In Vitro Study For The Effect Of Cinnamon Oil On Multi-Drug Resistant Staphylococcus aureus And Proteus mirabilis Strains.

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الخلاصة:

نُفّذتْ الدراسة لتَقييم تأثيرِ زيت الدارسين على بكتيريا أل*Staphylococcus aureus* و *Proteus mirabiliS* المتعددة المقاومَة للمضادات الحياتية والمعَزولة مِن ْعينات المرضى المصابين بالتهابات الاذن الوسطى ِ. 50 نموذج جُمِعتْ من مستشفى الحكيمِ التعليمي في محافظةِ النجف، 20عزلة من هذه العزلات تَبَيَّن بأنها كَانَت *Staphylococcus spp.* وتسع عزلات تبين بانها *Proteus mirabilis spp.* وبدِراسَة أخرى لمستوى النوعَ تم اختيار ستّة مِنْ هذه العزلات تَبَيَّن بأنها كَانتْ من نوع *Staphylococcus aureus* و*Proteus mirabilis* المتعددة ألمقاومة للمضادات الحياتية .أظهرت نَتائِجَ اختبار حسّاسيةِ المضادات الحيوية بأنّ أغلب هذه العزلات ُ كَانتْ مقاومة جداً (ألنسبة ألمئوية للمقاومةِ كَانتْ 40-90 %) للمضادات الحيوية المستعملة. تأثير زيت الدارسين على هذه العزلات أختبر عن طريق إجراء الاختبارات التالية طريقةِ انتشار القرص، اختبار التركيز التثبيطي الأدنى و اختبار تركيز الحَدّ الأدنى المبيد للجراثيمِ وقد أظهرت النَتائِج تشابه في التأثير المضاد للمكروبات بين هذا الزيت والمضاد Ciprofloxacin. استنتجت الدراسة بأنّ زيت الدارسين يُمْكِنُ أنْ يُستَعملَ ُ كمضاد مكروبي فعّال حتى في حالة *Staphylococcus aureus* و *Proteus mirabilis* المتعددة المقاومَة للمضادات الحياتية (MDR) .

Abstract :

The study was carried out to assess the effect of cinnamon oil on multi-drug resistant (MDR) strains of *Staphylococcus aureus and Proteus mirabilis* isolated from patient infected with otitis media. 50 specimens were collected at AL-Hakeem Teaching Hospital in AL-Najaf governorate, 20 isolates were found to be *Staphylococcus spp.* and *Proteus spp.* on further studying for the species level only six of these isolates were found to be multi-drug resistant *Staphylococcus aureus* and *Proteus mirabilis*. The results of antibiotic sensitivity testing showed that most of these isolates were highly resistant (resistance percentage was 40-90%) for the used antibiotics. The antimicrobial effect of cinnamon oil on these

isolates was tested via performing Disk Diffusion Method, Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) tests. The results showed a similarity in the antimicrobial actions between this oil and Ciprofloxacin antibiotic . The study concluded that Cinnamon Oil could be used as an effective antimicrobial even in case of difficult treated MDR *Staphylococcus aureus* and *Proteus mirabilis.*

Introduction

The spread of drug resistant pathogens is one of the most serious threats to successful treatment of microbial diseases. Down the ages essential oils and other extracts of plants have evoked interest as sources of natural products. They have been screened for their potential uses as alternative remedies for the treatment of many infectious diseases (Tepe, et al., 2004). World Health Organization (WHO) noted that majority of the world's population depends on traditional medicine for primary healthcare. Medicinal plants and their Essential oils have been shown to possess antibacterial, antifungal, antiviral insecticidal and antioxidant properties (Burt ,2005).

Plants essential oils (also called volatile oils) are aromatic oily liquids obtained from plant materials (flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits and roots). They can be obtained by expression, fermentation or extraction but the method of steam distillation is most commonly used for commercial production (Sylvestre, et al., 2006). Essential oils are complex mixers comprising many single compounds. Chemically they are derived from terpenes and their oxygenated compounds. Each of these constituents contributes to the beneficial or adverse effects. (Suhr and Nielsen, 2003)

Cinnamon which is native to India and Sri Lanka (Ceylon) and now it is cultivated in many tropical countries including Mexico considers one of the most important medicinal plants the scientific name for Cinnamon is Cinnamomum verum it belongs to the family Lauraceae, its medicinal parts include the outer bark, inner bark,

leaves and essential oil the active principles in those parts are the Volatile oils (cinnamaldehyde, eugenol, cinnamic acid, weitherhin), Mucilage, Diterpenes and Proanthocyanidins (Soliman and Badeaa, 2002). Most of the Cinnamon extracts are safe and having little side effects their essential oil contains both antifungal and antibacterial principles that can be used as antibiotics and to prevent food spoilage due to bacterial contamination (Dragland , et al., 2003) it is also possesses anti-diabetic property (Broadhurst, et al., 2000). For these reasons The present study was conducted to evaluate the ability of cinnamon oil to inhibit the growth of different multi-drug resistant *Staphylococcus aureus* and *Proteus mirabilis* **i**solates with different resistance patterns, We examined in vitro the cidal and/or static effects of cinnamon oil on MDR *Staphylococcus aureus* and *Proteus mirabilis*, thus in order to be recommended as a safe and suitable treatment which could be used clinically.

