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RESEARCH ARTICLE

DETERMINATION OF OPTIMAL CONDITIONS TO EXTRACT ANTIBACTERIAL SUBSTANCES FROM *PRUNUSARMENIACE* SEEDS

***Alaa Abdul Hussein Al-Daamy**

Department of Clinical Laboratory, College of Applied Medical Sciences, University of Karbala, Karbala, Iraq

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ABSTRACT

The present study aims to determine the optimum extraction conditions for *Prunus armeniace* seeds and test their effectiveness against a group of pathogenic bacteria. Five solvents used are acetone, ethanol, methanol, ethyl acetate and disitilled water to obtain of crude extract of *Prunus armeniace* seeds, which tested the effectiveness of antibacteria on five types of bacteria are *Pseudomonas eroginosa, Pseudomonas oryzihabita, Proteus varaplis, Klebsila pneumonia* and *Staphylococcus aureus* to determine the most efficient solvent extraction of them, and then was use of a series of concentrations of the solvent is more efficient 0, 20, 40, 60, 80 and 100% to determine the most efficient concentration of solvent optimization, and then was determined minimum inhibitory concentration(MIC) of the extract more efficient. The results of the current study showed that the most efficient in the extraction solvent is ethanol that they were the diameters of inhibition zone are 13, 15, 16.66, 16.33 and 19.33 mm for the types of bacteria above, respectively. The results showed that the concentration of ethanol was 100% in the optimal inhibition of bacteria, amounting to diameters of inhibition zone at this concentration of 30, 31.66, 12.16, 24.16 and 30.66 mm for each of the bacterial species above, respectively. The results also found that MIC of *Pseudomonas eroginosa, Pseudomonas oryzihabita* and *Proteus varaplis* is 23 mg/ml, but MIC of *Klebsila pneumonia* is 16 mg/ml, while the MIC of *Staphylococcus aureus* is 13 mg/ml. The optimal conditions for extracting the most effective composites against pathogenic bacteria from *Prunus armeniace* seeds are using ethanol solvent with concentration of 100%.

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INTRODUCTION

For over thousands of years now, natural plants have been seen as a useful source of medical agents with proven potential of treating infectious disease and with lesser side effects compared to the synthetic drug agents, more intensive studies for natural therapies, the use of plant compounds for pharmaceutical purpose has gradually increased in world, because of the side effects and bacterial resistance against the antibiotics , the scientists developed new drugs from national sources such as plants, which have been extensively used as alternative treatment for disease (Sumitra and Yogesh, 2010; Manoj *et al*., 2010), as antibacterial (Kumar *et al*., 2007; Arthanari *et al*., 2009). In general, bitter *prunus armeniace* used in folk medicine in the treatmentofskin disease andparasiticdisease, the medical value of plants founds in some chemical substances that produce a definite physiological activity on human body, the most important of these bio active substances of *prunus armeniace*seeds are alkaloids, flavonoids, tannins, and phenolic compounds(Cai *et al*., 2004). Also contains moisture, protein, carbohydrate, fatty acids, amino acids, metals : K, Mg, Mu, Po 4-2, ash, glycosides(Sabah and Mazen, 2010). *Prunus armeniace*kernel is known for containing amygdalin, a toxic cyanogenic glycoside, amygdalin is hydrolyzed by B-glucosidase into d-glucose, benzaldehyde, and prussic acid (hydrogen cyanid) (Walker and Krieble, 1990). The aim of our experment was to study the antibacterial activity for extrat of *prunus armeniace*seeds with the crust by many solvents and with different dilution of this solvent, on five species of bacteria.

