The Effects of *Aloe vera* Gel, Sesame Oil and Camphor Oil on *Pseudomonas aeruginosa* Isolated from Burnt Patients

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Abstract

Three isolates of *P. aeruginosa* were isolated from burnt patients. The ability of these isolates for adhesion and formation of slime layer were tested, the result showed that all isolates were able to adherence on the smooth surface. The sensitivity of *P. aeruginosa* isolates for antibiotics were tested , all isolates were sensitive to Gentamycin, Piperacillin and Amikacin Ciprofloxacin, and resist to Tetracyclin, Amoxicillin, Cephalexine , Ceftriaxone. Ciprofloxacin and Amikacin were found effective against *P. aeruginosa* isolates with MIC values of 3.8 µg/ ml for Ciprofloxacin and 0.244 µg/ ml for Amikacin The antibacterial effect of Different concentrations of *Aloe vera* gel, Sesame Oil and Camphor Oil against *P. aeruginosa* were determined, Camphor was highly effective with Concentration inhibit bacteria value of 10% followed by Sesame Oil (20%) and *Aloe vera* gel (>75%). The combinations of *Aloe vera* gel, Sesame Oil and Camphor Oil and antibiotics (Ciprofloxacin and Amikacin) showed that the efficacy of the two antibiotics (Ciprofloxacin and Amikacin) against *P.aeroginosa* isolates was improved in the presence of *Aloe vera* gel, Sesame Oil and Camphor Oil.

Key words *: Aloe vera* gel, Sesame Oil , Camphor Oil , *Pseudomonas aeruginosa*.

تأثير هلام *الالوفير (Aloe vera)* وزيت السمسم وزيت الكافور على بكتريا (*Pseudomonas aeruginosa*) المعزولة من مرضى الحروق

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الخلاصة

تم الحصول على ثلاث عزلات من بكتريا *P.aeroginosa* من مرضى الحروق . اختبرت قابلية هذه العزلات على الالتصاق فاظهرت النتائج ان جميع العزلات قابلة للالتصاق على السطوح الملساء . اختبرت حساسية العزلات للمضادات الحياتية فظهر ان جميع العزلات حساسة لمضادات الجنتامايسن والبيراسيلين والاميكاسين والسبروفلوكساسين ومقاومة لمضادات التتراسايكلين والاموكسيسلين والسيفالوكسين والسفترياكسون , ووجد ان مضادي السبروفلوكساسين والاميكاسين اكثر فاعلية ضد العزلات حيث بلغت قيمة التركيز المثبط الادنى لمضاد السبروفلوكساسين ٣،٨ مايكوغرام / مل ولمضاد الاميكاسين ٠،٢٤٤ مايكوغرام / مل. تم تحديد التاثير المضاد للبكتريا لتراكيز مختلفة من هلام الالوفيرا ووزيت السمسم وزيت الكافور فكان زيت الكافور اكثر فاعلية حيث بلغ التركيز المثبط للبكتريا ١٠% يليه زيت السمسم (٢٠%) وهلام الالوفيرا (< ٧٥%) . عند دمج المضادات بتراكيز تحت التركيز المثبط الادنى وتحت تحت التركيز المثبط الادنى مع هلام الالوفيرا ووزيت السمسم وزيت الكافور اظهرت المضادات تحسنا ملحوظا في تاثيرها على العزلات البكتيريه .

الكلمات المفتاحية :الوفيرا ، زيت السمسم ، *Pseudomonas aeruginosa* ، زيت الكافور .

Introduction

In spite of considerable advances in the treatment of burns, infection continues to pose the greatest danger to burn patients. Approximately 73 per cent of all death within the first five days post-burn have been shown to be directly or indirectly caused by septic processes [(1)]. The common pathogens isolated from burn patients include *Pseudomonas aeruginosa*, *Staphylococcus aureus* , *Klebsillea* spp, and various coliform bacilli. Fungi (*Candida albicans*, *Aspergillus fumigatus*) can also cause infection [(2)]. *Pseudomonas aeruginosa* is an opportunistic Gram negative pathogenic bacterium. This bacterium, in hostile conditions such as colonization in burned skin surface, produces large amounts of exopolysaccharide that bind with water and form gels [(3)]. Infections caused by *P. aeruginosa* are often severe and life threatening and are difficult to treat because of the limited susceptibility to antimicrobial agents and the high frequency of an emergence of antibiotic resistance during therapy [(4)]. Accumulation of resistance after exposure to various antibiotics and cross-resistance between agents may result in multidrug-resistant (MDR) *P. aeruginosa*[(5)].The spread of drug resistant pathogens is one of the most serious threats to successful treatment of microbial diseases,therefore, essential oils and other extracts of plants have evoked interest as sources of natural products [(6)].