Materials and Methods:

The present study was carried on in the Department of Microbiology, Medicine collage, kufa university in the period from 1/8/2005 to 5/4/2006. The specimens were collected at AL-Hakeem Teaching Hospital in AL-Najaf governorate. Isolation of pathogenic bacteria from otitis media specimens and identification to the species level was performed by standard methods (Macfaddin 2000). The antimicrobial sensitivity testing was done by Kirby-Bauer disc diffusion method standardized as per NCCLS(2002). Antibiotics were selected according to WHO model list of essential drugs the chosen antibiotics included Ciprofloxacin, Cefotaxim, Cephalexin, Amoxicillin, Rifampin, Clarethromycin, Cephalothin, Gentamicin, Carbencillin, and Kanamycin. Extended spectrum β-lactamase (ESBL-producing strains) were screened by the double-disk synergy test and confirmation tests recommended by the National Committee for Clinical Laboratory Standards (NCCLS). Results were interpreted according to NCCLS 2002 standard tables.

Cinnamon Oil Disk Preparation:

The disks were prepared according to the **Barry (1976)** in which empty sterilized discs (Whatman no.1, 6mm diameter) were impregnated with 50 µL per disk with different concentrations

(1:100, 1:50, 1:25, 1:12.5 and 1:6.25 mg/ml) of cinnamon oil and kept in a sterile container in the refrigerator to be used during one-month. The disks were placed on the cultured Mueller-Hinton agar surface and all Petri dishes were sealed with sterile laboratory parafilm to avoid eventual evaporation of the test samples. The plates were left for 30 min at room temperature to allow the diffusion of oil, and then they were incubated at 37°C for 18 h after the incubation period, the zone of inhibition was measured. Inhibition of bacterial growth in the plates containing test oil was judged by comparison with growth in blank control plates.
The MICs were determined as the lowest concentration of oil inhibiting visible growth of *Staphylococcus aureus* and *Proteus mirabilis* on the agar plate the procedure was done according to the method recommended by the National Committee for Clinical Laboratory Standards (NCCLs, 2002).

Results and Discussion:

New and safe antimicrobial agents are needed to prevent and overcome severe bacterial infections and the problems of bacterial resistance. (Harry, et al., 2005). Plants essential oils and extracts especially cinnamon have been used for many thousands of years, in pharmaceuticals, alternative medicine and natural therapies. It is necessary to investigate this plant scientifically to improve the quality of healthcare. For these reasons the present study was conducted to evaluate the effect of cinnamon oil on multi-drug resistant *Staphylococcus aureus* and *Proteus mirabilis* with different types of antimicrobial resistance.

Twenty isolate of *Staphylococcus spp.* and nine isolates of *Proteus spp.* used in this study were isolated from 50 specimens collected from patients infected with otitis media (Table 1). Six of these isolates were fully identified for the species level and characterized as *Staphylococcus aureus* and *Proteus mirabilis spps.*

Table 1: The number of Strains *Chosen from Staphylococcus aureus* and *Proteus mirabilis Isolates.*

No. of Strains	Types of bacteria
1	*Staphylococcus aureus*
2	*Staphylococcus aureus*
3	*Staphylococcus aureus*
4	*Proteus mirabilis*
5	*Proteus mirabilis*
6	*Proteus mirabilis*

Antibiotic sensitivity test was performed for all strains using 10-different antibiotic disks including Ciprofloxacin, Cefotaxim, Cephalexin, Amoxicillin, Rifampin, Clarthromycin, Cephalothin, Gentamicin, Carbencillin, and Kanamycin. The results of Antibiotic sensitivity test were recorded for the 6-chosen strains, the results showed a high degree of resistance in most of the isolates, the total resistance for all strains ranged from (40% - 90%) (table 2). Carbencillin and Cephalothin recorded a high resistance percentages (100%) in comparison to a lower percentages (ranged from 0-33.3%) recorded for Ciprofloxacin, Gentamicin, and Kanamycin.

Table 2: Antibiotic Sensitivity Testing for the 3- *Staphylococcus aureus* and 3- *Proteus mirabilis* isolates.

