

INHIBITORY EFFECT OF TANNIC ACID EXTRACTED FROM GRAPE SEEDS AND POMEGRANATE PEELS ON SOME MICROORGANISMS

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ABSTRACT

The study included extraction of tannic acid from grape seeds and pomegranate peels , their content of tannic acid were 4.1 and 27.6 g/100g (dry weight), respectively .The quality tests which done on extracted tannic acid from both sources showed positive results, where the increasing tannic acid concentration leads to increase killing effect on studied microorganisms especially at highest concentration (2%) .These results showed that extracted tannic acid from both sources can be used as preservation material in food processing and component of cosmetic materials and pharmacological drugs.

INTRODUCTION

Tannins are water soluble polyphenols that present in many different parts of plants(Akiyama *et al.*,2001), such as Oak tree galls, Peels of banana and pomegranate fruits, fruits of date tree and apple, seeds of coffee and grape as well as in many tropical plants (Huang *et al.*, 1993) .Tannins also play an important role in acceptability of food products and their chemical structures are different, though they have a distinguishing astringent taste (Chung *et al.*,1998) .Tannins compounds classified into two groups: hydrolyzable tannins, and; non hydrolyzable tannins. The first one is ester of phenolic acids with sugars (Scalbert,1991) .Tannic acid was identified according to Deyeux and Segeux in1795 (The Birtish Pharmaceutical Codex, 1911). It is Gallotannin which is ester of gallic acid with glucose and then classified as hydrolyzable tannins (Miranda *et al.*, 1996) .Tannins compounds were used in many industrial applications, such as juices clarification and antioxidant material when added to many food products and beverages (Khan *et al.*, 2000), also in oil refining and in preservation of marine meat fish and in manufacture of blue ink (Huang *et al.*, 1993) and a component of cosmetic products and pharmacological drugs due to their activity against chronic dysentery, stimulating healing and treatment cholera and its best antidote against poisonous mushrooms (Chung *et al.*, 1993). Chung *et al.* (1998) and Scalbert (1991) reported that tannic acid has inhibition effect on many different species of food born bacteria like *Aeromonas hydrophila*, *Escherichia coli*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Salmonella typhi* and *Enterococcus faecalis* . Heil *et al.* (2002) observed that tannins compounds have inhibition act on some fungi. Many studies had done on extraction of some chemical compounds from plants to identify their quantity, quality properties and inhibition effect on microorganisms (Cowan, 1999), but they didn't take it in consideration to use by products of food processing like pomegranate peels and grape seeds as a source of tannic acid. The main objective of this research is to extract tannic acid from grape seeds and

pomegranate peels as a by products of local fruits, and determination their quantity, quality properties and inhibitory effect on some microorganisms.

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MATERIALS AND METHODS

1- Samples preparation: The study was done on seeds of local grape fruit (*Vitis vinifera var.local*) and peels of pomegranate fruit (*Punica granatum var.local*). Fruit samples were taken from local market of Mosul city, Iraq, cleaned by water, then the grape seeds were extracted by electrical rotary juicer machine, washed by water and scoured by hand to separate tissue and peel of fruits. Pomegranate peels were obtained by removing seeds handly , grape seeds and pomegranate peels were dried separately in drying oven at 55°C until their moisture content reached 5% , then milled twice separately to obtain homogenized powder and packaged in vacuum plastic containers , thereafter stored in dark place at 4°C until different tests were done (Heil *et al.* , 2002) .

2- Tannic acid extraction: Tannic acid was extracted from grape seeds and pomegranate peels according to the procedure of Mole and Waterman (1987), extraction of tannic acid from grape seeds was modified because of their high content of oil , extraction was done by taking 30 g of grape seeds powder added to it 200 ml ethanol using soxhlet distillation at 40-60°C for 24 hours , the extract was evaporated by using vacuum rotary evaporator to remove solvent ,then 100 ml distilled water was added to the residue , oil layer was separated by separation funnel , followed by filtration . Ethyl acetate was added to filtrate many times, later acetate layer was separated and evaporated by using vacuum rotary evaporator until gaining semisolid product (tannic acid) to use it in different tests. Extraction of tannic acid from pomegranate peels was done by taking 20 g of their powder ,and adding 200 ml distilled water then extracted by soxhlet distillation at 100°C for 24 hours , ethyl acetate was added for many times . Acetate layer was separated by separation funnel and evaporated by vacuum rotary evaporator until gaining semisolid product (tannic acid) to use it in different tests.

