Antibacterial Effect of Seed Extracts of Cardamom (Elettaria cardamomum) against Staphylococcus aureus and Proteus mirabilis

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Abstract

In this study, twenty isolates of Staphylococcus aureus and twenty others of Proteus mirabilis were collected from Hawler Teaching hospital during the period 10th January/2008 to 10th March/2008 in Erbil city-Iraq. The isolates were identified according to cultural, morphological, and biochemical properties. The susceptibility of isolates to antibiotics (Cefixime, Cefotax, Ciprophloxacin and Doxycillin) was tested, the resistance results for them, were (Cefixime, Cefotax and Doxycillin), and (Ciprophloxacin and Doxycillin) for S. aureus and Proteus mirabilis respectively. The antibacterial effects of seed extracts of cardamom (Eltttaria cardamomum) on more resistant isolates of both species were examined by well diffusion technique, all extracts have antibacterial property but methanolic extract was better than ethanolic and aqueous extracts; MIC and SMIC were determined, and used as curing agents to eliminate the antibiotic resistance genes and removing the swarming phenomenon in case of (Proteus mirabilis). It cleared from this study that methanolic extract was better than ethanolic and aqueous extracts where the curing percentage was (3.33%-70%) while the curing percentage of ethanolic extract was (3.33%-66.6%) which it was better than aqueous extract (0.00%-3.33%) in Staphylococcus aureus, while in Proteus mirabilis the results of plasmid curing was very weak where methanolic extract cured only 3.33%.

Key Words: Antibacterial activity, Elettaria cardamomum, Staphylococcus aureus, Proteus mirabilis, well diffusion technique, plasmid curing, antiswarming, MIC.

Introduction

staphylococcus aureus is a gram positive bacterium responsible for sever morbidity and mortality world wide. It is one of the leading causes of human infections in the skin and soft tissues, bones and joints, abscesses and normal heart valves [1]. While Proteus mirabilis is gram negative and generally associated with high morbidity and mortality [1]. Both bacteria became resistant to a large number of antibiotics, via transferring resistance genes on bacterial plasmid from another bacterium by transferring processes that occurred in bacterial world, or by transposons [2]. Therefore WHO has been suggesting the need to find some new antibiotics or new approaches to overcome this problem [3].Plant extracts have been used for a wide variety of purposes for many thousands of years. A renewed interest in "natural preservatives" appears to be stimulated by present food safety concerns, growing problems with microbial resistance and arise in production of minimal processed food. Many researches have documented the antifungal and antibacterial effects of plants [4]. At present, it is estimated that about 80% of the world population rely on botanical preparation as medicines to meet their health need. Herbs and spices are generally considered safe and proved to be effective against certain ailments [5]. The plant of cardamom (Elettaria cardamomum) of the Zingiberacea family is one of the world's very ancient and expensive spices [6], mainly grown in Sir Lanka and South India. The seeds of their ripe fruits are used medicinally, as a spice, and also as a flavouring agent in curries, coffee and cakes, particularly in the Arab countries. Some is used in the manufacture of liqueurs and a relatively small quantity in pharmacy, chiefly in the form of compound tincture of cardamom [7]. Cardamom seed yields 4% of volatile oils containing a high proportion of Terpinyl acetate and cincole and small quantities of other monoterpenes, including alcohols and esters [8]. [9], in 1982 reported the presence of over 150 compounds in cardamom aroma. Many of these compounds are commonly found in cardamom oil [7].

Methods and Materials , Bacteria under study

Twenty isolates of S. aureus and 20 isolates of P. mirabilis were obtained from Hawler Teaching hospital in Erbil city-Iraq, during the period 10th January/2008 to 10th March/2008. All isolates of two species were diagnosed morphologically, culturally and by some biochemical tests [10]. Bacterial isolates were maintained and preserved on nutrient agar slants. For every experiment, freshly prepared sterile nutrient broth (10ml) was inoculated from the slants and incubated at 37°C for 24 hours.