****Corresponding author: Alaa Abdul Hussein Al-Daamy,***
Department of Clinical Laboratory, College of Applied Medical Sciences, University of Karbala, Karbala, Iraq

MATERIALS AND METHODS

Microorganisms: We took five pathogenic bacteria, which isolated from patients in Al-zahraa teaching hospital, karbala provinica. These bacteria numbered as 1,2,3,4 and 5. These bacteria are :

1- *Pseudomonas eroginosa*
2- *Pseudomonas oryzihabita*
3- *Proteus varaplis*

4- *Klebsila pneumonia*
5- *Staphylococcus aureus*

The extraction prosses: We take the dry seeds of *prunus armeniace* with crust obtained from local farms to the holy city of Karbala.Blended into powder by electric blender. Then we take 100 gm of this powder and distributed to five beakers each beaker containing 20 g of powder, then it was put 100 ml of solvent 70% (the ratio of extraction is 1 gm : 5 ml) as was the use of solvents are five:acetone, ethanol, methanol, ethyl acetate and distilled water.Each beaker was labeled with its solvent name and incubating it in shaking incubator overnight with 150 cycle/minute at 37ºC. This method used for extraction were discribed by Al-Daamy *et al.* (2015). After 24 hrs of shaking, the extract purified by cotton and gauze in other beaker (the filtration must be quietly with pressing the gauze to obtain all the substance) then pour the pre collected substance of each beaker in tubes and centrifuge them in 3000 rpm for 5 minutes. Then pour the substance in the tubes in glass Petri dishes carefully without moving the precipitated material , and labele each Petri dish with the name of solvent.Let the dishes to be dry (these take some time if the weather is cold and the temperature is less than 37ºC). After the drying of all dishes we scrabbled each dish to obtain the extract powder. After that we weight each extract powder and record it, to calculate the percentage of extraction and what is the best solvent used (we saved it in separated containers labeled with solvent name).

Determination of optimal solvent for extraction: From the pre collected powder we weight 0.05 gm from each container and put it in separated 5 tubes, each tube contain extract for certain type of our 5 solvents). Add on each tube 2 ml of ethanol 70% and shaking the tube until the powder meltin, the final concentration of extract in each tube equal25mg/ml. In addition to our five tubes we add a sixth tube also contains 2 ml for control (70% ethanol without any extract). We prepare 30 Petri dishes of muller hinton agar and pour 20ml in each dish and drill the media 3 wells with dimeter 5mm to each well.After activation of bacteria in nutrient broth we culture it in the prepared dishes.In each Petri dish add 100µl of each activation bacteria and spread it by sterilized spreader , then add 50µl plant extract in each well, in the negative control we add just the 50µl ethanol 70%, also we used 50 µg/ml Gentamycin as a positive controle. After culturing we incubate all the dishes in incubator for 24 hrs. In 37ºC.Then examine the inhibition zone of each well in each dish, the results were recorded to determine the best solvent that has high activity against examined bacteria (Al-Daamy *et al.*, 2014). Determine the optimal concentration of the best solvent:After determine the best solvent which is ethanol. We take five beakers with different concentrations of ethanol [20, 40, 60, 80, 100]% each concentration in separated beaker. For each beaker we add 20 g of *Prunusarmeniace* extract powder, and repeat the same process of extraction method which described above and record the results. In the next step, take five tubes and put 0.05 gof dry extract in each tube, then add 2ml of 70% ethanol to each tube to obtain on the final concentration 25 mg/ml. After that, we prepared 30 Petri dishes with muller hintone agar and follow the 3 tests method to determine the best concentration of ethanol which has largest inhibition zone (Al-Daamy*et al.*, 2014).

Determine the minimal inhibition concentration (MIC): Prepare two flasks :

- First flask have 50ml nutrient broth media.
- Second flask have 25ml nutrient broth and added to it 0.625 g from ethanol 100% dry extract to obtain on 25mg/ml concentration in this flask from extraction.

Then take 26 glass tubes and label it with numbers from 0 to 25. On each tube put the extract from *prunusarmeniace* with ethanol 100% concentration which is equal to the number of the label of the tube. For example: in tube number 25 put only 2ml from flask 2 which represents 25mg/ml concentration. In tube number 24 put 1.92 ml from flask 2 and 0.08 ml from flask 1 which represents 24mg/ml concentration,so that with other tubes, in the tube number 0 put only 2 ml from flask 1to obtain of 0 mg/ml in this tube.The distribution between flask 1 and 2 in these tubes according to the law C1 V1 = C2 V2. After that, take micro titter plate with 96 wells and numbered the wells from 0 to 25, twice for each type of bacteria (each type of bacteria have 52 wells twice for each number), then added 150 µl from each tube in two wells of the same number on each type of bacteria. Then added 50 µl of each type of bacteria (after dilute it with both media and take from the 3rd dilution comparison with McFarland solution) in all the 52 wells from 0 to 25 concentrations. After that cover the plates and incubate them in incubator for 24 hrs. in 37ºC. Then examine the plates to see growth or no growth of bacteria and determine the minimal inhibition concentration from extract for each type of bacteria (Stephen and Cavalieri, 2005).

