extract study of gas chromatography-mass spectroscopy (GC-MS) Sample was directly injected into panel. Helium was the carrier flow rate was 1mL/min The oven temperature was 3°C/min from 60°C to 220°C. Other conditions were as defined in GC. The mass spectrum was taken from 40–600 Daltons.

2.4 Phenotypic methods for β-lactamase enzymes :

Under the CLSI guidelines, the output of β -lactamase was determined using a modified Hodge test [12]and as described elsewhere [13]

2.5 Estimation β-lactamase and anti- β-lactamase Assay :

β-Lactamase activity was assessed spectrophotometrically by hydrolysis of speciefic substrate [14].

Antibacterial activity of *S. costus* extract, fractions, and isolated compounds was calculated using a revised Clinical and Laboratory Standards Institute (CLSI) protocol against Gram-negative *K. pneumoniaea* and standard E. coli ATCC 25923. The bacteria were grown to the required bacterial growth temperatures of 36 for 16 hours to produce phase cultures. was found to be three times that of the previously-determined values (MBC: minimum inhibitory concentration and MIC: minimum bactericidal concentration) the final concentrations for the analysis of the minimum inhibitory concentration (MIC) was 1.9 to 4000 g/mL and the bactericidal concentration (MBC) was 11–4600 g/mL**.**

2.6 Statistical analysis :

All the data was analyzed using graph pad prism, version 8.0. In order to compare the different classes (before and after treatment), **we used impaired T-test.**

3.Result and disscusion :

3.1 Diagnosis of *K. pneumoniae*

Klebsiella pneumoniae appeared as large , pink , mucoid , Convex , Opaque and lactose fermentation colonies on macConky agar Figure 1 . Then , Microscopic diagnosis gave as short, plump, straight rod shape (gram negative bacillus) bacterium. Catalase , Citrate , Urease , Voges Proskauer gave positive result , while oxidase , indole , pigment gave a positive result.

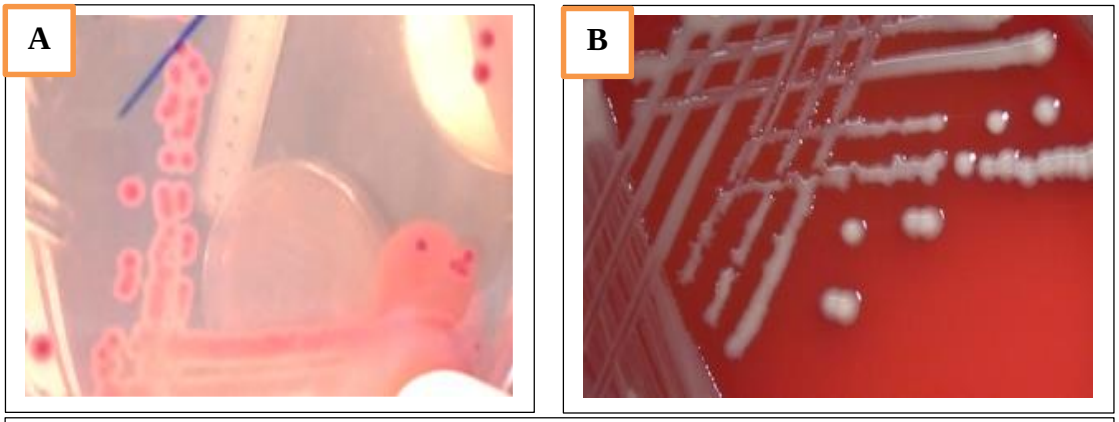
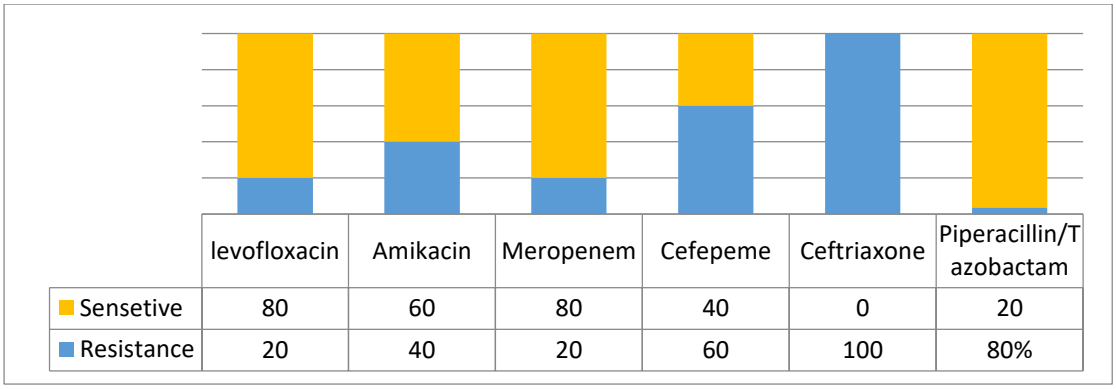