Antimicrobial Sensitivity test

Nutrient agar was used as growth medium, after sterilization and cooling the medium at 45°C, final concentration (30 µg/ml) of antimicrobial agents (Cefotax (Cet), Cefixime (Cef), Ciprophloxacin (Cip) and Doxycillin (Dox)) was added to the medium and poured into sterile petri dishes. After solidification, the plates were inoculated by streaking method with isolates then incubated at 37°C for 24 hours; the results were recorded next day [11].

Plant Extraction

The plant seeds of cardamom were obtained from local market of Erbil city-Iraq, and then washed, dried by dry air. The seeds were ground by electrical grinder, (100gm) of plant powder were weighted and soaked in (500ml) of sterilized double distilled water, absolute ethanol, and absolute methanol to obtain three different extracts according to the solvent used, and placed on magnetic stirrer left to mix by

magnetic bar at room temperature for (72) hours, then filtrated by muslin cloth, then by filter paper. The above step was repeated 3-5 times to residue, until a clear colorless supernatant extraction liquid was obtained indicating that no more extraction from the plant material was possible. The prepared extract was also evaporated to dryness and stored in the refrigerator at 5 ˚C till used [12].

Antimicrobial Activity Test of Plant Extracts

The well diffusion technique was applied to determine the antimicrobial activities of the tested plant extracts [13]. Molten (45˚C) sterile nutrient agar (20ml) was poured over base plates in sterile standard Petri dishes, after solidification the plates were inoculated with (0.1ml) of the standardized inoculum of each tested organism. The inoculated plates were allowed to dry in the incubator at 37˚C for 20 minutes. A standard cork borer of 6mm was used to cut uniform wells on the surface of nutrient agar and the determined concentration of each extract was injected in the wells. The plates incubated at 37˚C for 24 hours. The zones of inhibition were measured (mm).

Determination of MIC

The Minimum Inhibitory Concentration (MIC) of medicinal plant extracts was determined by turbidity method (spectrophotometeric method) at 600 nm, and serial dilutions were prepared for each plant extract (500-5000) µg/ml [14], the sub MIC (is less than the MIC) of medicinal plant extracts was determined and used as curing agents. In addition to the control sample that consists of (10ml) of nutrient broth and (0.1ml) of over night culture of bacterial suspension then incubated at 37˚C for 24 hours [15].

Motility and swarming tests

The isolates of Proteus mirabilis were treated with MIC of plant extracts and inoculated in semi solid agar and on nutrient agar and incubated for 24 hours at 37˚C [10].

Plasmid Curing test

Plasmid curing test was done by using the alcoholic and watery extracts of cardamom, sub MIC (SMIC is less than the MIC, in this study is less with200 µg/ml than the MIC) of the plant extract with (0.1ml) of overnight bacterial suspension were added to (10ml) nutrient broth then incubated at 37˚C for 24 hours. Next day (0.1ml) of it was spread on nutrient agar plate and incubated for 24 hours at 37˚C, next day 30 colonies transferred to antibiotic agar plate, after incubation for 24 hours at 37˚C, the viable colonies were calculated, then percentage of curing colonies were calculated [15].