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Aloe vera which is a member of liliaceae family(400 different species) with its origin in African continent , has been used to treat various skin conditions such as cuts, burns and eczema. It is alleged that sap from *Aloe vera* eases pain and reduces inflammation. Evidence on the effects of wound healing, however, is contradictory[7].Aloe vera leaf gel can inhibit the growth of the two bacteria *Shigella flexneri* and *Streptococcus pyogenies* [8].Sesame oil, derived from sesame seeds. The seed oil of Sesame spp was found to contain certain natural antibacterial agents that were effective against common skin pathogens, such as S*taphylococcus* and S*treptococcus* bacteria, as well as common skin fungi including the athlete's foot fungus[9]. Essential oils (also called volatile oils) are aromatic oily liquids obtained from plant materials (flowers, leaves, buds, seeds etc) ,compounds. Essential oils have been shown to possess antibacterial, antifungal,antiviral, insecticidal and antioxidant properties, Camphor essential oil is extracted from the *Cinnamomum camphora* (also known as *Laurus camphora*) of the Lauraceae family and dried rosemary leaves (*Rosmarinus officinalis*), in the mint family, contain up to 20% camphor. It can also be synthetically produced from oil of turpentine, Camphor oil can be used in the treatment of nervous depression, acne, inflammation, arthritis, muscular aches and pains, sprains, rheumatism, bronchitis, coughs, colds, fever, flu and infectious diseases[10, 11].This study, aim to detect of antibacterial activity of *Aloe vera* gel, Sesame Oil and Camphor Oil against clinical isolates of *P. aeruginosa* obtained from burns patients well as the potentials of their effect in combination with different antibiotics.

Material and Methods

Isolation and identification of isolates

Specimens were collected as wound swabs from burns patients. The specimens were cultured on MaCconkey agar and the isolated colonies were subcultured on Cetrimide agar and MaCconkey agar. Identification of *P. aeruginosa* based on gram stain, colony morphology and biochemical tests (oxidase test, catalase test , triple sugar iron (TSI) fermentation, color, pyocyanin pigment production on King A medium and an ability to grow at 4°C and 42°C) [12]. The ability of isolates for adhesion and formation of slime layer were tested according to Christensen *et al.*[13] .10ml of tryptone soya broth were inoculated with a loopful of organisms from overnight blood plate culture then incubated overnight (18-24) hours at 37ºC. The culture tube were then emptied of their contents and stained by adding 10ml of safranin stain solution. Each tube was then gently rotated to ensure uniform staining of any adherent material on the inner surface and the contents gently decanted. The tubes were then placed upside down to drain. A positive result was indicated by the presence of an adherent layer of stained material on the inner surface of the tube or visible film lined the walls of the tube. Ring formation at the liquid-air interface was not considered indicative of slime production.