3- Determination of tannic acid: Tannic acid content of grape seeds and pomegranate peels was determined according to McMurrough and McDowell (1978), by using DMCA (4-Dimethylamino-cinnam aldehyde) reagent .The absorbance was done at 640 nm by spectronic-20

4- Quality tests: These tests were done to insure that extracted material from grape seeds and pomegranate peels was tannic acid according to The British Pharmaceutical Codex (1911) and Shriner *et al.* (1964) .These tests were included solubility in water, ethanol, ethyl ether, chloroform and benzene and acidity test with litmus paper and formation white crude with gelatin or albumin and formation colored precipitate with potassium chromate , lead or cupper acetate, ferric chloride, potassium cyanide and purity test when treated with calcium chloride to distinguish tannic acid from gallic acid.

5- Microbiological tests: Tested strains: Different microbial strains were chose according to their role in food processing. Some of these microbial strains caused undesirable effect and food deterioration ,such as, gram positive or negative bacteria, different optimum growth temperature , spore former , or non spore former bacteria and different shapes of bacteria. The studied microorganisms, included:

1. *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Lactobacillus bulgaricus* (*L.delbruekii* sub sp. *bulgaricus*) and *Streptococcus thermophilus* (*Str. salivarius* sub sp. *thermophilus*).

2. *Saccharomyces cerevisiae* and *Aspergillus niger*.

The pure cultures were obtained from the Department of Food Science and Biotechnology, College of Agriculture and Forestry, Mosul University. The media that used for the growth of microorganisms were milk starch agar ,mannitol salt agar , macconkey agar ,MRS agar and potato dextrose agar .These media were prepared according to Harrigan and MacCance (1976) and Diliello(1982),the microbial cultures were activated at 37°C for 24 hours before test by using nutrient broth for *Bacillus subtilis*, macconkey broth for *E. coli*, nutrient broth and skim milk for lactic acid bacteria, brain heart infusion for *Staph. aureus* and malt broth for fungi which incubated at 28°C for 4 days .The suitable dilution of activated microorganisms were prepared by using physiological solution (distilled water + 0.1 % peptone in test tubes which contained 9 ml ,by using dilution 10^{-4} , 10^{-5} , 10^{-6}) . The concentrations of tannic acid that added to nutrient media were 0.5, 1.0, 1.5 and 2.0 %. To study the resistance of studied microorganisms was done by putting 1 ml of each dilution in two replicate and putting it in sterilized petri dish that contained sterilized nutrient agar media and tannic acid, mixed together with cyclic motion ,then lefted until the media sold then incubated at 37°C for 3 days for bacteria and 5days at 28°C for fungi .

The growing colonies number were determined and multiplied by dilution reciprocal to obtain total numbers of studied microorganisms .% of reduction ratio of studied microorganisms was determined by using this equation:

$$= \frac{\text{No. of microorganisms without tannic acid} - \text{No. of microorganisms with tannic acid}}{\text{No. of microorganisms without tannic acid}} \times 100$$

