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The Antimicrobial Activity of Peel and Seeds Extracts of Red Grapes

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ABSTRACT

Phenolic compounds in the grapes has multi-functional activities including anti-microbial activities. The present study assessed the antibacterial and anti-fungal activity of the locally available red grape which collected from local markets in Erbil city. The phenolic compounds were extracts from Seeds and Peels. Agar well diffusion method was utilized for the antimicrobial activity assessment. The results showed that the highest inhibition zone was observed by peel and Seed extracts against *Bacillus cereus* which were 13mm and 16mm respectively. In contrast the lowest inhibition zone was obtained from seed and peel extracts against *Klebsiella Pneumoniae* which were 3mm and 7mm respectively. Also the inhibition zone of peel and Seed for *S. typhi* were 7mm and 9 respectively. However, the grape extracts showed no inhibitory activity against *Candida albicans and Sacchromyces cervisiae*. Also the data showed that the highest minimum inhibitory concentration of the peel and seed extracts were against *Escherichia coli* which were 460 and 225 (mg/ml) respectively. Whereas the lowest minimum inhibitory concentration of the peel and peed extracts were against *B. cereus* which were 20 and 30 (mg/ml). In conclusion *B. cereus* seemed to be more sensitive for Peel and Peel extracts. Whereas, *P. aeroginose* and *K. Pneumoniae* showed the lowest sensitivity against seed and peel extracts. Furthermore, regarding the minimum inhibitory activity, the data showed that the gram positive bacteria were more sensitive to the seed and peel extracts. But, the fungal species seemed to be resisting the phenolic compounds in peel and seed extracts.

الفعالية الحيوية للمضادات المايكروبية لمستخلصات القشرة و البذور في العنب الاحمر

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الخلاصة

المواد الفينولية في العنب تمتلك وظائف عديدة تشمل مضادات البكتيرية. تقيم هذه الدراسة فعالية المضادات للبكتيريا والاعفان في العنب الموجودة محليا في اربيل. استخلصت المواد الفينولية من القشرة والبذور. استخدمت طريقة الحفر في الوسط الزراعي (Agar well diffusion) لتقيم فعالية مضادات البكتيرية. تبين النتائج ان اعلى منطقة تثبيط لوحظت من قبل مستخلصات من القشرة وبذور العنب ضد كل من Bacillus cereus ب 13 ملم و 16ملم على التوالي. في حين اقل منطقة التثبيط لوحظت من قبل مستخلصات القشرة والبذور ضد كل من Klebsiella Pneumoniae ب 3ملم و 7ملم على التوالي . ايضا لم تظهر مستخلصات العنب اي فعالية التثبيطية ضد *Candida albicans* و *Sacchromyces cervisiae* . تبين النتائج ان اعلى تركيز التثبيط من القشرة والبذور ضد *Escherichia coli* ب 460 و 225(ملغم/مل) على التوالي. ولكن اقل تركيز التثبيط كان ضد *B. cereus* ب 20 و 30 (ملغم/مل) . وعلى العموم لدى مستخلصات العنب فعالية تثبيطية ضد البكتيريا اعلى مما هو عليه للاعفان. اضافة الى ذلك فان البكتيريا موجبة للصبغات اكثر حساسية لمستخلصات العنب مقارنة بالبكتريا سالبة للصبغات.

الكلمات المفتاحية:
العنب الاحمر فعاليات التثبيطية مستخلصات القشرة و البذور، اقل فعالية تثبيطية.

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Introduction:

Grape (*Vitis Vinifera*) is one of the main crops cultivated and widely consumed globally (Ranjitha C.Y and T.R, 2014). Grapes have classified into groups including wine grapes, raising and table grapes ((Xia et al., 2010). Grapes are a main source of poly-phenolic compounds essentially epicatechin , monomeric catechin and gallic acid. Also, anthocyanins, hydroxycinnamic acids, flavanols and flavonol glycosides are abundantly present in the grape (Kabir et al., 2015). The aforementioned chemicals have been stated to have many health benefits such as reducing the consequences of cardiovascular diseases, cancerous diseases and anti-ageing (Shanmuganayagam et al., 2007, Tsang et al., 2005) . Additionally, it has been stated grape extracts such as Seed and Peels have a wide application in pharmacology and therapeutic influences against apoptosis and inflammation (Sato et al., 2001, Li et al., 2001). Plant extracts including grapes extracts have been submitted to intensive investigations microbiologically. It has been discovered that they can damage microbial cells and inhibit the growth of microbes (Mirkarimi et al., 2013). However, local varieties need more investigations in terms of antimicrobial inhibitory activity against some human pathogenic microorganisms to be compared to global grape varieties. The aim of this research is to assess the antioxidants and antimicrobial activity of seed and peel extracts of red grapes against some pathogenic microorganism including *Staphylococcus aureus E. coli, Bacillus cereus, Salmonella typhi, P. aeroginose* and *K. Pneumoniae.* Also it is potential to utilize as a natural antimicrobial agent against some yeast such as *Candida albicans and Sacchromyces cervisiae.*

