



Studying the inhibitory effect of Alcoholic extract of inner Stratum of Oak Fruit (jaft) and hydro alcoholic extract of Summer bulb on Acinetobacter in vitro

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Abstract

Acinetobacter is an important nosocomial agent. In regarding to the increase of resistant bacteria and identified of side effect of antibiotics, using of plant drug with antibacterial effect is to appeal. This study aims to investigate inhibitory effect of alcoholic extract of oak inner stratum and hydro alcoholic extract of summer bulb on *Acinetobacter* in vitro. Oak inner stratum and summer bulb was collected and alcoholic and hydro alcoholic extraction was done. Inhibitory effect was carried out by disk diffusion and agar well diffusion method. Alcoholic extract of jaft had inhibitory effect, but hydro alcoholic extract of summer bulb don't have significant effect on this bacteria. The highest inhibitory effect of jaft was in 80µg/ml Concentration. The results showed that alcoholic extract of jaft has inhibitory effect on *Acinetobacter*, but hydro alcoholic extract of summer bulb don't have noticeable inhibitory effect.

Keywords: Alcoholic extract, summer bulb, jaft, *Acinetobacter*.

Introduction

Acinetobacter is a cocobasil bacter negative- none fermentative and aerobic. That is developmentally in hospital environment. And is one of the important opportunist's pathogens and is one of the important factors of various infections of hospital¹. Various types of *Acinetobacter*s have wide distribution in soil-water- surplus and human skin². Hospital infections can be important in death and economic damages. They are serious dangers because of difficulties in their treatments³. In a study in Germany, Waist et al, study various types of bacteria include: *Staphylococcus*, *Enterococcus*, *Escherichia coli* and *Acinetobacter* that were isolated from burn patients⁴. In recent years because of reciprocal growth and development of resistance microbial that is often because of resistant genes some antibiotics loss their effects⁵. Regarding to recent founds; today anti microbial power of antibiotics is limited⁶. In 21 century, that is century of comeback to nature and using plants to treatment, we see development in researches about medical plants and we see that using them is increased⁷. From late, western areas of Iran, use some plants to treatment the infections of burning. One of these is *nectaroscordum tripedale* that is subversion of lilase plant and is used to infection treatment in south and west areas of Iran, and in traditional medical it used as calmate in joint and bone pains⁸. *Quercus coccifera* with local name of (Jaft) is used to damage improvement infection and digestion pus^{9,10,11}. It found in western forests of Iran, an its effect on acinetobacter is not

studied. The present research, study the extract on acinetobacter in laboratory environment.

Material and Methods

30 distinctive cases of clinical infection ones, were used. ATCC19606 was considered as standard scale.

Hydro alcoholic extract of summer bulb: After collection and dryness of *nectaroscordum tripedale* far from direct sun ray, first, its leaves and stem were grinded and 10 g of it was mixed to 40ml water and 60 ml ethanol and insert on shocker to 2 hour. This mixture after filtering was concentrated by remove device, and finally by 37 c was drought by ben mari¹².

Alcohol extract of Jaft: After collecting and washing, *Quercus coccifera* was grinded and was mixed with 250 ml ethanol 80% and was shaking 48 hour in room temperature. Then the extract was filtered tow times and its alcohol was evaporated by rotary device and pure extract was equity and its salvation 10% was provide in alcohol 80% and maintained in glass¹³ and temperature 4^c.

Antimicrobial activity tests: Antibacterial activity of extract was experimented using disk diffusion¹⁴. Then minimum inhibitory concentration and minimum bactericidal concentration were appointed using macro dylotion method and NCCLS standard, by this modify that DMSO by final concentration of 1% was used as amolcifaire^{15,16}.

Antimicrobial activity tests: Some holes (6mm) in moler-hinton plates were created. From 24 hour planting of microbial reciprocals, suspensions of 0/5 tiff (Mac far lend) in serum physiology (0/85 Nacl) was provide and was inseminated uniformly by cottony sterile. Extract with 250 ml density was solved in distilled water, and was strolled by filters of . /2, and 100 µm of extract was infused in each hole. In disk method, some blank disks were inserted on vacuum plates and extracts by destiny of 5, 10, 20, 40, 80 mg were infused over them. The plates were transferred to incubator 37°C for 24 hour. Then areola of none growth around disks was studied and their diagonal was measured and recorded. Each test was repeated three times¹⁶.