RESULTS AND DISCUSSION

1- Tannic acid content: Table (1) reveals that the tannic acid content of grape seeds is 4.1 g / 100g (dry weight), which is less than what Hulme (1971) reported where the content of total tannin was 4.5 g /100 g , and higher than what Rabak and Shrader (1981) found in that the content of tannic acid was 1.55 %. This variation can be explained on the differences between studied species of grape fruits , genetic factorial , extraction method , season of taking samples and breeding circumstances which have a significant effect on chemical constitutes of fruit. The ratio of seeds to grape fruit was between 3 - 4% , therefore the amount of tannic acid from 1000 g fruit was between 120 - 160 g which is a little amount comparing with the result mentioned by Dalaly and Al-Hakim (1987) which indicated that total tannin content of tea leaves was between 7-15 % (wet weight) and between 30 -70 % of tannic acid in Oak tree galls (Mohammed *et al.*, 1988) , thus , grape seeds is not considered as an important source of tannic acid . The result in the same table shows that the tannic acid content of pomegranate peels is 27.6g/100g (dry weight) , this result is higher than the findings of Dalaly and Al- Hakim (1987

) which showed that total tannin content of pomegranate peels was 20%

Quality tests	Tannic acid content (dry weight)	
	Grape seeds	Pomegranate peels
	4.1 g/100g	27.6 g/100g
1.Solubility		
a-water	+	+
b-ethanol	+	+
c-chloroform	-	-
d-benzene	-	-
2-Acidity		
Litmus paper indicator	red	red
3-Colored precipitate		
a-ferric chloride	blue	blue
b-potassium cyanide	blue	blue
c-potassium chromate	brown	brown
d-lead acetate	brown	brown
e-cupper acetate	brown	brown
4-Crude test		
a-gelatin	+	+
b-albumin	+	+
5-purity test		
calcium chloride	pink	pink

.Accordingly ,it was concluded that pomegranate peels are important source of tannic acid comparing with grape seeds .

2 – Quality tests: All quality tests that done on extracted material from grape seeds or pomegranate peels gave positive results ,which indicate that the extracted material from both sources is tannic acid . The extracted material gave total solubility in water (1 part : 1 part) and with ethanol (10 parts : 1part) and insoluble in ethyl ether or in chlorophorm or in benzene and produced red

Table (1): Quality tests and tannic acid content of grape seeds and pomegranate peels
+: Positive result -:Negative resul

color with litmus paper (acidity test) while formed blue precipitate with ferric chloride or with potassium cyanide and produced brown precipitate with potassium chromate or with lead or cupper acetate and produced pink color with calcium chloride to insure that the extracted material was pure and neither hydrolyzed nor contaminated. These results agreed with the British Pharmaceutical Codex (1911) and Schmidt (1956) and Shriner *et al* . (1964).

3 -Inhibition effect on microorganisms: Results in figure (1) showed that increasing tannic acid concentration caused decreasing in bacterial numbers and increasing reduction ratio .The *Staph. aureus* is the most sensitive bacteria to tannic acid especially at 2%.It's initial number was 13×10^5 decreased to 4×10^5 c.f.u/ml ,and the reduction ratio was 69%.The initial number of *E.coli* was 33×10^4 then decreased to 16×10^4 c.f.u/ml ,and the reduction ratio was 51 % ,and *Bacillus subtilus* with its initial number 68×10^6 decreased to 47×10^6 spores /ml ,and the reduction ratio which was 30% is lesser sensitive .

Figure (2) showed that the effect of tannic acid concentrations on numbers of *Str.thermophilus* (*Str. salivarius* sub sp. *thermophilus*) and *Lact. bulgaricus* (*L. delbruekii* sub sp. *bulgaricus*) .Using 2 % of extracted tannic acid caused decreasing initial numbers of *Str. thermophilus* from 52×10^4 to 26×10^4 c.f.u/ml , the reduction ratio was 50% .The initial number of *Lact . bulgaricus* was decreased from 3×10^4 to 16×10^3 c.f.u/ ml , the reduction ratio was 46 % .

