ANTIBACTERIAL ACTIVITY OF APRICOT KERNEL EXTRACT CONTAINING AMYGDALIN

Salih A. Al-Bakri, Zeid A. Nima, Raya R. Jabri, Esra'a A. Ajeel
Biotechnology Division, School of Applied Science, University of Technology, Baghdad- Iraq.

Abstract

Amygdalin was extracted from apricot kernels, the chemical characteristics of amygdalin are: white, crystalline, and inodorous powder, slightly soluble in cold water, very soluble in hot water, alcohol and acetone, and insoluble in ether. It has a pH of 7 in saturated aqueous solution. Amygdalin purity in the extract was estimated to give 99.5%. Amygdalin was identified in the extract by FT-IR.
The sensitivity of some species of pathogenic bacteria included Pseudomonas aeruginosa, Serratia marcescens, Staphylococcus aureus, and Streptococcus pyogenes to different concentration of amygdalin (2%, 4%, 6%, 8%, and 10%) was tested to find out the role of amygdalin as antibacterial agent.
The experimental results of bacterial sensitivity to different concentrations of amygdalin, showed sensitivity of tested bacteria to all concentrations of amygdalin except concentration 2% amygdalin, bacterial species were resistant to it.

النشاط المضاد للبكتريا لمستخلص لب المشمش الحاوي على الامكدالين

صالح عبد الرضا البكري, زيد عبد المجيد نعمة, ريا رعد جبري, اسراء عطية عجيل
فرع التقنيات الاحيائية ، الجامعة التكنولوجية, قسم العلوم التطبيقية، بغداد- العراق.

الخلاصه

تم استخلاص الامكدالين من لبِ المشمش، و كانت الخصائص الكيميائية للامكدالين: مسحوق ابيض بلوري عديم الرائحة ، قابل للذوبان قليلاً في الماء البارد، قابل للذوبان جداً في الماء الحار والكحول والأسيتون، وعديم الذوبان في الأيثر. الرقم الهيدروجيني له 7 في المحلّول المائي المشبع. تم قياس نسبة نقاوة الامكدالين في المستخلص و وجد انها 99,5 %. كما تم الكشف عن الامكدالين باختبار FT-IR.

تم قياس حساسية كل من الانواع البكترية الممرضة *Pseudomonas aeruginosa* و *Serratia marcescens* و *Staphylococcus aureus* و *Streptococcus pyogenes* إلى تراكيز مختلفة من الامكدالين (2%, 4%, 6%, 8%, 10%) لغرض اختبار دور الامكدالين كمادة مضادة للبكتريا.بينت النّتائج المختبرية حساسية البكتريا إلى كل تراكيز الامكدالين عدا تركيز2 % فقط كانت مقاومة له.

Introduction

Over the past several years, the medical community has become increasingly concerned over the ability of certain bacteria to develop resistance to antibiotics [1,2]. Accordingly, there is a danger of losing the battle against certain pathogens (organisms causing diseases) by using the antibiotics in the treatment [1]. The development of drug resistance in human pathogens against commonly used antibiotics has necessitated a search for new antimicrobial substances from other sources including plants [3]. The interesting on the topic of antimicrobial plant extracts has been growing. Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic diseases as well as infectious diseases [4]. The medical value of plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive compounds of plants are alkaloids, flavanoids, tannins and phenolic compounds [5]. Apricot kernel has been used in folk medicine [6]. It is known for containing amygdalin, a toxic cyanogenic glycoside. Amygdalin is hydrolyzed by β-glucosidase into d-glucose, benzaldehyde, and prussic acid (hydrogen cyanide) [7]. This study deals with the antibacterial activity of different concentrations of apricot kernel extract containing amygdalin against some tested bacterial species.

Materials and Methods

- **Extraction of Amygdalin from Apricot Kernels**

After collection of Apricot kernels *Prunus armeniaca* (were collected from local markets); shells, husks, rocks, and leaves were eliminated; kernels were milled to separate the oil. The product from milling was dried to get dried powder. Apricot kernel extract was prepared by percolation method which consists of evaporation of solution containing 25g of kernel powder of apricot and 200 ml of ethyl alcohol and 4% citric acid for 150 min. The precipitate was filtered, left to dry, and weighted [8, 9].