Antibiotic sensitivity test

The sensitivity of bacteria to antibiotics (Tetracyclin , Amikacin ,Gentamycin ,Ciprofloxacin ,Amoxicillin ,Piperacillin ,Cephalexine, Ceftriaxone) were tested by using disk diffusion test were performed for all the isolates by the method recommended by Clinical and Laboratory Standard Institute (CLST)[14]. A suspension of each isolate was made so that the turbidity was equal to 0.5 McFarland standard and then plated onto Nutrient agar plate. Antibiotic disk was applied to each plate. After incubation at 37°C for 24 h, zone size was measured. The minimal inhibitory concentrations (MICs) and sub minimal inhibitory concentrations (half of MIC) of Ciprofloxacin and Amikacin were determined. This test was achieved according to Morello *et al.*[15] , as following: Sterile tubes of Mueller-Hinton broth were prepared; each tube contented 2ml of sterile Mueller-Hinton broth. A serial of two-fold dilutions of antibiotics were prepared by adding of 2ml of antibiotic stock solution to the first tube of Mueller-Hinton broth, mixed the contents, then transferred of 2ml from this tube in to a second tube, mixed the contents of the second tube and transferred of 2ml to a third tube. The dilution process was continued until reach to the last tube. After the contents of the last tube mixed well, discarded 2ml of broth so that the final volume in all tubes was 2ml. From the Nutrient agar plate culture of bacterial isolate the suspension of *P. aeruginosa* was prepared in 5ml of normal saline that equivalent to McFarland 0.5 standard, 0.1ml of the bacterial suspension was transferred to the each of the serial of antibiotic-broth tubes. Each tube was shacked gently to mix the tube contents and placed in the incubator at 35ºC for 18-24 hours.The experiment was included the following control tubes:

- A tube contents sterile broth (Sterility control).
- A tube contents broth and bacterial isolate (Growth control).

- A tube contents antibiotic and sterile broth.

After the incubation the tubes were examined for the presence or absence of turbidity, the lowest concentration that inhibits the visible growth of bacteria was determined as MIC.

Antibacterial activity of Aloe vera gel, Sesame Oil and Camphor Oil

The *Aloe vera* gel was gotten from the leaves by pushing the plant leaf by fingers and the gel collected in sterile tube. Sesame Oil and Camphor Oil were obtained from local market. Different concentrations (5-75) % of *Aloe vera* gel, Sesame Oil and Camphor Oil were prepared by using the solvent DMSO (Dimethyl sulfoxide). The antibacterial effect of these different concentrations against *P. aeruginosa* was determined according to Morello *et al.*[15]

The effect of combinations of antibiotics with Aloe vera gel, Sesame Oil and Camphor Oil

The effect of two different antibiotic concentrations (subinhibitory antibiotic concentrations and sub sub MICs of Ciprofloxacin and Amikacin in combination with sub MICs and sub sub MICs of *Aloe vera* gel, Sesame Oil and Camphor Oil against *P. aeruginosa* were determined [15]. The combination of antibiotics with *Aloe vera* gel, Sesame Oil and Camphor Oil were performed as mention in the table (1). The tubes contents and placed in the incubator at 35ºC for 18-24 hours. After the incubation the tubes were examined for the presence or absence of turbidity.

Table 1: The combination of antibiotics with *Aloe vera* gel, Sesame Oil and Camphor Oil

Tube No.	Antibiotics in combination				Final volume in the tube (ml)	Volume of *P. aeruginosa* (ml)	Final concentration in the tube (µg/ml)
	Ciprofloxacin or Amikacin		*Aloe vera* gel , Sesame Oil or Camphor Oil				
	Volume (ml)	Concentration (µg/ml)	Volume (ml)	Concentration (µg/ml)			
1	0.5	MIC	0.5	MIC	1	1	sub-MIC/sub-MIC
2	0.5	sub-MIC	0.5	sub-MIC	1	1	sub-sub MIC/sub-sub MIC