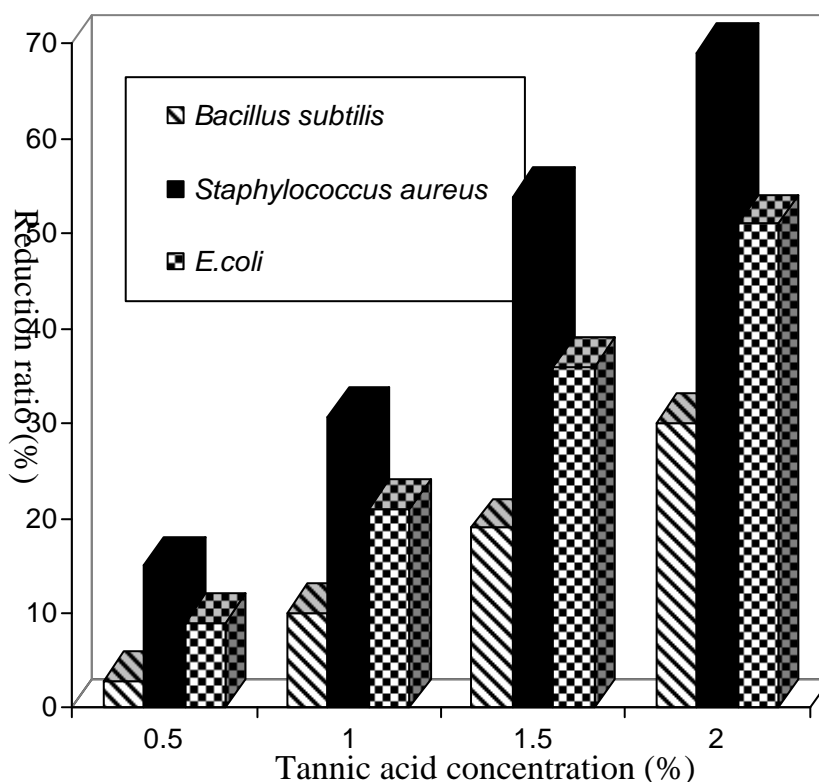


Figure (1): Effect of different tannic acid concentrations on number of *Bacillus subtilis*,
Staphylococcus aureus and *E. coli*

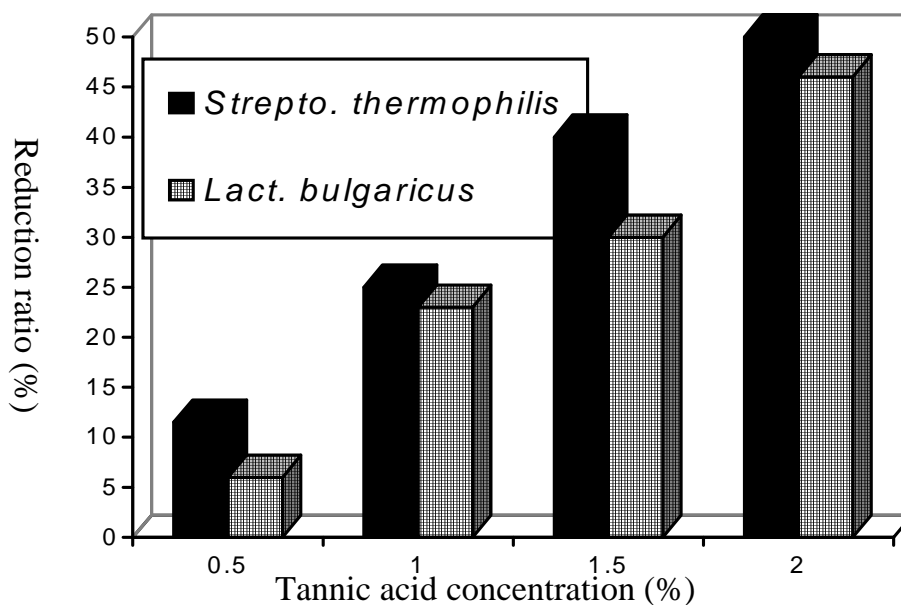


Figure (2): Effect of different tannic acid concentrations on number of *Streptothermophilis* and *Lact. Bulgaricus*

Figure (3)showed that using 2% tannic acid caused highly effect on numbers of studied fungi , where decreased the initial numbers of *Aspergillus*

niger from 46×10^5 to 38×10^5 c.f.u/ml . The reduction ratio was 17%, and caused decreasing