Strains	Cipro.	Cefot.	Cephal.	Amox.	Rifa	Clarth.	Cephalo.	Gent.	Carb	Kana.	Resistance Percentage
1	S	R	R	R	R	R	R	R	R	R	**90**
2	R	R	S	S	S	S	R	S	R	S	**40**
3	S	R	S	R	S	R	R	S	R	S	**50**
Resistance Percentage	33.3	100	33.3	66.6	33.3	66.6	100	33.3	100	33.3	
4	S	S	R	R	R	R	R	S	R	S	**60**
5	S	S	R	R	R	R	R	S	R	R	**70**
6	S	R	R	R	R	R	R	R	R	R	**90**
Resistance Percentage	**0**	**33.3**	**100**	**100**	**100**	**100**	**100**	**33.3**	**100**	**33.3**	

R=Resistant, S=Sensitive, *Cipro.*= Ciprofloxacin, *Cefot.*= Cefotaxim, Cephal.= Cephalexin, *Amox.*= Amoxicillin, Rifa,= Rifampin, *Clarth.*= Clarethromycin, *Cephalo.*= Cephalothin, Gent.= Gentamicin, Carb= Carbencillin, Kana.= Kanamycin.

All strains were further tested for the production of extended spectrum β–lactamase enzyme according to the NCCLs (2002) recommendations, it was found that strain-1and 6 was able to produce extended spectrum β–lactamase enzyme. Extended-spectrum β-lactamase (ESBLs) producing strains emerge by point mutation from non-extended-spectrum precursors these enzymes are derived through a single amino acid substitution or a few amino acid substitutions from the parental enzymes TEM-1, TEM-2, and SHV-1. these enzymes are able to Resist the effect of extended-spectrum cephalosporins specially 3rd and 4th generation cephalosporins (Radice, et al., 2001).

Disc diffusion method was used to evaluate the zone of microbial growth inhibition at various concentrations of cinnamon oil (table 3). The zones varied from 2mm-25mm in diameter, strains 2,3,4, and 5 were highly susceptible to the oil in comparison to a lower susceptibility recorded by strains 1 and 6.

Strain-1 and 6 which is ESBLs-producing strain as mentioned previously proved to be sensitive to the effect of cinnamon oil in which the growth was inhibited with a zone of 12mm in diameter, this zone considers a good result if we compare it with the results of antibiotic sensitivity testing in which it formed 90% resistance for the tested antibiotics (table 2). Depending on these results we can say that cinnamon oil antibacterial effect is almost close to the effect of clavulonic acid and aztreonam combined cephalosporins (those used in the treatment of ESBLs producing strains)

Similar results were recorded by (Prabuseenivasan, *et al.,* 2006) who found that cinnamon oil (in concentration of 1:5 mg/ml) was significantly able to inhibit the growth of many pathogenic bacteria with various zone diameters especially *K. pneumoniae* (27.5 mm), *P.aeruginosa* (33.3 mm), *B. subtilis* (29.9 mm), *P. vulgaris* (29.4 mm), and *S. aureus* (20.8 mm) pathogens used in this study were standard strains (show no resistance). As well as those reported by (Simic ,et al., 2004) their study suggested that cinnamon oil was very effective against pathogens even in moderate concentrations.

(Table 3):Inhibition Zone Diameters Using *Cinnamon Oil* Disks.

Strains	Diameter of Cinnamon Oil Inhibition Zones(mm)				
	1:100*	1:50*	1:25*	1:12.5*	1:6.25*
1	0	0	3	9	12
2	0	0	9	12	25
3	0	0	5	8	15
4	0	2	7	12	17
5	0	2	6	9	14
6	0	0	2	6	13

*mg/ml

Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC) of Cinnamon Oil were determined and compared with the results of antibiotic sensitivity testing (table 4). The results revealed that cinnamon oil showed maximum activity with MIC values ranging from 1:25 mg/ml to 1:12.5 mg/ml in most of the tested strains (1,2,3,4,5 and 6) while the MBC was 1:6.25 mg/ml. By comparing the results of table 4 with the results of disk diffusion test (table 2) the oil was found to be strongly bactericidal specially the concentration 1:6.25 proved to be a cidal concentration to almost all tested strains and its action was similar to the action of ciprofloxacin. The oil was able to inhibit the growth of most tested strains with moderate MIC values. Similar results were reported by (Prabuseenivasan, 2006) and (Mau, et al., 2001) who stated that Cinnamon oil showed promising inhibitory activity even at low concentration the antibacterial activity of cinnamon oil was probably due to their major component, cinnamaldehyde and their properties could be multiple.

(Table 4): Determination of MIC of Cinnamon Oil at Various Concentrations.

Strains	Minimum Inhibitory Concentration (MIC)				
	1:100	1:50	1:25	1:12.5	1:6.25
1	+	+	+	+	−
2	+	+	+	−	−
3	+	+	+	−	−
4	+	+	−	−	−
5	+	+	+	−	−
6	+	+	+	−	−

(+):growth
(−) :no growth

In conclusion, cinnamon oil showed antibacterial activity against six of the tested strains so it could be consider as a good source of antibacterial agent and it can be used as antibacterial supplement in the developing countries even in the treatment of MDR *Staphylococcus aureus* and *Proteus mirabilis*. In addition to in vivo studies clinical trials would be needed to justify and further evaluate the potential of this oil as an antibacterial agent in topical or oral application.

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