Statistical Analysis: Statistical analysis included random complete design (RCD) with 2 replicates. 0.05 is the level of probability that used to identify a significant differences. The significant differences between the averages was also tested by using the test less significant difference (LSD) at the level of probability of 0.05 (Dequ and Tessema, 2005).

RESULTS

The results in Table (1) shows that the percentage of substances using extraction are [7.5, 5, 5.05, 12.05, 11.5]% by using each of solvents [Acetone, ethanol, methanol, ethyl acetate, distilled water] respectively. In these results unclear (show) that the maximum percentage of extraction is utilization ethyl acetate in 12.05%, and the minimal percentage of extraction is utilization ethanol in 5%.

Table 1. The percentage of materials extracted from *prunus armeniaca* seeds

Extraction solvent (100ml)	Origin weight of powder (g)	Weight of extract (g)	Percentage of extract materials %
Acetone	20	1.50	7.5
Ethanol	20	1.00	5
Methanol	20	1.01	5.05
Ethyl acetate	20	2.41	12.05
Distilled water	20	2.30	11.5

The results in table (2) shows the extract of *prunus armeniace* seeds with ethanol have a larger diameter inhibition zone on all types of bacteria 13, 15.5, 16.66, 16.33 and 19.33 mm for bacteria *pseudomonas eroginosa, pseudomonas oryzihabita, proteus varaplis, klepsila pneumonia,* and *Staphylococcus aureus*; respectively. With significant differences ($P < 0.05$) comparison withgentamycin and also between other solvents using. In contrast, the results show that ethyl acetate extract showed no inhibitory effective against all bacterial species tested.

Table 2. Inhibition zone (mm) to extract *Prunusarmeniaca* seeds against bacteria

Bacteria	Extraction Solvent(70%)						$LSD_{0.05}$
	Gentamycin 10 мg/ml	Acetone	Ethanol	Methanol	Ethyl acetate	Distil water	
Pseudomonas eroginosa	21.66 ± 0.88	0 ± 0.0	13±1.15	10 ± 0.0	0 ± 0.0	12.33± 2.33	2.82
Pseudomonas oryzihabita	20.33 ± 0.88	11.5 ± 1.32	15.5 ± 1.44	11.33 ± 0.6	0 ± 0.0	11.66± 0.33	2.31
Proteus varaplis	12.5 ± 0.28	10.5 ± 0.76	16.66 ± 1.09	0 ± 0.0	0 ± 0.0	10.5± 0.28	1.43
Klebsila pneumonia	17.66 ± 0.33	0 ± 0.0	16.33 ± 2.92	0 ± 0.0	0 ± 0.0	13 ± 1.25	3.28
Staphylococcus aureus	17 ± 0.57	0 ± 0.0	19.33 ± 0.88	13.66 ± 1.58	0 ± 0.0	10.66± 0.33	1.99

The numbers refer to mean± standared error LSD is Least Significance Differences

Table 3. Inhibition zone (mm) of extract *Prunusarmeniaca* seeds against bacteria by using dilutions series of ethanol

Bacteria	Ethanol Ratio(%)						$LSD_{0.05}$
	Gentamycin 10 мg/ml	20 %	40 %	60 %	80 %	100 %	
Pseudomonas eroginosa	21.66 ± 0.88	16.16± 1.33	16.16±3.65	18.33±0.83	22.66±3.65	30.0±2.33	5.82
Pseudomonas oryzihabita	20.33 ± 0.88	10.66± 1.42	16.16 ± 2.16	18.5±1.5	23.33±4.95	31.66±1.66	6.26
Proteus varaplis	12.5 ± 0.28	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	11.83± 1.09	12.16±0.72	1.38
Klebsilapneumonia	17.66 ± 0.33	0 ± 0.0	0.0 ± 0.0	11.16±0.44	16 ± 1.5	24.16±3.63	4.08
Staphylococcus areus	17 ± 0.57	14 ± 0.57	14.5 ± 0.76	16.5±1.75	24.16± 0.83	30.66±2.04	3.12

