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The Effect Of Ethanolic Extract From Purslane Leaves on Pathogenic Bacteria Isolated from Liver Transplantation Patients

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Abstract: Portulaca oleracea is a herbaceous weed belongs to the family of *Portulacaceae*..Protein from ethanolic extract of purslane leaves used instead of immunosuppressive drugs taken by liver transplantation patients.Firstly we prepared ethanolic extract of purslane leaves to study the effect of the alcoholic extract on isolated bacteria as *Klebsiella* species and *Staphylococcus Aureus* in wound samples and the lowest percentage of species was *Pseudomonas* species and *Proteus* species then SDS-PAGE technique and electroelution by migration of proteins out of gel slices into solution and determinion of minimum inhibitory concentration (MIC) of electroeluted protein extract and finally Susceptibility test for bacterial pathogens . **Objective**: The current paper's objective is to study the effect of ethanolic extract from purslane leaves on pathogenic bacteria isolated from liver transplantation patients. **Results:** For *Staphylococcus aureus*,Meropenem and Amikacin were reported as the maximum inhibition zone of 27mm.For *Proteus* sp. the maximum inhibition zone of 30mm was demonstrated Mikacin and Gentamicin.For *Pseudomonas* sp. Meropenem was recorded maximum inhibition zone of 30mm. The minimum inhibition concentration(MIC) effect for *klebsiella, pseudomonas*, and *Proteus* were at concentration of 0.25 g/ml and for *staphylococcus aureus* it was at concentration of 0.35 g/ml.

keywords: Medicinal plants,*Purslane,*Antibacterial activit

Introduction

When medical therapy has failed and there is acute or chronic end-stage liver disease, liver transplantation is needed. Individuals who experience hepatic decompensation, such as hepatic encephalopathy, should be treated medically. Prospective liver transplant candidates should also begin a thorough evaluation process. [1]. Bacterial infections are a leading cause of morbidity and mortality in patients receiving solid organ transplants (SOT) [2] . A series of diverse multidrug-resistant (MDR) infections that are responsible for significant morbidity and mortality in transplant recipients have surfaced within the past 20 years [3]. Generally speaking, the degree of clinical severity at the time of infection and the overall level of immunosuppression have an impact on how well patients recover from bacterial infections. In these circumstances, the failure to initiate proper empirical antibiotic therapy is more common and may be a factor in the greater death rate [4]. Post-transplant immunosuppression In the absence of immunosuppressive medication, the recipient of a liver transplant will reject it, as is the case with most other allografts.Because of this, it is strongly advised to undergo pharmacological immunosuppression, immunosuppression for underlying liver illnesses, and immunizations against diseases that can be prevented by vaccination both before and after liver transplantation.Compared to the general

population, transplant recipients exhibit lower vaccine reluctance [5]. Pathogenic bacteria are bacteria that can cause disease [6]. The number of these pathogenic species in humans is estimated to be fewer than a hundred. By contrast, several thousand species are part of the gut flora present in the digestive tractPathogenic bacteria are also the cause of high infant mortality rates in developing countries [7]. Antibiotics are utilized in intensive farming to encourage animal growth as well as in the treatment of human illness. Antibiotic resistance in bacterial populations may be rapidly developing as a result of both applications [8]. Certain bacterial illnesses can also be treated with phage treatment, which uses bacteriophages [9]. Antimicrobial resistance is a complicated system and the causes vary from person to person depending on the bacterial strains present and the resistance mechanisms that have been established. The rise of antibiotic resistance to newly developed antibiotics supports the need for innovation,monitoring antibiotic use, prevention, diagnosis, and a quick decrease in drug abuse of these substances. Antimicrobial protocols will need to be changed to ensure that these medications are only administered when all other therapeutic options have failed. The clinical efficacy of antibiotics gradually declines due to widespread bacterial antimicrobial resistance, threatening human health [5]. The spread of "superbugs" that are now resistant to several antibiotics is a serious issue. These pathogens include *S. aureus, K. pneumoniae, P. aeruginosa, Acinobacter baumannii,* and *Enterobacter* species .Methicillin-resistant *Staphylococcus aureus* (MRSA) and *K. pneumoniae* had the greatest infection rates of the pathogenic bacteria that were isolated from patients [4].

