In-Vitro Antibacterial Activity Of Proanthocyanidins Against Some Of Pathogenic Bacterial Isolates

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الخلاصة

أجريت هذه الدراسة لقياس الفعالية التثبيطية للبروانثوسيانيدين المستخلص من بذور العنب *Vitis* (*vinifera*) ضد عدد من العزلات البكتيرية المرضية اربعة عزلات من *Salmonella typhimurium* sero var 1,2,3,4 وعزلة واحدة لكل من *Pseudomonas aeruginosa* و *Klebsiella alexanderi* و*Staphylococcus aureus* و*Escheria coli* ، وذلك بطريقة الانتشاربالحفر في الوسط الصلب (مولر هينتون-أجار) ، ومقارنة هذه الفعالية مع عشرة مضادات حيوية مختلفة :
Ciprofloxacin, Tetracycilin, Gentamicin, Ampicillin, Doxycycline, Cephalexin, Ceftiaxone, Augmentine, Aztreonam, Erythromycin). كما تم تحديد قيم MIC لعدد من المضادات الحيوية تجاه هذه العزلات البكتيرية .اظهر اختبار فحص الحساسية لعشرة مضادات حيوية ان بعض العزلات مقاومة بنسبة 100% لكل المضادات المستخدمة في الفحص . فيما كانت العزلات الاخرى حساسة لبعض المضادات .
اظهرت النتائج ان مركب البروانثوسيانيدين قد اظهر فعالية تثبيطيه جيدة ضد بعض العزلات في جميع التراكيز ولم تظهر العزلات الاخرى أي استجابة الا في التراكيز العالية مقارنة بالتراكيز الأقل اذ تأثرت عزلات *typhimurium* *Salmonella* sero var 1,2,3,4 و*Escheria coli* تأثرا كبيرا بالبروانثوسيانيدين واعطى التركيز mg/ml 500 اعلى فعالية في التثبيط.

ABSTRACT

The study was conducted to measure the antibacterial activity of Proanthocyanidins from grape (*Vitis vinifera*) seed extract against isolates of pathogenic bacteria : *Salmonella typhimurium* sero var 1, *Salmonella typhimurium* sero var 2, *Salmonella typhimurium* sero var 3, *Salmonella typhimurium* sero var 4, *Pseudomonas aeruginosa* , *Klebsiella alexanderi, Staphylococcus aureus* ,and *Escheria coli.* by agar diffusion method on (Muller-Hinton agar) and compared the activity of compound with 10 different antibiotics (Ciprofloxacin, Tetracycilin, Gentamicin, Ampicillin, Doxycycline, Cephalexin, Ceftriaxone, Augmentin, Aztreonam, Erythromycin). MIC (Minimum inhibitory concentration) to Doxycycline, Cephalexin, Ceftriaxone, Aztreonam , were also detection , the result showed that some isolates have highly level of resistance for most antibiotics , While other isolates were sensitive to some antibiotics .
The results showed the Proanthocyanidins have a good inhibitory effect against some isolates , at all concentrations, While other isolates have no response with the compound except high concentrations, the *Escheria coli* and *Salmonella typhimurium* sero var 1,2,3,4 isolates showed high effect with Proanthocyanidins and the concentration 500 mg/ml was more inhibitory activity .

INTRODUCTION

Functional ingredients of grape seeds (*Vitis vinifera*) include several flavonoids with a phenolic nature such as flavanols (catechin and epicatechin), procyanidins and phenolic acids. Recognition of such health benefits of catechins and procyanidins has led to the use of grape seed extract as a dietary supplement [1] . All these constituents have been reported to exhibit antioxidant activity in vivo and in vitro as they can scavenge a lot of superoxide radicals and thereby play an important role in the inhibition of carcinogenesis and act as antibacterial agent [2]. Also, these constituents can protect against DNA damage caused by free

radicals [1],[3] . Phenolics in grapes have been reported to inhibit oxidation of human low density lipoproteins (LDL) *in vitro*[4].

Proanthocyanidins are naturally occurring plant metabolites widely available in fruits, vegetables, nuts, seeds, flowers, and bark [5]. Also known as procyanidins, these substances are the main precursors of the blue-violet and red pigments in plants. These compounds are part of a specific group of polyphenolic compounds the flavonoids.Flavonoids is further categorized by sub-groups. Proanthocyanidins belong to the category known as condensed tannins, one of the two main categories of plant tannins [6].