Table 4. Minimum Inhibitory Concentration of ethanolic extract *Prunusarmeniaca* seeds

Extract Concentration (mg/ml)	Types of bacteria				
	Pseudomonas eroginosa	*Pseudomonas oryzihabita*	*Proteus varaplis*	*Klebsila pneumonia*	*Staphylococcus aureus*
1	+	+	+	+	+
2	+	+	+	+	+
3	+	+	+	+	+
4	+	+	+	+	+
5	+	+	+	+	+
6	+	+	+	+	+
7	+	+	+	+	+
8	+	+	+	+	+
9	+	+	+	+	+
10	+	+	+	+	+
11	+	+	+	+	+
12	+	+	+	+	+
13	+	+	+	+	-
14	+	+	+	+	-
15	+	+	+	+	-
16	+	+	+	-	-
17	+	+	+	-	-
18	+	+	+	-	-
19	+	+	+	-	-
20	+	+	+	-	-
21	+	+	+	-	-
22	+	+	+	-	-
23	-	-	-	-	-
24	-	-	-	-	-
25	-	-	-	-	-

+ means growth - means not growth

Figure 1. Inhibition zone of extract *Prunus armeniaca* seeds against bacteria

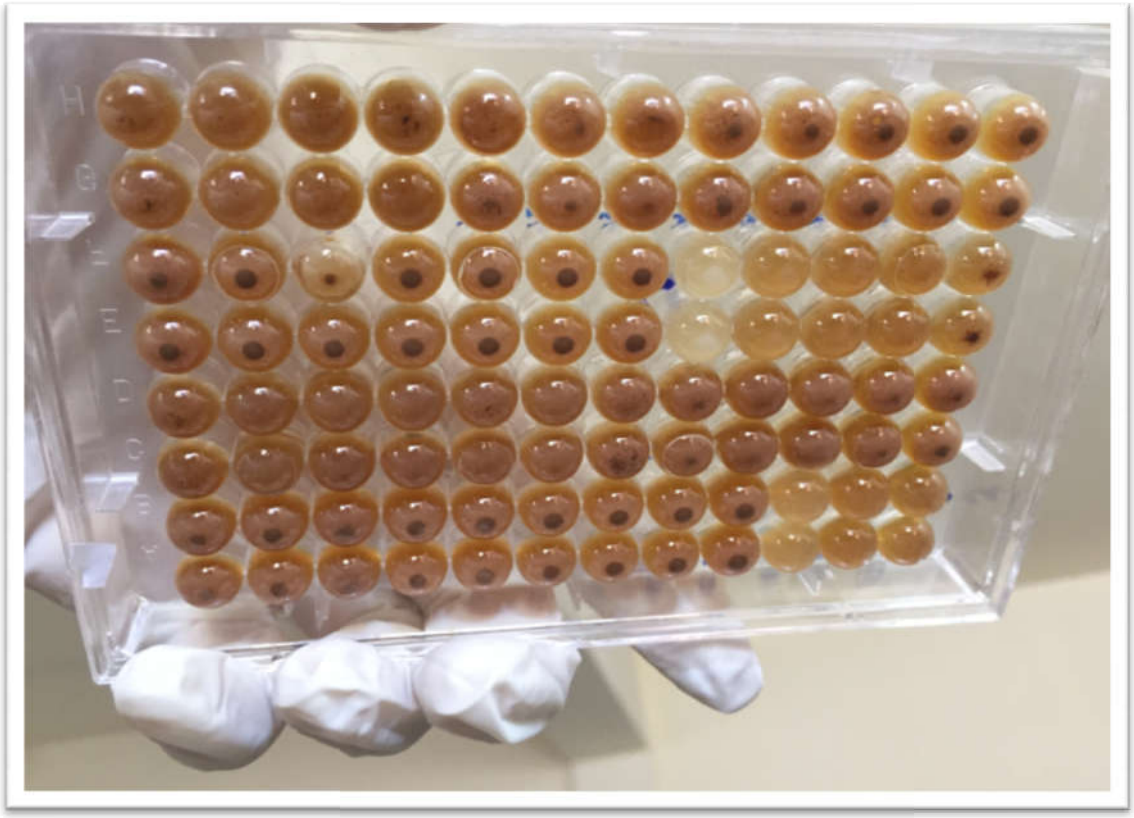

Figure 2. Minimum Inhibitory Concentration(MIC) to ethanolic extract

The results in Table (3) shows the extract of plant with 100% ethanol have a larger diameter inhibition zone on all types of bacteria 30.0, 31.66, 12.16, 24.16, 30.66 mm for bacteria *Pseudomonas eroginosa, Pseudomonas oryzihabita, Proteus varaplis, Klebsila pneumonia, Staphylococcus areus*; respectively, with significant differences ($P < 0.05$) comparison with gentamycin, also with significant differences ($P < 0.05$) comparison with other solvents. The results in Table (4) shows that the minimum inhibition concentration (MIC) for bacteria (*Pseudomonas eroginosa, Pseudomonasoryzihabita***,** *Proteus varaplis*) is 23 mg/ml, while for*Klebsila pneumonia* is 16mg/ml and for *Staphylococcus areus* is 13mg/ml.

DISCUSSION

In one study, showed that *prunus armeniace* seeds have antibacterial activity against *staphylococcus aureus* and haven't on *escherichiacoli*, but use 80 % ethanol (Aljamali, 2013). In other study, tested the activation of this plant on some type of species, but use only kernels of *prunus armeniace* seeds without crusts, and use the 35% ethanol as a solvent on some types of bacteria*Serratia marcesceus, Streptococcus pyogenes, Pseudomonas aeruginosa, Staphylococcus aureus* (Al-Bakri *et al*., 2010). Other researches showed the antibacterial activity of bitter apricot seeds in Iran on other types of bacteria S*almonella typhi, Salmonella typhi* A*, Salmonella typhi* B *, Escherichia coli, Staphylococcus aureus*, However, using 80% methanol (Abtahi *et al*., 2008). Other search in Pakistan