- **Evaluation Test of Extract Purity**

The purity of amygdalin product in the extract was determined by dissolving 100 mg of product powder in 100 ml distilled water. The absorbance of the solution was read in ultraviolet light (UV) spectrophotometer between 230u and 280u. The percentage of amygdalin quantity in the product powder was calculated according to the reading of maximum absorbance, and absorbance of traced tangent, and the constant equivalent to 595 was utilized as divisor [10].

- **Vibrational Microspectroscopy of Amygdalin by FT-IR**

The vibrational spectra of amygdalin in powdered and solution states were examined by FT-IR [11].

- **Suitable Solvent for Preparation of Amygdalin Concentrations**

Different solvent of water, and alcohols (ethanol) of 5%, 10%, 20%, 25%, 30%, 35%, 40%, and 50%were used to check the solubility of 10% amygdalin and select the suitable one for preparing different concentrations (2%, 4%, 6%, 8%, and 10%) of amygdalin [12,13].

- **Antibacterial Activity Test**

The antibacterial activity of amygdalin against different species of bacteria, *Pseudomonas aeruginosa*, *Serratia marcescens*, *Staphylococcus aureus*, and *Streptococcus pyogenes* (the bacterial species are obtained from the labs of Biotechnology Division at University of Technology) has been examined by preparing different concentrations of amygdalin (2%, 4%, 6%, 8%, and 10%) that were dissolved in 35% ethanol. All the concentrations were added into the wells (each plate containing five wells, each well is 3-4mm diameter) of each of the four bacterial cultivated plates (the cultivated medium was nutrient agar (HIMIDIA)). The plates were incubated at 37°C for 24 hr. Then the antibacterial activity and the MIC (minimum inhibitory concentration which inhibits bacterial growth at low concentration) of amygdalin were recorded by using a ruler to measure the formed inhibition zone, and the results were compared to a control of 35% ethanol [12, 13].

Results and Discussion

- **Extraction and Identification of Amygdalin**

Amygdalin was extracted from apricot kernels in boiling water containing ethyl alcohol. Extraction at boiling temperature degree increases the efficiency of amygdalin extraction without the influence of amygdalin hydrolysis enzymes, β-glucosidases [8]. Also the extraction with the presence of citric acid may prevent the epimerization of amygdalin into neoamygdalin [8, 14].

After filtration, drying, and crystallization of the extract to form powder, the chemical characteristics of amygdalin powder were determined as: white, crystalline, inodorous powder, slightly soluble in cold water, very soluble in hot water, alcohol and acetone, and insoluble in ether. It has a pH of 7 in saturated aqueous solution [15]. The net weight of amygdalin powder was 3g. Therefore the net percentage of amygdalin in the apricot kernels (25g) was 12%. The purity of extract was evaluated by reading the absorbance in UV light between 230nm and 280nm. After calculating the following equation: $(700 - 110) / 595 * 100 = 99.15\%$ (where 700 is the maximum absorbance, 110 is the absorbance of the traced tangent as shown in figure 1, and 595 is the constant equivalent) the result was 99.15% which indicates the purification of the amygdalin extract.

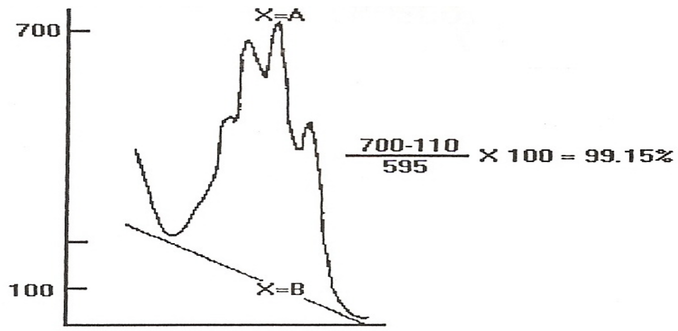

Figure 1. UV-Visible Spectrum for Amygdalin
Where: A= maximum absorbance, B= absorbance of traced tangent

Amygdalin has been identified in the extract in two states, solid and solution by FT-IR. The results in figure 2 revealed that the bands at 3078 cm^{-1} in the solution and 3060 cm^{-1} in the solid states are due to C-H stretching vibrations of the aromatic ring of the amygdalin. The bands at ~ 2880 cm^{-1} are due to aliphatic C-H stretching vibrations [15].