Methods and Materials:

Collection and preparation of grapes samples:

Samples of Red grapes: Locally available Red grapes *(Vitis vinifera)* were collected from local markets in Erbil city on August 2015. The grapes were crushed and seeds were separated. The seeds were washed well using clean water and dried in oven. The seeds were crushed in a blender and used in the experiment.

Extraction Method:

The phenolic compound was extracted by using a solvent prepared from ethanol and water in a ratio (90:10) respectively then acidified with 0.4% hydrochloric acid (37%) at pH values of 2.5. 100ml of acidified solvent was added to 100g of sample and homogenized in a blender for 2min. Then the content was transferred to a 400 ml container and covered with parafilm in a dark place overnight in the fridge; the mixture was then filtered under vacuum using filter paper after storage and washed with acidified solvent. Later 250 ml was collected from the filtrate solution, and 125 ml of acidified ethanol was added to it, filtered again and the residue washed with solvent to a total of 450 ml solution, the extract was transferred to a 500 ml volumetric flask and the volume was made up with the solvent (Lees and Francis, 1972).

Collection of microorganism:

Microorganisms: *Staphylococcus. aureus, Escherichia coli, Bacillus. cereus, Salmonella typhi, Pseudomonas aeroginose, Klebsiella Pneumonia, Candida albicans* and *Saccharomyces cerevisiae* were collected From Biology department, College of Science, Salahaddin University and Rzgari Hospital-Erbil. The microorganisms were grown in nutrient broth (Himedia) at 37ºC for 18 hrs. The turbidity of the growth was compared with McFarland standard (tube no. 0.5) to reach approximately 1.5 × 108 CFU/ml.

Assessment of Microbial Activity :

The antimicrobial activity of the grape extracts was assessed using agar well diffusion method. Serial dilutions were prepared from of grape extracts. The inoculums microbial was uniformly spread using sterile cotton swab on a sterile Mueller Hinton agar (Himedia), added 50 µl of serial dilution were added to the wells (6 mm diameter holes, 20 mm apart from one another).

They were incubated for 24 hrs at 37°C, under aerobic conditions. Results represent the inhibitory action of microbial growth around the well and calculated in mm(Nirmala and Narendhirakannan, 2011) .

Minimum Inhibition Concentration (MIC):

The MIC represents the lowest grape extracts concentration which visibly inhibited the growth of the microbes at 37 °C for 18 hrs. The MIC was calculated using liquid micro dilution method. Firstly, the microbial inoculums were prepared as explained earlier. Then, the serial dilutions of grape extracts dissolved in methanol (the preparation method was explained earlier) , to each well added 50 µl of microbial inoculums. The MIC defined as: The lowest concentration of the extracts which inhibited the visible growth after incubation for 24 at 37 ℃ (Nirmala and Narendhirakannan, 2011).

Determination of percentage inhibition of diameter growth

The inhibition percentage of the grape extracts was calculated after discovering the inhibition zone according to the following equation (Himratul-Aznita W.H., 2011)

$$Inhibition\ percentage\ \% = \frac{Diameter\ of\ the\ control - diamter\ of\ the\ sample}{Diameter\ of\ the\ control} * 100$$

Results and discussion :

The effect of grape seeds and peel extracts on the activity of some pathogenic gram positive and negative bacteria was found as showed in table (1). Regarding the peel extracts, it is obvious that the maximum inhibition zone was observed against B. *cereus* which was 13 mm inhibition percentage 76%. In contrast, seed extracts had the minimum inhibition zone on both P. *aeroginose* and K. *Pneumoniae* which were 3mm and 3mm with the inhibition percentage 87% and 87% for both of them. Similarly, seed extracts had the highest inhibition zone on B. *cereus* which was 16 mm with the inhibition percentage 72%. On the other hand the lowest inhibition was observed against K. *Pneumoniae* which was 7 mm with the inhibition percentage 82%. It can be seen that the gram positive bacteria were more sensitive than the gram negative bacteria for both extracts. In a study about the influence of antimicrobial activity of grape fluid and pomace against some pathogenic bacteria and yeasts, it was found that the pomace which includes peel and seeds extracts inhibited the growth of some gram negative bacteria (Oliveira et al., 2013)). This is congruent with our finding. Also it was found that the seed extracts were more effective than other grape parts. It has been stated that the grape seed extracts seemed to possess more inhibitory activity than other grape component extracts (Xia et al., 2010). This could due to the effectiveness of phenolic compound in the seed s against pathogenic bacteria.