Figure 1 : *Klebsiella pneumoniae* on culture media (A) macConky agar , (B) blood agar

3.2 Susceptibility Test :

The data shown in Figure 2 demonstrate that K. pnumonea clinical isolates are highly resistant to the majority of the antibiotics tested.. This work revealed that all *K. pnumonea* clinical isolates had 100% resistance to ceftriaxone . This study also showed a highest resistance to PIP/TAZO (80%) , Cefepime (60%) , Amikacin (40%) , and meropenem =levo (20%). The emergence of *A. baumannii* carbapenem-resistant organisms in Iraq has become a major therapeutic challenge .



	levofloxacin	Amikacin	Meropenem	Cefepeme	Ceftriaxone	Piperacillin/Tazobactam
Sensetive	80	60	80	40	0	20
Resistance	20	40	20	60	100	80%

Figure 2 Antibiotic resistance of K. pneumonae clinical isolates.

3.3 Phenotypic and Estimation of β- lactamase enzymes assay :

According to modified hodge test , this test gave positive result for all isolates .All isolates of *K. pneumonea* were investigated on their ability to produce *β*- lactamase by [14]. It was discovered that all isolates formed *β*- lactamase at a rate of 100% Figure 3. The ability of isolates to produce *β*- lactamase varied greatly, with some being high, and others moderate as figure 1 . β-Lactamases are bacterial enzymes, some of which are necessary for pathogen resistance to β-lactam antibiotics. They are divided into four groups (A, B, C, and D). Many researches reported β- lactamase first time in Al-Anbar governate , such as Mohammed and Al-meani , 2020 reported carbapenemase (KPC , OXA-48 , and VIM) in K. pneumoneae [6] and A. bauamanii[7] , while Mohammed obaid , 2020 reported Ampc cephalosprinase in K. pneumoneae [15]. On other hand , Awad and Laith documented Oxacillinase in *A. bauamanii* [16]

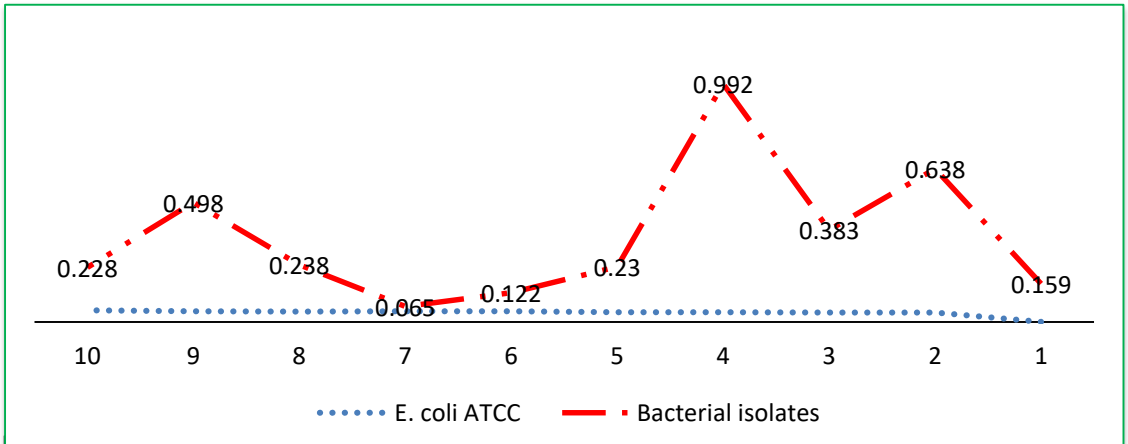


Figure3 : β- lactamse Activity in K. pneumoneae isolates .

3.4 Gas chromatography-mass analysis

The GC/MS analysis of the ***Saussurea costus*** is presented in figure. Five major compounds were identified , These compounds were identified as Costunolide (38.3 %), Rutin (15.33%), Pentadecanoic acid (6.54%), Oliec acid (4.77%), and Caproic acid (3.22%) Figure 4 , (table1).