Portulaca oleracea (P.Oleracea) is a herbaceous weed that belongs to the family of Portulacaceae. Purslane is found all over the world including the temperate countries,and is commonly called “Rejlah” in Egypt [10]. The seeds have important medicinal value, since they can lower blood glucose. It is entirely considered to have antiphlogistic, bactericidal, anti-diabetic, calming and refreshing properties. Purslane has nutritive value and biological activity such as antioxidant and antidiabetic, many researchers consider it as ‘Power Food’ of the future [11].A wide spectrum of medical and pharmacological properties of *P. oleracea* like antidiabetic, anti-inflammatory, and anticancer activities [12]. It is known to have higher nutritional value than major cultivated vegetables with considerable amount of β-carotene, ascorbic acid and α-linolenic acid. Its curative value is evident from its use for treatment of pain and edema. Purslane has sturdy regenerative properties on a cellular level and is encumbered with antioxidants, beta carotene, vitamin C, and vitamin E, and also contains a high volume of omega-3 fatty acids, which aids to diminish folds [8].

All these components are influential antioxidants that can help to enhance the appearance and even avoid coming signs of aging by reducing UV-induced damage. Purslane is a key to a countless anti-ageing skin care and diminishes irritation in the skin, progress overall family flow and excite cell repair which diminutions the appearance of scars and wrinkles. Purslane contains other chemical elements including urea, calcium, iron, phosphorous, manganese and omega-3-acids whose concentration in purslane is the highest found in leafy vegetables [13, 14].. In addition, purslane is reported to be rich in ß-carotene and it’s used as a healthy food for patients with cardiovascular diseases. Amino acids exist in purslane leaves are the following: Alanine, Methionine, Phenylalanine and Tyrosine with M.wt 89,149,165 and 181 kDa respectively.

Materials and methods

2.1 Bacterial isolates collection

A total number of 30 surgical wounds samples were collected from 40 patients (30 positive and 10 negative).Patient attending in Gastroenterology Center (GEC), Mansoura University, Egypt. Patients who received antibiotic treatment systemically within the previous 72 hour were excluded from the study. The surgical wound samples were collected with sterile swaps under complete aseptic conditions. All the samples were kept in an ice box and were transferred to the microbiology lab within 2 hr for isolation of bacterial species to test bacterial infection.

2.2 Bacterial isolation and identification

Surgical wound swab specimens have been suspended directly in 1 ml sterile nutrient broth inoculated onto blood agar medium by Continuous streaking method. They have been incubated at 37°C for 24 hours in aerobic condition. No growth after 48hr. Isolated bacteria were purified by subculture on nutrient agar and then identified according to the colony characters, microscopic examination by Gram stain and biochemically identified by VITEK 2 compact 15 **(**Biomerieux, France). Bacterial species were resvoir at -20̊C by using nutrient broth /glycerol (v/v) until bacteriology studies.

2.3 Antibiotic Susceptibility Test

According to CLSI guidelines 2010 all the bacterial isolates identified were tested against different antibiotic discs according to Tenedencial [15]. The antimicrobial activities of Amoxicillen /Clavulanic acid 2: I (AMC), Meropenen (MEM), Cephalexin (CL), Cefoperazone (CEP), Cefoperazone\ Sulbactam (CES) Piperacillin (PRL),Rifampin (RA) , Vancomycin (VA) Penoxacin (PEF),Amikacin (AK), Clindamycin (DA) and Cefepime (FEP) were carried out using the disc diffusion method. The results have been expressed as diameter of inhibition zones **(Table 1)** and have been recommended by National Committee for clinical laboratory standards (CLSI guidelines 2010) [16].

Table (1) Diameters of inhibition zone for commercial antibiotics according to CLSI and EUCAST.

	Antibiotic	Disk Potency	Resistant	Intermediate	Susceptible
1	Pefolaxcin	5	<23	_	>24
2	Amikacin	30	<14	15-16	>17
3	Meropenen	10	<19	20-22	>23
4	Piperacillin	100	<17	18-20	>21
5	Cefoperazone	75	<24	25-31	>32

2.4. Plant materials

Fresh Purslane leaves (*Portulaca oleracea)* plants were gathered from a private fields (El-Sharqia Governorates, Egypt) before the flowerring period during November 2021. They were dried to obtain the extract from the maceration with soaking method.

2.5. Preparation Method of the Alcoholic Extract

Plant leaves were properly dried and pulverized into a coarse powder in a grinder. Each of 20 gm ground powder was soaked in 200 ml of ethanol 95% for 24 hr (shaking occasionally with a shaker), and then the materials were filtered (Whatman No. 1 filter paper).The filtrates were evaporated using rotary evaporator unit and dried at 50-40°C for two days (Stuart RE300D8, England). 0.97 gm of the dried extracts have been dissolved in 10 ml of Dimethyl sulfoxide (Demso) solution.