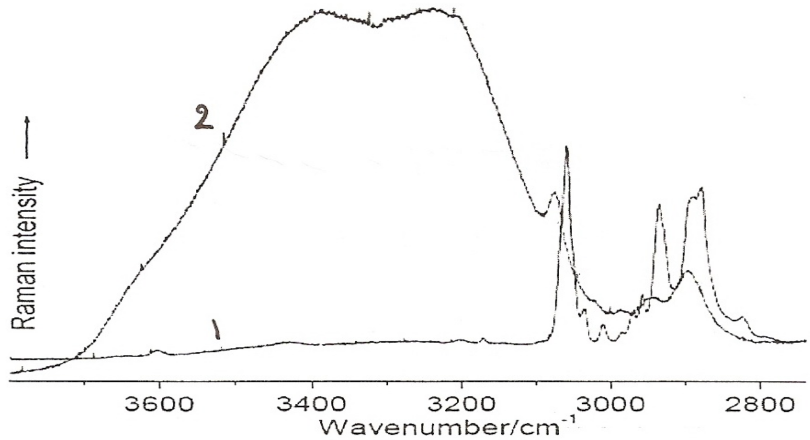

Figure 2. Viabrational Microspectroscopy of Amygdalin by FT-IR.
Where: 1 = Amygdalin Powdered State, 2 = Amygdalin Solution State

All the results of extraction, evaluation test of purity, and identification; revealed the obtaining of crude extract of amygdalin.

- **Preparation of Amygdalin Concentrations**

Because the study was seeking a solvent for dissolving amygdalin, water and different alcoholic concentrations were chosen for testing the solubility; alcoholic concentrations from60% and more were not used in this study because of their antimicrobial activity [16].

The solubility of amygdalin in the used solvents is illustrated in table 1.The results showed that amygdalin could dissolve in concentrations of ethanol between 35% - 50%. Accordingly 35% ethanol is a suitable solvent can be chosen for preparing amygdalin different concentrations (2%, 4%, 6%, 8%, and 10%).

Table 1: Amygdalin Solubility in Solvents

Amygdalin (%)	Solubility of Amygdalin								
	Solvents								
	Water	Ethanol							
		5%	10%	20%	25%	30%	35%	40%	50%
10%	-	-	-	-	-	-	+	+	+

Where: - = unsoluble, + = soluble

- **Antibacterial Activity of Amygdalin**

The antibacterial activity of five different prepared concentrations of amygdalin (2%, 4%, 6%, 8%, and 10%) and 35% of ethanol, the later used as a control, against *Pseudomonas aeruginosa*, *Serratia marcescens*, *Staphylococcus aureus*, and *Streptococcus pyogenes* were recorded after 24 hr. of incubation.

Table 2 showed that 35% ethanol, control group, had no effect on all tested bacterial types while there were obvious inhibition zones had been formed around all tested bacteria when they were treated with the different concentrations of amygdalin except 2% amygdalin which bacterial species exhibited resistance to it.

Table 2. Bacterial Sensitivity to Different Concentrations of Amygdalin

Bacteria	Inhibition Zone/mm					
	Control	Amygdalin (%)				
	35% Ethanol	2%	4%	6%	8%	10%
Pseudomonas aeruginosa	R	R	13	16	25	29
Serratia marcescens	R	R	10	12	14	17
Staphylococcus aureus	R	R	15	17	20	24
Streptococcus pyogenes	R	R	16	17	19	23