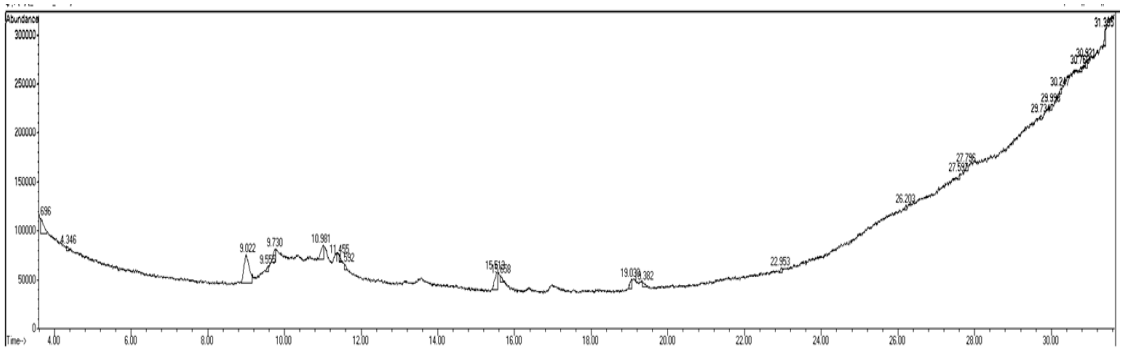
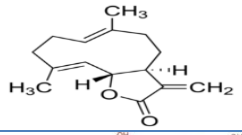
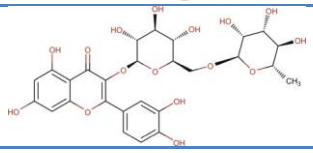
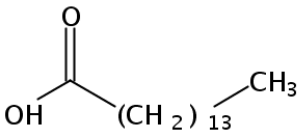


Figure 4 :Saussurea costus **analysis**

Table1 Percentage of components of *Saussurea costus* .

Active compouns	Area	R. time	Chemical structure
Costunolide	28.3	5.25	
Rutin	15.88	2.28	
Pentadecanoic acid	6.54	3	
Oliec acid	4.77	2.5	
Caproic acid	3.22	2	

Saussurea costus is a Himalayan medicinal plant that aids in the treatment of a variety of ailments. Several reports have shown that costunolide has antimicrobial activity. Antibacterial activity of costunolide against Mycobacterium tuberculosis H37Rv (M. tuberculosis) [17]. Additionally, it was discovered that costunolide was antimicrobial against

Staphylococcus aureus[18], *Escherichia coli*, and *Pseudomonas aeruginosa* [19]. Additionally, costunolide delayed the growth of Helicobacter pylori. Costunolide's therapeutic potential includes antioxidant, anti-inflammatory, anti-allergic, bone remodeling, neurodegenerative disease prevention, antimicrobial activity, alopecia suppression, lung disease prevention, and anti-diabetic impact. Costunolide, in particular, induces apoptosis, inhibits cell proliferation, TERT, angiogenesis, metastasis, and microtubule disassembly, among other mechanisms [20]. Figure 5

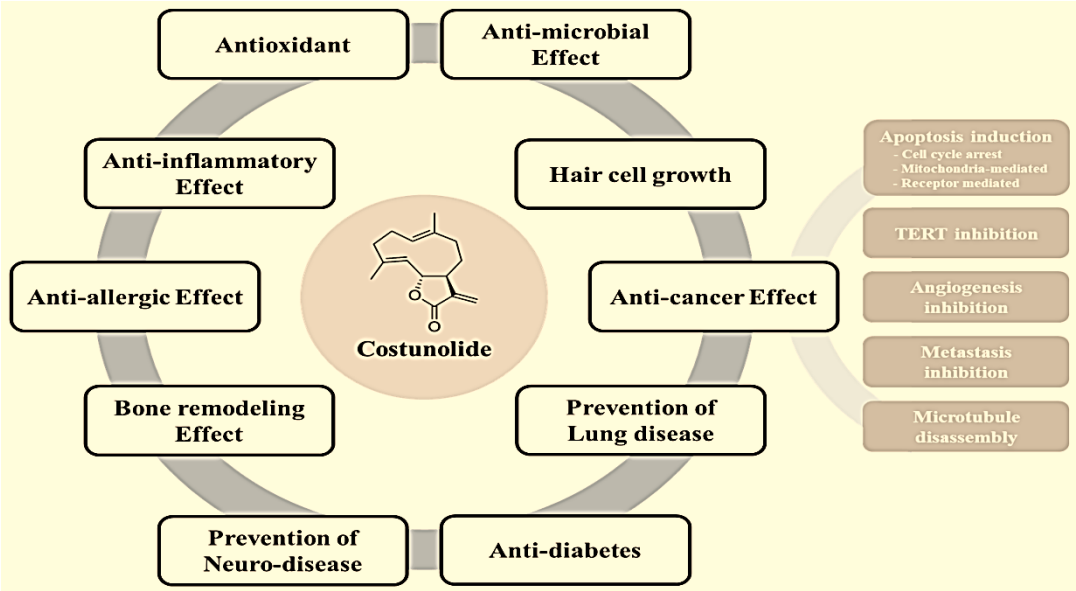


Figure 5 Bioactivities of costunolide.