Results and Discussion

The isolates of both species were identified according to morphological, cultural and some biochemical tests [10].The susceptibility of these isolates was tested against four antimicrobial agents (Doxycillin, Cefexime, Cefotax and Ciprofloxacillin), the resistance pattern is shown in Table (1). The antimicrobial activity of cardamom extracts (methanol, ethanol and aqueous) was examined against the most resistant isolate of both S. aureus and P. mirabilis. Results obtained by using well diffusion technique are summarized in Table (2). Methanolic extract of cardamom showed to be the most inhibitor extract against the tested isolates of both species, and this return to methanol solvent which extracts the most active components that found in plant [16]. The ethanolic extract activity of the plant under study was more potential than the watery extract and this refer to the extracted components in mentioned plant which more potential than in watery extract. These results agreed with those of [17], who found that ethanolic extract of tested plant, was better than watery extract of the same plant. On the other hand the inhibitory activity of all extracts was more affective against S. aureus than P. mirabilis, and this result which obtained by many researchers when testing medicinal plants against the G$\bar{o}$ve and G^+ ve where may be return to the physiology of the cell wall of both categories is not same (G$\bar{o}$ve bacteria have protective outer membrane that forms the outer layer of the cell envelop, serving as an efficient barrier against certain hydrophilic solutes and macromolecules [18 and 19]. The antibacterial effects were expressed as MIC of the three extracts of cardamom against the most resistant isolate of both species which are illustrated in Table (3). The alcoholic extracts (methanol and ethanol) were more active than watery extract. The quantity of the extract required to inhibit bacteria was low in broth dilution assay as compared to well diffusion assay. This is likely because of the difference in way of the two assays; in broth dilution the extract is directly incorporated into the broth therefore the bacteria are brought into direct contact with all components of the extract rather than relying on diffusion of components through the well. The concentration of the ingredients is always higher than next to the well and decreases gradually. Since bacteria give rise to a new generation every 18-20 minutes; therefore inoculated plates containing wells at 37˚C help bacteria to grow while components of the extracts need some time to diffuse through the disc and exert their effects. These factors point out lack of sensitivity of agar diffusion assay; therefore it is recommended that interpretation of the results of tests must be based on the comparison between dilution and diffusion method [3]. Methanolic extract of cardamom inhibited the swarming phenomenon of P. mirabilis but did not inhibit the motility of this isolate, while the rest extracts did not have any effect on swarming and motility of tested isolate. This may be due to the presence of phenolic groups of the chemical components in plant extracts that binds to the proteins and phospholipids of the outer membrane of the cell

wall and this will prevent development of the cells into swarmers [20].Table (1) showed that the two bacteria are resistant, and the cardamom extracts expressed antibacterial and curing agents as in Table (4), where methanolic extract is the most curing agent, it cured 3.33 to 70% (Cef, Cet and Dox) in S. aureus and 3.33% (Cip) in P. mirabilis, while ethanolic extract cured Cet with 3.33% and Dox with 66.6 in S. aureus but with no action in P. mirabilis. Aqueous extract cured only Dox with 3.33% in S. aureus, but no action on resistance genes in P. mirabilis, this may regard to ratio and kind of extracted components in each extract.Plant extracts playing a role in curing the resistance genes [15, 21 and22]. In conclusion, from this study we found that the extracts of cardamom seeds that prepared by using each of methanol, ethanol and water, have a strong inhibitory activity on tested pathogenic bacteria. According to this, using of cardamom as antibiotics and in food may be useful.

Table (1) Sensitivity of S. aureus and P.mirabilis to antimicrobials

Antibiotics	***S. aureus***	No. of Resistant Isolates	Resistant%	***P.mirabilis***	No. of Resistant Isolates	Resistant%
Dox	+	10	50	+	19	95
Cip	-	20	100	+	20	100
Cefotax	+	18	90	-	20	100
Cefixime	+	15	75	-	20	100

+:- Resist , - :- Sensitive

Table (2) Antibacterial activity of Cardamom extracts against S. aureus and P. mirabilis

	S. aureus			***P. mirabilis***		
Cardamom Extract Conc.µg/ml	Methanol	Ethanol	Water	Methanol	Ethanol	Water
	Inhibition zones (mm)					
500	-*	-	-	-	-	-
1000	-	-	-	-	-	-
2000	8	7	-	-	-	-
3000	12	8	-	-	-	-
4000	13	9	8	7	7	-
5000	13.5	11	10.5	11	8.5	-

*:- No effect

Table (3): MIC determination of Cardamom extracts against S. aureus and P. mirabilis

S. aureus		***P. mirabilis***	
Cardamom / µg/ml			
Methanol	2000	Methanol	2500
Ethanol	2000	Ethanol	2500
Water	4000	Water	4000