2.6.Antimicrobial activity of *P.Oleracea* extract

Ethanolic extracts of *P.Oleracea* were diluted by DEMSO to obtain concentration 0.5,0.4,0.35,0.25,0.15 g /ml.Antibacterial activity has been carried out using well diffusion method as per NCCLS [17] .Petri dishes that contain 30 ml of nutrient agar media inoculated with 1 ml of bacterial isolates standard inoculums ($1*10^{8}$ CFU/ml) for each bacterial strains.Agar wells have been made by using a sterile cork borer (7 mm diameter).Each well has been filled with 200 microliter of the tested plant extract and the plates have been incubated at 37°C for 24hr .Diameters of inhibition zone of < 10mm zone has been considered as low sensitivity,10-14 mm as medium,15-19 mm as high sensitivity and 20 mm as extreme sensitivity as per the standard for pharmacology of traditional Chinese medicine[18].

2.7. Separation of Extract by Sodium dodecyl sulfate Polyacrylmide Gel Electrophoresis (SDS-PAGE) and Electroelution

Determination of protein content in the ethanolic extract by the Follin-Lowry method based on the protein concentration the whole protein sample displayed by a color modification are planned using colorimetric techniques (SHIMADZU UV-1800 spectrophotometer).A blue-purple color multipart is formed from the phenolic cluster of tyrosine and tryptophan residues (amino acid) in a protein which advance revealed immersion in the region of 660 nm wavelength with the Folin-Ciocalteau reagent containing sodium tungstate molybdate and phosphate. Thus, the

amount of color depends on the amount of these amino acids that fluctuate for different proteins. The incubation time is awfully grave for a reproducible assay. The Bovine Serum Albumin (BSA) is used universally as standard in most proteins evaluation, as of its low cost, high purity and ready accessibility. The method is sensitive down to about 10 µg/ml and is possibly the most widely used for protein assay despite its being only a relative process Amino acids that exist in ethanolic cutting of purslane leaves are the following: Alanine, Methionine, Phenylalanine and Tyrosine with M.wt 89,149,165 and 181 KDa individually were prerpared and purified as the method described by for sepration of proteins according to their molecular weights by (SDS-PAGE) which applied with some modification. Around 100 µg of protein test sample laterally with 10 µl of buffer comprising bromophenol blue as tracing was loaded. A marker was loaded into the gel to find out the molecular weight of the bands. The gels were run at a continuous voltage of 200 V until the bromophenol blue marker reached the bottom of the gel followed by staining in Coomassie brilliant blue for V.5 Molecular weights of proteins were determined according to the method of This procedure was based on calculating the passage reserves covered by each protein starting from the top of the determining gel and divided on the distance traveled by the tracking dye, gave relative mobility of the proteins known as (Rf) The logarithm of standard molecular weights were planned in terms of their Rf values.Rf of unknown protein was calculated from the calibration curve.A strip at one side of the electrophoresed preperative gel was cut and stained with coomassie brilliant blue R-250 according to method described by **(5)**,The electrophoresed gel was dripping in excess of staining solution overnight. On the next day the gel was rinsed with distilled water and destained with excess amount of destaining solution for several times until the excess stain was satisfactorily removed. After destaining, the strip is placed beside the unstained preparative gel and a band containing the wanted proteines was cut(89,149,165,181 and 204 KDa).The stainless strip containing the wanted protein was placed in a dialysis sheath with sufficient electroelution buffer volume and protein was eluted from the gel by electroelution with a constant volt of 300 v for 4 hours. Electroeluted protein was dialysed against one litre of phosphate buffer saline (PBS), Ph7.2 sudden at 4ºC with constant stirring. After dialysis step, the electroeluted protein was rigorous using 50ml polyethylene glycol for 15 minutes at room temperature from each protein.

2.9. Antimicrobial activity of electroeluted protein

Test for a partial list of amino acids those purslane leaves contain are the following Alanine, Methionine, Phenylalanine, Tyrosine and Tryptophan with M.wt 89,149,165,181 and 204 kDa respectively. Antibacterial activity has been carried out using well diffusion method as per NCCLS .Petri dishes that contain 20 ml of nutrient agar media inoculated with 1 ml of bacterial isolates standard suspensions ($1*10^{8}$ CFU/ml) for each bacterial species .Agar wells have been made by using a sterile cork borer (7 mm diameter).Each well has been filled with 100 µl of the electroeluted protein and the plates have been incubated at 37ºC for 24hr .