Where: R = Resistant

The inhibition zone increased proportionally with the increase in amygdalin concentration (Table 2). These results disclose the activity of amygdalin as antibacterial [17]. The antibacterial activity of amygdalin might be brought upon hydrolysis of amygdalin into d-glucose, benzaldehyde, and prussic acid (hydrogen cyanide) by β-glucosidase [17, 7 , 18, 19, 20] which may be found in the tested bacteria [21]. The study suggested that the tested bacteria might consume amygdalin as a source of sugar which can be obtained from decomposition of amygdalin by β-glucosidase [7, 21]. It seems that hydrogen cyanide (HCN), one of amygdalin decomposition products, had played an important role in the inhibition of the tested bacterial growth; the toxicity of hydrogen cyanide is caused by cyanide ion, a result of partial ionization of hydrogen cyanide [22]. Cyanide ion halts cellular respiration (electron transport chain) by inhibiting of an enzyme called cytocrome C oxidase located in the bacterial membrane [22]. Many researchers have proved that bacteria cannot metabolize HCN or adapt to it. In contrast to some fungi that have HCN-inducible enzyme, formamide hydrolyse (FHL), which detoxifies cyanide to formamide [23, 24, 25, 26].

Benzaldehyde, another product of amygdalin decomposition, [7, 18, 19, 20] is supposed to inhibit the growth of the tested bacteria by its reaction with phenolic compounds that might be present in the extract to form phenolic benzaldehyde, a toxic compound [17, 27]. It seems that amygdalin has antibacterial activity. The antibacterial activity may brought by amygdalin decomposition by bacterial enzymes to give hydrogen cyanide and benzaldeyde, the toxic compounds which killed the bacteria**.**

The results of this report are in agreement with the study of (Abtahi, *et.al.* 2008) [17]. They reported the antibacterial activity of bitter apricot extract against several bacterial strains. They supposed that the antibacterial activities might be brought by amygdalin, alkaloids, flavanoids, tannins and phenolic compounds.

Conclusion

The experimental results indicate the extraction of amygdalin from apricot kernels and revealed its role in the inhibition of bacterial growth as antibacterial. The antibacterial activity of amygdalin might represented by its decomposition by bacterial β-glucosidase to liberate hydrogen cyanide and benzaldehyde, the main players in this story. Hydrogen cyanide might inhibit the bacterial growth through holding the electron transport chain needed for bacterial respiration. Benzaldehyde might exhibit its toxicity by its reaction with phenolic compounds present in the extract to give phenolic benzaldehyde which killed the tested bacterial species.