Table (4): Plasmid Curing % of S. aureus and P. mirabilis isolates

S. aureus			
Cardamom SMIC µg/ml	Methanol	Ethanol	Water
	Dox Cip Cef Cet	Dox Cip Cef Cet	Dox Cip Cef Cet
	70.00 - 3.33 3.33	66.6 - 0.00 3.33	3.33 - 0.00 0.00
P. mirabilis			
Cardamom SMIC µg/ml	Dox Cip Cef Cet	Dox Cip Cef Cet	Dox Cip Cef Cet
	0.00 3.33 - -	0.00 0.00 - -	0.00 0.00 - -

-:- Sensitive

References

1. W. Levinson and E. Jawetz, Medical Microbiology and Immunology. 6th edit McGraw-Hill, New York. 2000.
2. G. Zajicek, The Antibiotic Paradox. H. H. Humphrey Centre for Experimental Medicine and Cancer Research, Hebrew University Hadassah Medical School, Jerusalem 91220, Israel. 1996.
3. A. Hannan, S. Saleem, S. Chaudhary, M. Barkaat and M. U. Arshad, Antibacterial Activity of Nigella sativa against Clinical Isolates of Methicillin Resistant Staphylococcus aureus, Journal of Ayub Medical College of Abbottabad, 20(3): 72-74 (2008) .
4. R. Irkin and M. Korukuoglu, Control of Some Filamentous Fungi and Yeasts by Dehydrated Allium Extracts, Journal of Consumer Production and Food Safety, 4: 3-6 (2009).
5. R. Hema, S. Kumaravel and N. Elanchezhiyan, Antimicrobial Activity of Some of the South- Indian Spices and Herbals against Food Pathogens. Journal of Pharmacology, 3(1): 38-40 (2009).
6. S. Lwasa and F. Bwowe, Exploring the Economic Potential of Cardamom (Elettaria cardamomum) as an Alternative and Promising
12. Antibiotics. Ph.D. Thesis. University of Manchester, UK (1976).
13. J. B. Harborne, T. J. Mabray and H. Mabray, Phsiology and Function of Flavonoids. Acadimic press. NewYork (1975).
14. M. Kivanc, and B. Kunduhoglu, Antimicrobial Activity of Fresh Plant Juice on the Growth of Bacteria and Yeasts. Journal of Qafqaz University, 1: 26-53 (1997).
15. R. Cruickshank, J.D. Duguid, B. P. Marmion and R. H. A. Swain, Medical Microbiology, the Practice of Medical Microbiology. 12th ed., vol. 1 Churchill Livingstone, London and New York. 180-188 (1975).
16. H. F. Salah, Effect of Some Medicinal Plant Extracts on Antibiotic Resistance by Plasmid of Escherichia coli Isolated from Different Sources. M.Sc. Thesis, College of Science Education. University of Salahaddin. Erbil-Iraq (2007).
17. M. M. Cowan, Plant Products as Antimicrobial Agents. American Society for Microbiology, 12(4):564-582 (1999).
18. A. A. Tayel and W. F. El-Tras, Possibility of Fighting Food Borne Bacteria with Herbs and Spices Extracts. Journal of Food Safety, 10: 92-97 (2009).
Income Source for Uganda's Smallholder Farmers. African Crop Science Conference Proceedings-Eygpt, 8: 1317-1321 (2007).
7. J. S. Ajarem and M. Ahmed, Effects of Perinatal Exposure of Cardamom (Elettaria cardamomum) on The Post- natal Development and Social Behavior of Mice Offspring. Journal of King Suud University, 4(2): 34-57, (1992).
8. N. Abo - Khalwa and J . Kubo, Chemical Composition of the Essential Oil of Cardamom Seeds (Elettaria cardamomum) , Proc. Saudi of Biology Science, 10: 297-305 (1987).
9. V.S. Govindarajan, S. Narasimhan, K.G. Raghuveer and S. Lewis, Cardamom- Production, technology, chemistry and quality. CRC Critical Reviews in Food Science and Nutrition, 16: 229-305 (1982)
10. R. M. Atlas, L. C. Parks, and A.E. Barown, Laboratory Manual of Experimental Microbiology. Mosby-Year Book, Inc., U.S.A (1995).
11. A.R. Al-Najjar, Studies on the Effect of Surfactants on the Antimicrobial Activity of Several