MIC and MBC determination: In Moler- Hiton environment, serial dilutions were provided from extracts. Next an experiment was provided from 24 hour planting of microbial reciprocal in suspensions by destiny 106 cfu in Moler- Hiton environment, and 1 ml was added to test tubes. Final amount of inoculums in each tube was 5*10⁵ cfu and final destiny of extract in test tubes was 5- 10- 20- 40 and 80 mg. also a tube as negative control and a tube as positive control were added to test. The tubes were transferred to incubator 37c and were studied after 24 hour. Minimum of extract destiny that prevents bacteria growth was considered as MIC. Each test was repeated three times. To determine MBC, 10 µl of each tests was planted on Moler-Hiton plates. Then plates were inserted in 37°C to 24 hour. After this time, colonies of each plate were accounted and number of them was evaluated in volume scale. Minimum amount of extract that in it /99 of total cells were killed was considered as MBC.

Results and Discussion

Results of inhibitory effect: Hydro alcoholic extract of summer bulb had not interesting effect on acenitobacter and had a poor effect on these bacteria only in maximum destiny.

Results of inhibitory effect of alcoholic extract of inner stratum of oak fruit (jaft): This study had Shawn that alcoholic extract of inner stratum of oak fruit (jaft) has an interesting effect on acinetobacter in all destinies (table 1).

MIC and MBC to alcoholic extract of inner stratum of oak fruit (jaft): Accepted results of alcoholic extract of inner stratum of oak fruit (jaft) for MIC and MBC was 60 and 80 mg.

MIC and MBC to Hydro alcoholic extract of summer bulb: Since the effect of this extract was so limited in high destinies, so MIC and MBC not determined.

Discussion: Current chemical drugs using to infection treatment have side effects- determine drug resistance and high economical costs to societies and families, while facilitating in using medicinal plants and common believing to them prepare an appropriate condition to use them. In past decades only

plants were used as medicine. By rapid growth of synthesis medicines sciences, they become alternative to medical plants. Experience shows that synthesis medicines have many inappropriate effects and it be cleared that almost all material have harmful effects¹⁴, so today using medical plants is in most attention. In this research impact of inhibitory effect of alcoholic extract of inner stratum of oak fruit (jaft) and hydro alcoholic extract of summer bulb on Acinetobacter was studied. Regarding to existence saponins in hydro alcoholic extract of summer bulb¹⁵, it was expectation that extract of this plant has inhibitory and killing effect on acinetobacter, but results of this research indicate a poor inhibitory effect of hydro alcoholic extract of summer bulb on acinetobacter. Also in study of Panahi et al, poor effect of this extract on candida albicans was determined.¹⁷ probability existence saponins in hydro alcoholic extract of summer bulb was not enough that had inhibitory and killing effect on acinetobacter. From compounds of oak fruit we can refer to oil material- various sugars- corset and amid an¹⁸, that regarding to inhibitory effect of candida albicans¹⁹ and viruses it was expectation that extract of Jaft has inhibitory and killing effect on acinetobacter. Results of this research shows that inhibitory and killing effect Jaft on acinetobacter is in compared to ethanol extract of one other plant (flower) on Escherichia coli bacteria²⁰ that probably because of effective compounds include tanen in structure of alcoholic extract of Jaft. Regarding to a research by Ebrahimi et al, that was study the antimicrobial effect of various factors of oak against Escherichia coli bacteria²¹. In other study by Ebrahimi et al²² that was study the antimicrobial activity of alcoholic extract of oak fruit. And it indicate that effect of this extract on staphylococcus aureus, staphylococcus epidermidis and Escherichia coli was determined. Although alcoholic extract of Jaft has a poor impact in compare to methanol extract of plants²³.

Table-1
Result of inhibitory effect of extracts

Extract	concentration	Zone diameter	
		Disk diffusion	Embedded well
Hydro alcoholic extract of summer bulb	5	0	0
	10	0	0
	20	5	5
	40	8>	8>
	80	12>	10>
Standard sample	80	10>	8>
Alcoholic extract of inner stratum of oak fruit (jaft)	5	5>	0
	10	8>	>5
	20	12	12
	40	15	15
	80	20	18
Standard sample	80	17>	18

All of concentrations in milligrams and the diameter in millimeters

Conclusion

Regarding to above results it can be claim that hydro extract of Jaft has a powerful inhibitory impact on acinetobacter bacteria, and hydro alcoholic extract of summer bulb hasn't interesting effect on this bacteria.