Table 1: The inhibition diameter of the seed and peel extracts against some pathogenic bacteria

Bacteria isolates	**Grape extracts**			
	Peel extracts (mm)	Inhibition percentage (%)	Seed extracts	Inhibition percentage(%) (mm)
Staphylococcus aureus	10	79	13	76
Escherichia coli	4	86	8	81
Bacillus cereus	13	76	16	72
Salmonella typhi	7	82	9	80
Pseudomonas *aeroginose*	3	87	9	80
Klebsiella Pneumoniae	3	87	7	82

Regarding the antifungal activity of the both seed and peel extracts against two neither different species *Candida albicans* nor S. *cervisiae* are presented in the table (2). It is obvious that there were no effect of the seed and peel extracts on the fungi and the inhibition percentage was zero% for both fungi and for both extracts. A study was conducted on the phenolic compound in the red and white wine, it was found that *Candida spp* resisted the phenolic compound and the inhibition zone was zero (Papadopoulou et al., 2005). This is in congruent with our findings.

Table 2: The anti-fungal activity of seed and peel extracts

Yeasts isolates	Grape extracts			
	Peel extracts	Inhibition percentage %	Seed extracts	Inhibition percentage %
Candida albicans	-	0	-	0
Sacchromyces cervisiae	-	0	-	0

Minimum inhibitory concentration (MIC) data for peel and seed extracts is shown in the table (3). Regarding peel extracts, it can be seen that the highest MIC is seen again *E coli* which was 460 (mg/ml), where the lowest MIC was seen against *B. cereus* which was 30 (mg/ml). On the other hands, for seed extracts the highest MIC was seen against *E coli* which was 225 (mg/ml) but the lowest MIC was 20 (mg/ml) against *B. cereus*

This can refer to the fact that the grape seeds and peel extracts could be used against both gram positive and negative bacteria. This is in agreement with finding of the previous studies. For instance, in a study about grape polyphenol as natural bioactive, it was concluded that the there is a bactericidal activity in grape constituents extracts against both gram positive and negative bacteria (Kabir et al., 2015). In another study about the antimicrobial acitivty of grape seed extracts against some urinary tract infections bacteria such as S. *aureus , K. pneumoniae and E. coli*. It was also noted that the seed extracts were more effective against the bacteria so that they can be used as antibacterial agent(Ranjitha C.Y and T.R, 2014). It was also stated that film coating containing grape seed extracts could be effective against *S. Typhi* (Sivarooban et al., 2008). On the other hand, it was worth mentioning that gram positive bacteria *(B. cereus)* seems to be more sensitive to seed and peel extracts comparing to gram negative bacteria *(E coli)*. This was in agreement with other studies (Papadopoulou et al., 2005, Jayaprakasha et al., 2003). The reason could be attributed to the chemical structure and complexity of the bacteria cell wall. Therefore the less sophisticated cell wall structure in gram-positive bacteria makes it more susceptible to the anti-microbial (El Darra et al., 2012).

Table 3: Minimum inhibitory concentration (mg/ml) of the seed and peel extracts against some pathogenic bacteria

Bacteria isolates	Minimum inhibitory concentrations of Grape extracts(mg/ml)	
	Peel	Seed
Staphylococcus aureus	70	35
Escherichia coli	460	225
Bacillus cereus	30	20
Salmonella typhi	250	125
Pseudomonas aeroginose	45	35
Klebsiella Pneumoniae	40	32

Conclusions:

To summarize, the study assessed the antibacterial and anti-fungal activity seed and peel extracts of locally available red grapes. The results referred that the seed and peel extracts inhibited the growth of microorganisms. *B. cereus* seemed to be more sensitive for peel and eel extracts. Whereas, *P. aeroginose* and *K. Pneumoniae* showed the lowest sensitivity against seed and peel extracts. Furthermore, regarding the minimum inhibitory activity, the data showed that the gram positive bacteria were more sensitive to the seed and peel extracts. But, the fungal species seemed to be resisting the phenolic compounds in peel and seed extracts.

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