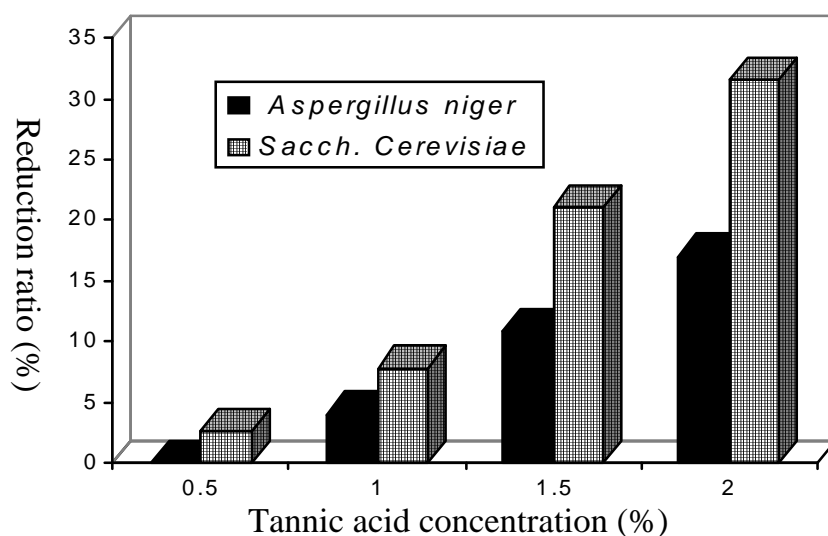


Figure (3): Effect of different tannic acid concentrations on number of *Aspergillus niger* and *Sacch. cerevisiae*

initial numbers of *Saccharomyces cerevisiae* from 38×10^4 to 26×10^4 c.f.u /ml , the reduction ratio was 31.5% .

It was concluded from these results that the most resistant bacteria to tannic acid is *Bacillus subtilis* , because of their content of hard wall spores and low moisture content in addition to their content of dipicolinic acid which binding with calcium ions. This agrees with Chung *et al.* (1998).

These results indicated that the most microorganisms resistance to tannic acid is *Aspergillus niger* and *Saccharomyces cerevisiae* and this due to their complex chemical structure of their walls comparing with the structure of bacteria walls (Saleh and Bassam , 1982) . It was observed from figure 1, 2,3 that the most effective concentration of tannic acid is 2% . This caused reduction in number of *Staph. aureus* , *E. coli* , *Str.thermophilus* , *Lact. bulgaricus* , *B. subtilis* *Saccharomyces cerevisiae* and *Aspergillus niger* in ratio 69,51,50,46,30,17 and 31.5 % , respectively. These results agree with Scalbort (1991) who had observed that tannic acid inhibited the oxidative phosphorylation pathway and microbial enzyme which hydrolyse the substrate that needs during growth such as proteolytic , lipolytic enzymes and carbohydratase and observed its binding with proteins by formation polymer compounds with hydroxyl groups.

التأثير المثبط لحامض التانيك المستخلص من بذور العنب وقشور الرمان في بعض الاحياء المجهرية

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الخلاصة

تضمنت الدراسة استخلاص حامض التانيك من بذور العنب وقشور الرمان والذي كانت كميته ٤ و ٢٧.٦ غم/١٠٠ غم (على اساس الوزن الجاف) ، على التوالي . اوضحت الاختبارات النوعية على حامض التانيك المستخلص من كلا المصدرين انها كانت ايجابية ، ادت زيادة تركيز حامض التانيك الى زيادة قابلية التثبيط للاحياء المجهرية التي تم دراستها بصورة خاصة عند استخدام التركيز الاعلى (٢%). لقد اوضحت هذه النتائج ان حامض التانيك المستخلص من كلا المصدرين يمكن استخدامه كمادة حافظة في تصنيع الاغذية ومادة صيدلانية وعلاجية .

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