References

1. Wang H, Guo P, Sun H, Wang H, Yang Q, Chen M, et al. Molecular epidemiology of clinical isolates of carbapenem-resistant Acinetobacter spp. from Chinese hospitals, *Antimicrob Agents Chemother*, **51(11)**, 4022-8 (2007)
2. Gerner-Smidt P. Taxonomy and epidemiology of acinetobacter infections, *Rev Med Microbiol*, **6**, 186-97(1995).
3. Riyahi Zaniani F, Ghazvini K, Sadeghian A, Reihani R, Bagheri M, Darban Hosseini M. Resistent Acinetobacter Wound Infection in Orthopaedics, *Iranian Journal of Orthopaedic Surgery*, **8(1)**, 40-3 (2010)
4. Dunbar J: Review of the bum cases treated in the Glasgow Royal infirmary during the past hundred years, with some observations on the present day treatment-Glasgow, *Med. J.*, 122-239 (1934)
5. Ebrahimi A, Khayami M, Nejati V. Evaluation of the Antibacterial and Wound Healing Activity of Quercus persica. *GMUHS Journal*, **18(1)**, 11-17 (2012)
6. Cowan M. Plant products as antimicrobial agents. *Clin Microbiol Rev*. **12(4)**, 564-582 (1999)
7. Samsam shariat H. Medical plants. 1st ed. Isfahan: chahar bagh, (2006)
8. Panahi J, Havasian MR, Pakzad I, davudian A, Jalilian F, Jalilian A. In Vitro Inhibitory Effect of Alcoholic Extract of Inner Stratum of Oak Fruit (Jaft) on Candida Albicans, *J Pharm Biomed Sci.*, **3(1)**, 5-8 (2013)
9. Pfaller, M.A. and D.J. Diekema, Epidemiology of invasive candidiasis: a persistent public health problem, *Clin Microbiol Rev*, **20(1)**, 133-63(2007)
10. Jabra-Rizk, M.A., W.A. Falkler, and T.F. Meiller, Fungal biofilms and drug resistance. *Emerg Infect Dis*, **10(1)**, 14-9(2004)
11. Glover D.D. and B. Larsen, Relationship of fungal vaginitis therapy to prior antibiotic exposure, *Infect Dis Obstet Gynecol*, **11(3)**, 157-60(2003)
12. Anonymous. The united states pharmacopeia, The national formulary. USP convention, InC. 2638-40 (2002)
13. Bosio K, Avanzini C, D'Avolio A, Ozino O, Savoia D. In vitro activity of propolis against Streptococcus pyogenes. *Lett Appl Microbiol*, **31(2)**, 174-7 (2000)
14. Egorov N.S. Antibiotics: A Scientific approach, Translated by Alexander Rosinkin, MIR Publishers. Moscow, (1985)
15. NCCLS. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically. Approved Standards – Fifth Edition NCCLS document M7-A5, Wayne, Pennsylvania, (2000)
16. Chitsaz M, Pargar A, Naseri M, Kamalinegad M, Bazargan M, Mansuri M, et al.. Essential oil composition and Antibacterial effects Hydroalcoholic extract Thyme essential oil thin (ziziphora clinopodiodes: LAM) On selected bacteria, *Daneshvar medicine*, **14(68)**, 15-22 (2007)
17. Panahi J , Havasiyan MR, Gheitasi S, Pakzad I, Jaliliyan A, Hoshmandfar R, Havasiyan M. The in Vitro Inhibitory Effects of the Aqueous Extracts of Summer Onion on Candida Albicans, *J Ilam Uni Med Sci.*, **21(1)**, 54-59(2013).
18. Bagiu RV, Vlaicu B, Butnariu M. Chemical Composition and in vitro antifungal activity screening of the allium ursinum (Liliaceae), *Int J Mol Sci.*, **13**, 1426-36 (2012)
19. Panahi J, Havasian MR, Pakzad I, davudian A , Jalilian F, Jalilian A. In Vitro Inhibitory Effect of Alcoholic Extract of Inner Stratum of Oak Fruit (Jaft) on Candida Albicans, *J Pharm Biomed Sci.*, **3(1)**, 5-8 (2013)
20. Sherafati Chaleshtari F, Sherafati Chaleshtari R, Momeni M. Antimicrobial effect of ethanol extract of Scrophularia (Scrophularia striata) on E. coli in vitro. *J Shahr kord Uni Med Sci.*, *Complementary Medicine Supplement*, 32-37 (2009)
21. Ebrahimi A, Khayami M, Nejati V. Comarsion of antimicrobial effect of different parts of Quercus persica against Escherichia coli O157:H7, *J of Ofoghe Danesh*, **18(1)**, 11-18(2012)
22. Ebrahimi A, Khayami M, Nejati V. Evaluation of antibacterial activity of Hydroalcoholic axtract fruit of oak Iranian to disk diffusion method, *J of medical plants*, **1(33)**, 26-34 (2009)
23. Hojati Bonab Z, Nikhah E. Evaluation of antioxidant and antibacterial effect of methanolic extract From Thyme (Thymus vulgar), Senna(Cassia angustifolia) and licorice(glycyrrhiza glabra), *Daneshvar medicine*, **19(100)**, 186-193 (2012)