Proanthocyanidins are high-molecular-weight polymers comprised of the monomeric unit flavan-3-ol (+)catechin and (-) epicatechin. Oxidative condensation occurs between carbon C-4 of the heterocycle and carbons C-6 or C-8 of the attached A and B rings [6] . The procyanidins B1-B4, characterized by the C4-C8 linkage, are the most common dimers, occasionally accompanied by corresponding C4-C6 linked isomers (B5-B8) [7] . The biological properties of flavnoids, including proanthocyanidins, have been extensively reviewed [8]. In addition to their free radical scavenging and antioxidant activity [9].Proanthocyanidins have been reported to have antibacterial, antiviral, anticarcinogenic, anti-inflammatory, anti-allergic, and vasodilatory actions [5],[10] Proanthocyanidins have also been shown to inhibit lipid peroxidation,platelet aggregation,capillary permeability and fragility, and to affect enzyme systems including phospholipase A2, cyclooxygenase, and lipoxygenase [5, 9, 10].

Resistance to antimicrobial agents has become an increasingly important and pressing global problem. Substantial investment and research in the field of anti-infective are now desperately needed if a public health crisis is to be averted. Structural modification of antimicrobial drugs to which resistance has developed has proven to be an effective means of extending the lifespan of various antibacterial agents including -lactams and quinolones [11].

The aim of this study is to investigate inhibitory activity of Proanthocyanidins extracted from (*Vitis vinifera*) in vitro on some of pathogenic bacterial isolates and to compare with the effect of some antibiotics.

MATERIALS AND METHODS

Preparation of Proanthocyanidins:

Proanthocyanidins was prepared as described by [12] .

Test microorganisms

Eight isolates of pathogenic bacteria : 4 isolates of *Salmonella typhimurium* 1,2,3,4 ,one isolate for *Pseudomonas aeruginosa* , *Klebsiella alexanderi* , *Staphylococcus aureus*و *Escheria coli.* were

collected from Biology department - labs / College of Science/Al Mustansiriya University the organisms were identified by standard microbiological techniques including colony morphology, and by Api Staph, Api20 E biochemical characteristics [13] **.**

The antimicrobial activity of Proanthocyanidins:

The antimicrobial activity of Proanthocyanidins was determined by agar well diffusion method against the isolates of bacteria in this method, pure isolate of 24hrs growth was cultured in Muller-Hinton Agar plate (Hi Media, Mumbai, India) by using sterile swab so as to achieve a confluent growth. The plates were allowed to dry and a sterile cork borer of diameter 8.0mm was used to bore five wells in each agar plates. Five concentration of the crude extract were made by dissolving (1) gram of the crude extract in (2) ml distilled water to obtain 500mg\ml filteredin Millipore filter paper and used as stock to prepare the other concentration (100,200,300,400,500) mg\ml .A 10µL volume of each concentration was applied by micropipette in the wells into Muller-Hinton Agar plate. Distilled water served as control [14] . The plates were allowed to stand for 1hr or more for diffusion to take place and then incubated at 37ºC for 24hrs. The zone of inhibition was recorded. Each experiment was performed in duplicate.

Sensitivity to antimicrobial agents

All isolates were tested against ten different antimicrobial agents including :

N0.	Antibiotics	Symbol	Concentration
1	Ampicillin	Am	10 mcg/disk
2	Augmentin	AMC	20/10 mcg/disk
3	Aztreonam	ATM	30 mcg/disk
4	Ceftriaxone	CRO	30mcg/disk
5	Cephalexin	CL	30mcg/disk
6	Ciprofloxacin	CIP	5 mcg/disk
7	Doxycycline	DO	30 mcg/disk
8	Erythromycin	E	15 mcg/disk
9	Gentamicin	CN	10 mcg/disk
10	Tetracycline	TE	30 mcg/disk

And this was done by taking100µl of bacterial suspension and streaked onto a Muller-Hinton agar plate using a sterile cotton swab. After 10-15minutes, antibiotic disks were put onto the agar plates using a sterile forceps then incubated at 37˚C for 24 hours. The diameters of inhibition

zones were measured and bacteria were determined as sensitive or resistant according to the standard measurements [15] .

RESULTS AND DISCUSSIONS

The study was performed for measuring the antibacterial activity of Proanthocyanidins which extracted from grape seed (vitis vinifera) seed, against number of pathogenic bacteria which isolated from different clinical sample (urine, skin, blood, stool). There were :4 isolate of *Salmonella: Salmonella typhimurium* sero var 1, *Salmonella typhimurium* sero var 2, *Salmonella typhimurium* sero var 3, *Salmonella typhimurium* sero var 4, and one isolate of *Pseudomonas aeruginosa* , *Klebsiella alexanderi*, *Staphylococcus aureus* ,and *Escheria coli.*

These isolates were tested for antibiotic susceptibility against 10 antibiotic which include : (Ciprofloxacin, Tetracycilin, Gentamicin, Ampicillin, Doxycycline, Cephalexin, Ceftriaxone, Augmentin, Aztreonam, Erythromycin), by disk diffusion method and the results of antibiotic susceptibility revealed that the isolates showed multiple drug resistance listed in table 1 : *Pseudomonas aeruginosa* isolate was highly resistance for all tested antibiotics in 100% .While the percentage of resistance to *Staphylococcus aureus* was 70% and sensitive to CN, CRO antibiotic , the percentage of resistance of the isolate *Salmonella typhimurium* sero var 1,2 and *Klebsiella alexanderi* was 60%, for the isolate 3,4 50%, and for the isolate of *E.coli* was 40%. , *E. coli* was less 50% and its sensitive to antibiotics TE, CN, CIP, CRO, ATM, CL, these results agree with other paper[16,17].