References

1. Soulsby, E. J. **2005**. Resistance to antimicrobials in humans and animals. *BMJ* **331** (7527):1219–20.
2. Ochiai, K.; Yamanaka, T.; Kimura, K. and Sawada, O. **1959**. Inheritance of drug resistance (and its tranfer) between Shigella strains and between Shigella and *E. coli* strains. *Hihon Iji Shimpor* **1861**: 34.
3. Erdogrul, O. T. **2002**. Antibacterial activities of some plant extracts used in folk medicine. *Pharm. Biol*. **40**: 269-273.
4. Diallo, D.; Hveem, B.; Mahmoud, M. A.; Betge, G.; Paulsen, B. S. and Maiga, A. **1999**. An ethnobotaniacal survey of herbal drugs of Gourma district. *Mali. Pharm. Biol*., **37**: 80-91.
5. Cai, Y.; Lue, W.; Sun, M. and Corke, H. **2004**. Antioxidant activity and phenolic comounds of 112 traditional Chinese medicinal plants associated with anticancer. *Life Sci*., **74**: 2157-2184.
6. El-Badawy, T. A.; Rahma, E. H.; El-Badawy, A. A.; Gomaa, M. A.; Lasztity, R. and Sarkadi, L. **1994**. Biochemical studies of some non-conventional sources of proteins. Part 4. Effect of detoxification treatments on the nutitional quality of apricot Kernels. *Die Nahrung*., **38**: 12-20.
7. Walker, J. W. and Krieble, V. K. **1990**. The hydrolysis of amygdalin by acids. Part I. *Journal of the Chemical Society*. **95**(11): 1369–1377.
8. Woo-Sang Joo,Ji-Seon Jeong; yogeun Kim; Yong-Moon Lee; Je-Hyun Lee and Seon-Pyo Hong. **2006**. Prevention of epimerization and quantitative determination of amygdalin in armeniacae semen with schizandrae fructus solution. *Archives of Pharmacal Research*, **29**(12): 1096-1101
9. Smith, D. J. and Weber, J. D. **1984**, Preparative and analytical separation of amygdalin and related compounds in injectables and tablets by reversed-phase HPLC and the effect of temperature on the separation. *J. Chromatogr. Sci*., **22:**94–98.
10. Dybowski, R. **1978**. Examination of amygdalin preparations. *Anal. Proc*., **18**, 316–318.
11. Thygesen, L. G.; Løkke, M. M.; Micklander, E. E.; and Siren B. **2003**.. Vibrational microspectroscopy of food. Raman vs. FT-IR. *Trends in Food Science & technology*, (14 ref.), pp. 50-57.
12. Winstanley, T.; Edwards, Cl; Limb, D.; Megson, K. and Spencer, R. J. **1994**. Evaluation of a Surfactant, Dispersol LN, as an Anti-Swarming agent in agar dilution susceptibility testing. *Journal of Antimicrobial Chemotherapy* **33:**353-356.
13. National Committee for Clinical Laboratory Standards. **1997**. *Specialty Collection: Susceptibility Testing*. SC21-L. M7-A4. NCCLS, Wayne, PA.
14. Fenselau C, Pallante S, Batzinger RP, *et al*. ,**1977**, Mandelonitrile beta-glucuronide: synthesis and characterization. *Science* **198** (4317):625–627.
15. Haas, P. **1964**. *An Introduction to the Chemistry of Plant Products*. Vol. II. Metabolic Processes. BiblioBazar L.L.C. p.181
16. Hibbard, J. S. **2005**. Analyses comparing the antimicrobial activity and safety of current antiseptic agents: a review. *J Infus Nurs* **28** (3):194–207.
17. Abtahi, H.; Ghazave, A.; Karimi, M.; Mollaghasemi, S. and Mosayebi, G. **2008**, Antimicrobial activities of water and methoanol extracts of bitter apricot seeds. *J. Med. Sci*., **8**(4):433-436.
18. Lerner, I. J. ,**1981**, Laetrile: a lesson in cancer quackery. *CA Cancer J Clin* **31**(2): 91–95.
19. Newmark J.; Brady, R. O.; Grimley, P. M.; Gal, A. E.; Waller, S. G.; and Thistlethwaite, J. R. **1981**. Amygdalin (Laetrile) and prunasin beta-glucosidases: distribution in germ-free rat and in human tumor tissue. *Proc. Natl. Acad. Sci*. U.S.A. **78**(10):6513–6516.

20. Newton, G. W.; Schmidt, E. S.; Lewis, J. P.; Conn, E. and Lawrence, R. **1981**. Amygdalin toxicity studies in rats predict chronic cyanide poisoning in humans. *West. J. Med.* **134**(2):97–103.
21. Hildebrand, D. C. and Schroth, M. N. **1964**, beta - Glucosidase Activity in Phytopathogenic Bacteria. *Appl. Microbiol.* ,**12**(6):487-491.
22. Organisation for the Prohibition of Chemical Weapons. **2009.** "*Hydrogen Cyanide*". *Organisation for the Prohibition of Chemical Weapons*".http://www.opcw.org/ about-chemical-weapons/types-of-chemical-agent/blood-agents/hydrogen-cyanide/
23. Fry, W. E. and Millar, R. L. **1971**. Cyanide tolerance in Stemphylium loti *Phytopathology* **61**: 494-500.
24. Fry, W. E. and Millar, R. L. **1971**. Development of cyanide tolerance in stemphylium loti. *Phytopathology* **61**:501-506.
25. Fry, W. E. and Millar, R. L. **1972**, *Cyanide* Degradation by an Enzyme from Stemphylium loti. *Arch. Biochem. Biophs*. **151**: 468-474.
26. Rust, L. A.; Fry, W. E. and Beer, S. V. **.1980**. Hydrogen Cyanide Sensitivity in Bacterial Pathogens of Cyanogenic and Non-Cyanogenic Plants. *Phyatopathology* **70**:1005-1008.
27. Friedman, M.; Henika, P. R. and Mandrell, R.E. **2002**. Antimicrobial activities of phenolic benzaldehydes and benzoic acids against campylobacter jejuni, escherichia coli, listeria monocytogenes, and salmonella enteric. *Journal of Food Protection*.**66** (10):1811-1821.