3.5 Effect of plant extact against β- lactamse :

This variation in the production of β-lactamases by K. pneumoniae may be due to the diversity of genes encoded β-lactamase and isolates sources from COVID-19 patients. The β-lactamase produced by *K. pneumoniae* were inhibited by K. pneumoniae and in strong statistical significance at P- value : <0.001 figure . Plant extract are complex combinations of several different compounds, each of which contributes to the biological effects of these oils[21]. Plant extract increases the cell membrane 's permeability and variations in their strength and differences in their oligomeric state and capacity to dissociate and insert into the cytoplasmic membrane are the product of target specificity. [22]

Aalayb and Ayou [20] studied the Saussurea extract had the highest antibacterial activity. Chang et al. [23] also looked into extracts of Saussurea with different solvents. The antimicrobial activity of Saussurea was investigated and compared to that of synthetic

antibiotics against L. monocytogenes, B. cereus, B. subtilis, S. aureus, S. choleraesuis, and V. parahaemolyticus . The Saussurea extract fraction have high activity against B. cereus and V. parahaemolyticus strains. Despite the fact that the current study is preliminary, it has laid the groundwork for further research into the isolation and purification of bioactive compounds found in this medicinal plant.

Thus, rutin has been demonstrated to improve the function of aminoflavones (Morin and other flavonoids), such as, against Salmonella enterica and other bacteria [24] . figure 6

Rutin is widely studied for antibacterial activity Escherichia coli has shown a profound effect on bacterial growth [25]. Proteus vulgaris, Shigella sonnei, and Klebsiella sp. have all been shown to be inhibited by rutin [26], which can be found in honey. Antibacterial effects of penicillin against Pseudomonas are also present Studies have been done to examine the in-situ antimicrobial polyphenols in food, and flavonoids play a promising role in food preservation [27]. DNAase IV was shown to have rutin-inase activity in an animal trial, the combination of rutin and other flavonoids had synergistic antibacterial activity Minimum inhibitory concentration for kaempol increased significantly after rutin was added [28].

Oleic acids have been shown to inhibit Bacillus megaterium growth at MICs of 0.2 and 0.05 mM, respectively. Pseudomonas phaseolicola _MIC)1.2 mM was also inhibited by oliec acids. [29].

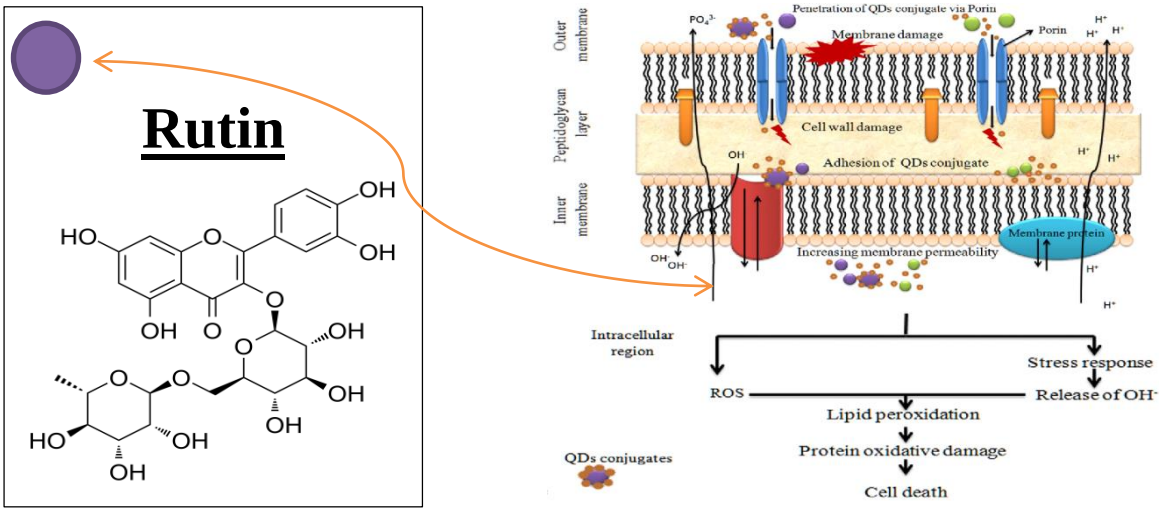


Figure 6 : Mechanism of Rutin

4. Conclusions :

Saussurea costus was appeared anti - **MDR-*K. pneumonea*** activity and exhibited **β-lactamase inhibition** .

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