19. N. A. Olasupo D. J. Fitzgerald, M. J. Gasson and A. Narbad, Activity of Natural Antimicrobial Compounds against Escherichia coli and Salmonella enterica serovar Typhimarurium. Letters in Applied Microbiology, 36: 448-451 (2003).
20. S. Afghani and I. Mugatash, Antibacterial Activity of the Spices Elettaria cardamomum.Skyline College, San Bruno CA (2008).
21. D. G. Smith, Inihibition of Swarming in Proteus spp. By Tannic Acid. Journal of Applied Bacteriology, 39: 29-32 (1974).
22. R. M. Shakibaiee, M. R. Heydar, M. Ahmed-Nezhad and M. Mohamadi, Plasmid Curing Activity of Five Plants Extracts on Multiple Resistant Plasmid Bearing Klebsiella pneumoniae- strains. Medical Faculty of Gulan University of Medical Science, 9: 33-34 (2000).
23. A. K. Khder, Effect of Thymus serphyllum and Mentha spicata Extract and Some Chemical Materials on Multidrug Resistant Proteus mirabilis. Proceeding of 4th INTERNATIONAL Conference of Biological Science (Zoology), 161-165 (2006).

تأثير الفعل المضاد لمستخلصات بذور نبات الهيل *Elettaria cardamomum* ضد بكتريا *Staphylococcus aureus* و *Proteus mirabilis*

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الملخص

تم جمع عشرون عزلة لجرثومة Staphylococcus aureus و عشرون أخرون لجرثومة Proteus mirabilis من مستشفى هولير التعليمي في مدينة أربيل– العراق خلال الفترة من ٢٠٠٨/١/١٠ الى ٢٠٠٨/٣/١٠. وشخصت العزلات وفقا للصفات المزرعية، الشكلية (مجهريا) والبايوكيميائية. وتم اختبار حساسية العزلات للمضادات الحيوية (سفيكزيم، سيفوتاكس، سيبروفلوكساسين ودوكسيسيلين) وكانت نتيجة المقاومة للمضادات تحت الدراسة (سفيكزيم، سيفوتاكس ودوكسيسيلين) و(سيبروفلوكساسين ودوكسيسيلين) لS. aureus و P. mirabilis على التوالي. وكان تأثير مستخلصات بذور نبات الهيل Elittaria cardamomum كمضاد للبكتريا على العزلات الاكثر مقاومة لكلا الجرثومتين بتقنية ال well diffusion حيث أبدت جميع المستخلصات فعالية ضد البكتريا وكان المستخلص الميثانولي الافضل كمضاد للبكتريا يليه المستخلص الايثانولي. كما تم تحديد تراكيز ال MIC \ SMIC وأستخدمت كعوامل حياد لأزالة جينات المقاومة للمضادات الحيوية لكلا الجرثومتين و كذلك أزالة صفة الانتشار swarming لجرثومة P. mirabilis وتبين من خلال هذه الدراسة أن المستخلص الميثانولي أكثرفعالية من المستخلصين الاخرين حيث كانت نسبة الحيادية (٣,٣٣%– ٧٠%) وكانت نسبة الحيادية للمستخلص الايثانولي (٣,٣٣%– ٦٦,٦%) بينما كانت نسبة الحيادية للمستخلص المائي (٠,٠٠%– ٣,٣٣%) في جرثومة S. aureus بينما كان تأثير المستخلصات كعوامل حياد على جرثومة P. mirabilis جدا ضعيف حيث أزال المستخلص الميثانولي صفة المقاومة للمضادات الحيوية بنسبة ٣,٣٣% فقط.