2.10 Antibacterial Activity of Amino Acids

Petri dishes that contain 20 ml of nutrient agar media inoculated with 1 ml of bacterial isolates standard suspensions (1*10 8 CFU/ml) each bacterial species .Agar wells have been made by using a sterile cork borer (5 mm diameter).Each well has been filled with 100 µl of the Amino acids and the plates has been incubated at 37ºC for 24hr.

3.1. Results and Discussion

A total number of 40 patients was considered in this study. The results in **Table (2)** showed that the number of positive specimens for bacterial growth was 30 from surgical wounds where the negative ones were 10 from surgical wounds.

Table (2) Number of positive and negative patient samples..

Bacterial Growth	No.of specimens	%of specimens
Positive	30	75
Negative	10	25
Total	40	100

Five different bacterial species were isolated; among them 4 were gram-negative and only *Staphaphylococcus aureus* gram positive.

The results in **Table (3)** showed that the no. of *klebisella* isolates was 13 from wounds species representing 43.3% of the total positive specimens. This is followed by *S.aureus* where the no of isolates was 10 from wounds specimens representing 33.3 % or total positive specimens. The recorded number of *Pseudomonas* isolates was 5 from wounds specimens representing 16.6%. The minimum number of species was reported for *proteus* specimens was 2 representing 6.6% of total positive species.

Table (3) Pathogenic Bacteria Isolated from Wounds Samples

Pathogenic Bacteria	No.	Percentage %
Klebisella sp.	13	43.3%
Staphylococcus sp.	10	33.3%
Pseudomonas sp.	5	16.6%
Proteus sp.	2	6.6%
Total	30	100%

3.2. Antibacterial Activity of Antibiotics used

The results in **Table (4)** indicate a more or a less similar inhibition effect for bacterial growth. For *E.coli*, Meropenem was reported as the most effective antibiotic where the clear zone diameter was 30 mm, while the lowest effective one was Clindamycin with inhibition zone of 10 mm. For *Staphylococcus* sp. Amikacin and Meropenem were recorded maximum inhibition zone of 27 mm, while the minimum inhibition zone (12 mm) was recorded in case of Nitrofurantoin and Petloxacin. For *Proteus* sp. the maximum inhibition zone (30 mm) was demonstrated with Amikacin and Gentamicin, while the minimum one (11 mm) was reported with Pepracillin and Rifamycin SV. For *Pseudomonas*, Meropenem was recorded maximum inhibition zoneof 30 mm, while the minimum inhibition zone (13 mm) was recorded for Pefloxacin. For *Klebsiella*, only Amikacin was reported to exhibit a poor inhibition eflect with 13 mm diameter of inhibition zone. Antibiotic resistance to ampicillin, amoxicillun and erythromycin was 100% present in all gram-positive and gram-negative bacterial isolates.[19] While Staphy lococcus isolates were sensitive to Vancomycin and varied (sensitive or resistant) against Tetracycline. Salmonella, and Pseudomonas isolates have shown great resistance against both antibiotics. B-lactams, such as penicillins (Ampicillin, Amoxicillin) and cephalosporins (Cefaclor). prevent the formation of the cell wall by inhibiting peptidoglycan polymerization, while glycopeptides (Vancomycin) combine with the cell wall. The behaviourof the same isolates, and the gram-positive isolates showed some resistance against Gentamycin. Amikacin was discovered to be able to exert an antibacterial effect on all bacteria. Quinolones (Nalidixic acid and Ciprofloxacin) bind to a bacterial complex of DNA and DNA gyrase, and blocking DNA replication is effective on both Gram-negative and some Gram-positive bacteria. Ribosome function is affected by aninogly olvcosides (Amikacin, tetracyclines (Tetracycline), macrolides (Eythromycin), and gentamycin and tobranycin, Previous studies with comparable findings included Tahnkiwale et al. [20], Samy et al. [21], and Macedo & Santos [22]. Others discovered that differences in the patterns of antibiotic sensitivity of the isolated organisms could be caused by a range of variables, including changes in pH values, the circumstances and timing of incubation, composition, the kind of culture media, the inoculum's size, the origin of the isolated organism, and possible variations in strain activity[23].Variations in antibiotic sensitivity may be influenced by thc composition of the bacterial cell wall and the permeability ofthe cell membranes to different antibiotics.