use butanol and methanol extract of kernal apricot seeds against 20 types of Gram-positive and 13 types of Gram-negative bacteria, the butanol extract was more effective in inhibiting the growth of Gram-positive, with the highest activity exhibited against *Micrococcus luteus*, the methanol extract were highly active against the Gram-Negative bacteria especially *Escherichia coli* (Erdogan – orhan and Kartal, 2011). In Brazil, one study show the antibacterial activity of bitter and sweet kernals of P*prunus armeniace* extract, however by use methanol and water extract against five bacteria species *Enterobacter aerogenes, Escherichia coli, Proteus mirabilis, Pseudomonas aeruginosa* and *Staphylococcus aureus*, it's show activity against *Escherichia coli, Proteus mirabilis* and *Staphylococcus aureus* only (Yigit *et al*., 2009). Other study in korea determined the antibacterial activity of oil apricot seeds against 16 bacteria by disk diffusion, agar dilution and gaseous contact methods), it's show that the essential oil exhibited avariable degree of anti microbial activity against range of bacteria (Lee *et al*., 2014). In comparison with, our test used *prunus armeniace* seeds with crust which consist of moisture, protein, fatty acids, phenols, amino acids, carbohydrates, metals: K, M, Mn, Po4-2, glycosides[6]. While in other search use kernal without crusts, which consist of amygdalin there is different composition, which amygdalin is hydrolyzed by B-glucosidase into d-glucose, benzaldehyde, and prussic acid (hydrogen cyanid) without composition of crusts(Walker and Krieble, 1990). Also other search use oil of apricot seeds which consist mainly of unsaturated fatty acids such as oleic acid and linoleic acid (Femenia *et al*., 1995). These differences in composition make the search differences. In addition to the solvents and concentrations and methods that used in each search.

Conclusion: The optimal conditions for extracting the most effective composites against pathogenic bacteria from *Prunus armeniace* seeds are using ethanol solvent with concentration of 100%.

Recommendations

Other studies on apricot seeds and drawn more than one step and try to purify the material, which owns the effectiveness of anti-bacterial pathogenesis.

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