Table-1 : Antibiotic sensitivity test.

Test organism	CIP 5mcg	TE 30mcg	CN 10mcg	AM 10mcg	ATM 30mcg	DO 30mcg	CL 30mcg	CRO 30mcg	AMC 20/10mcg	E **15**mcg
	Zone of inhibition[mm]									
Salmonella 1	S	S	S	R	S	R	R	R	R	R
Salmonella 2	S	S	R	R	S	R	S	R	R	R
Salmonella 3	S	S	S	R	S	R	R	S	R	R
Salmonella 4	S	S	S	R	S	R	S	R	R	R
Staphylococcus aureus	R	R	S	R	R	S	R	S	R	R
Pseudomonas aeruginosa	R	R	R	R	R	R	R	R	R	R
Klebsiella alexanderi	R	R	R	R	S	S	S	S	R	R
Escheria coli	R	S	S	R	S	S	S	S	R	R

CIP:Ciprofloxacin(5mcg),TE:Tetracycilin(30mcg),CN:Gentamicin(10mcg)، AM :Ampicillin (10mcg), ATM:Aztreonam (30mcg), DO: Doxycycline(30mcg), CL:Cephalexin(30mcg), CRO: Ceftriaxone(30mcg), AMC: Augmentin (20/10mcg) ,E:Erythromycin(15mcg).

MIC (Minimum inhibitory concentration) was also detected as showed in table 2 : the MIC of some antibiotics (CRO, DO, CL, ATM) were also determined using the two fold dilution methods . The isolates determent resistance or sensitive according to the (break point) value.

Table-2: MIC of the antibiotics against pathogenic bacterial isolates.

Antibiotic / Test organism	CRO R ≥ 64	DO R ≥ 16	CL R ≥ 14	ATM R ≥ 32
Salmonella 1	512	128	256	32
Salmonella 2	512	128	14	32
Salmonella 3	32	128	128	32
Salmonella 4	512	128	14	32
Pseudomonas aeruginosa	512	256	1024	1024
Staphylococcus aureus	64	8	128	1024
Klebsiella alexanderi	32	8	14	32
Escheria coli	32	8	14	32

The results revealed that the isolates of bacteria tested were grown in highly concentration of the antibiotic , this results agree with similar results obtained found the antibiotic have highly activity against most of isolates [18,19.20].

Antibacterial activity of Proanthocyanidin:

As shown in table 3 some isolates showed inhibition zone against Proanthocyanidin extract in all concentrations and zone of inhibition increased by increasing in the concentration of the Proanthocyanidin extract. Proanthocyanidin showed highly antibacterial activity against all isolate of *Salmonella typhimurium* and *Escheria coli* this activity increased with concentration as showed in table 3 .The inhibition zone was (24 – 33) in the concentration of 500 mg/ml , While other isolate showed less response against Proanthocyanidin, the inhibition zone was (10 – 27) in the other concentration (100, 200, 300, 400) mg/ml

The results agree with similar results found that Proanthocyanidins are also known to possess antibacterial, antiviral, anti-inflammatory, anti-allergic, and vasodilator properties [21].

Table-3: Antimicrobial activity of Proanthocyanidin from grape (*Vitis vinifera*) against bacterial isolates.

Test organism	Proanthocyanidin extract concentration mg\ml					
	100mg\ml	200mg\ml	300mg\ml	400mg\ml	500mg\ml	Control
	Inhibition zone [mm]					
Salmonella 1	Growth	13	15	17	24	Growth
Salmonella 2	Growth	13	15	20	30	Growth
Salmonella 3	Growth	17	20	27	33	Growth
Salmonella 4	Growth	14	16	21	25	Growth
Staphylococcus aureus	Growth	Growth	12	12	17	Growth
Pseudomonas aeruginosa	Growth	Growth	Growth	Growth	12	Growth
Klebsiella alexanderi	Growth	Growth	Growth	12	15	Growth
Escheria coli	Growth	10	20	25	30	Growth

*Inhibition zone (mm)

Previous investigations have reported that proanthocyanidin protects multiple target organs from drug- and chemical-induced toxicity. GSPE protects cells against acetaminophen-induced hepato- and nephrotoxicity, amiodarone-induced lung toxicity, doxorubicin-induced cardiotoxicity, and dimethylnitrosamine-induced spleen toxicity [22].

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