3.3Antibacterial Activity of *Portulaca Oleracea* extracts

Table (5) shows that the antibacterial activity of ethanolic extract of *P. Oleracea* has maximum inhibition zone (30 mm) on *Klebsiella Sp., Proteus sp.* and *Psendomonas aeruginosa* .The effect of 0.5g/ml concentration on *Staphylococcus aureus* was the inhibition zone (25 mm) .The minimum inhibition concentration(MIC) effect of the inhibition zone for *klebsiella*, *Pseudomonas*, and *Proteus* sp. were at concentration 0.25 g/ml and for staph it was at concentration 0.35 g/ml. Dhole stated that the ethanolic extract of *Portulaca*

oleracea exhibits good inhibitory activity against *Staphylococcus aureus*(32 mm) and *Pseudomonas aeruginosa* (30 mm) at concentration of 0.5g/ml. **(20)** reported that the ethanolic extract of *P. Oleracea* showed good inhibitory activity against gram positive and gram negative bacteria [24**].** reported that Ethanolic crude extract showed maximum effect on *Staphylococcus Aureus, Klebisilla Pneumonia,and Neurosporacrassaf*.[25] These results supported the folklore usage of the studied plant and suggested that this plant extract possess that would compound which would have an antimicrobial agent in the form of drugs for the therapy of infectious diseases caused by pathogens. According to **(25)** results showed that ethanolic extract of porlacea could inhibit pathogenic bacteria.[26].

As using of antibiotics has caused serious problems, such as multidrug-resistant bacteria, antibiotic Overuse, and antibiotic residues in food, and more,3 new materials, which can replace antibiotics to treat

bacterial infections are needed. Previous studies have shown that plants extracts can be

used to treat a variety of disorders including inflammatory conditions, bacterial infections, cancer, and other diseases. Results showed that the extracts of *P. Oleracea* have inhibited the growth of all strains of bacteria under study.The ethanolic extract could inhibit growth of bacteria also with order of sensitivity as following :*Proteus sp. ,Pseudomonas sp.,Staphylococcus sp.* then *Klebisella sp.*

Table (4) Inhibition effect for bacterial growth

Antibiotics BacteriaIsolated	AK	AMC	CL/PRL/ DA/PEF/RA	FEB	CEB	MEM	VA
Staphylococcus sp.	27(S)	20(I)	R	20 (I)	18(I)	27(S)	21(S)
Proteus sp.	30(S)	R	R	20 (I)	R	21(S)	R
Pseudomonas sp.	19(S)	R	R	20(I)	R	30(S)	R
Klebsiella sp.	R	R	R	R	R	R	R

Table (5) Effect of ethanolic extract Portulaca Oleracea on the growth of some Pathogenic Bacterial Strains (inhibition zone in mm).

Bacteria	Diameter of Inhibition Zone (mm)				
Staphylococcus aureus	26	20	15*	R	R
Proteus sp.	30	24	20	14*	R
Pseudomonas sp.	32	25	22	136*	R
Klebisella sp.	28	25	22	15*	R

MIC *

Table (6) Effect of amino acids extracted from *Portulaca Oleracea* on growth of some Pathogenic Bacterial Strains (inhibition zone in mm).

Amino acids	*Pseudomonas Sp.*	*Klebisellia Sp.*	*Proteus Sp.*	*Staphylococcus Sp.*
alanine	**28**	**20**	**17**	**R**
Tyrosine	**R**	**26**	**20**	**28**
Methionine	**22**	**26**	**20**	**28**
Phenylalanine	**24**	**24**	**20**	**18**

3.4Antibacterial Activity of Aminoacids

The Results in **Table (6)** Indicate the most and amore or less similar inhibition effect for bacterial growth.For *Pseudomonas* sp. The Most effective Amino acids are Alanine and Phenyl alanine and the lowest effect in tyrosine.For *Klebisella* sp. The most effective is phenylalanine the more inTyrosine and Methionine then Alanine. For Proteus sp. The most effect iis in phenylalanine and the lowest in alanine and equal in tyrosine and Methionine.For Staphylococcus sp. The most in tyrosine and methionine and resistant in alanine .

Conclusion

The current paper's objective is to study effect of ethanolic extract from purslane leaves on pathogenic bacteria isolated from liver transplantation patients. For *Staphylococcus aureus*, Meropenem and Amikacin were reported as the maximum inhibition zone of 27mm.For *Proteus sp*.The maximum inhibition zone 30mm was demonstrated Mikacin and Gentamicin. For *Pseudomonas* sp. Meropenem was recorded maximum inhibition zone of 30mm. The minimum inhibition concentration (MIC) effect of the inhibition zone for

klebsiella sp., pseudomonas sp, and *Proteus sp*.were at concentration 0.25 g/ml and for *staphylococcus sp.* it was at concentration of 0.35 g/ml. Based on the result obtained during this study, we recommend the application of some medicinal plants like Purslane *(Portulacea olearcea)* against pathogenic bacteria isolated from patients admitted to Mansoura University Hospitals..

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