Results and Discussion

Three isolates of *P. aeruginosa* were isolated from burnt patients , a series of biochemical tests were used for identification . The isolates were positive for oxidase and catalase test and growth at4°C and 42°C (table 1). The ability of these isolates for adhesion and formation of slime layer were tested, the result showed that all isolates were able to adherence on the smooth surface (fig 1, table 2). Burn patients were most commonly infected with *P. aeruginosa* , it is opportunistic pathogen responsible for nosocomial infections, Anjuman and Mir [16] reported that there is a significant increase in the number of *P. aeruginosa* isolated from pus followed by urine. Among, 4409 burn patients samples were evaluated for microbial culture, in which 2810 (63.7%) were culture positive, the most predominant isolates in all samples was *P.aeruginosa* (47.7%)[17].The sensitivity of *P. aeruginosa* isolates for antibiotics were tested , all isolates were sensitive to Gentamycin, Piperacillin and Amikacin Ciprofloxacin, and resist to Tetracyclin, Amoxicillin, Cephalexine , Ceftriaxone (table 3) . Ciprofloxacin and Amikacin were found more effective against *P. aeruginosa* isolates with MIC values of 3.8 µg/ ml for Ciprofloxacin and 0.244 µg/ ml for Amikacin(table 4). Gram negative pathogens such as *Pseudomonas aeroginosa*, and *Klebsiella pneumonia* have become multi-drug resistant [18] . According to Japoni *et al.*(3) study, *P. aeroginosa* was resistant to ciprofloxacin (27.1%), ceftazidime (15.7%), cefepime (2.9%), imipeneme (67.1%), piperacilin (14.3%). The antibacterial effect of Different concentrations of *Aloe vera* gel, Sesame Oil and Camphor Oil against *P. aeruginosa* were determined, Camphor was highly effective with Concentration inhibit bacteria value of 10% followed by Sesame Oil (20%) and *Aloe vera* gel (>75%) (table 5) .A *Vera* gel consists of 99.3% water. The remaining 0.7% is made up of solids with glucose and mannose constituting for a large part. These sugars together with the enzymes and amino acids in the gel give the special properties as a skin care product. The gel stimulates cell growth and as such enhances the restoration of damaged skin. It moisturizes the skin because it has a water holding capacity. This moist on the skin and also has a

cooling effect, it had shown that Aloe vera leaf gel can inhibit the growth of the two bacteria *Shigella flexneri* and *Streptococcus progenes*. Specific plant compounds such as anthraquinones and dihhydroxyanthra-quinones, as well as saponins have been proposed to have direct antimicrobial activity [8,19]. The GC-MS phytochemical screening of methanolic extract showed the presence of carboxylic acids and phenolic groups in essential oils especially some of the most potent antioxidants like Sesamol, Sesamolin and Sesamin. Both the methanolic and ethanolic extracts have broad spectrum antimicrobial effect against all the tested micro-organisms except *S.pneumoniae, Candida albicans* and *S. aureus* respectively, while the aqueous extract exhibited no inhibitory effect on *S. aureus* and *S. pneumoniae* except on *C. albicans*[20]. The camphor oil which extracted from fallen leaves of Cinnamomum camphora by the method of distillation had antibacterial effect against *E. coli , P.vulgaris ,S. aureus* in some extent, and the minimum inhibitory concentration(MIC) was between 0.125 to 0.25 g/mL,but the oil from fallen leaves extracted by acetone had almost no antibacterial effect against these bacteria [21].The effect of combinations of *Aloe vera* gel, Sesame Oil and Camphor Oil and antibiotics (Ciprofloxacin and Amikacin) showed additive effect on the growth of the *P.aeroginosa* (table 6 and table 7) . The efficacy of the two antibiotics against *P.aeroginosa* isolates was improved in the presence of *Aloe vera* gel, Sesame Oil and Camphor Oil. The enhancement in the killing effect (additivity) of the antibiotics at sub MIC and sub sub MIC values, suggests that Sesame Oil and the ketonic group in Camphor Oil and *Aloe vera* gel compounds can improve the efficacy of antibiotics. The antimicrobial effects of *Aloe vera* have been attributed to the plant's natural anthraquinones: aloe emodin, aloetic acid, aloin, anthracine, anthranol, barbaloin, chrysophanic acid, ethereal oil, ester of cinnamonic acid, isobarbaloin, and resistannol[22].In relatively small concentrations together with the gel fraction, these anthraquinones provide analgesic, antibacterial, antifungal, and antiviral activity; in high concentrations, they can be toxic[23]. Saponins, which contain glycosides, are soapy substances that have both cleansing and antiseptic properties[24].

Table 2: The results of biochemical tests of *P. aeruginosa* isolates

Biochemical tests	*P.aeruginosa* 1	*P.aeruginosa* 2	*P.aeruginosa* 3
gram stain	- ve*	- ve	- ve
catalase test	+	+	+
oxidase test	+	+	+
growth at4°C and 42°C	+	+	+
triple sugar iron (TSI) fermentation formation of gas formation of H_2S	 - -	 - -	 - -
adherence on the smooth surface	+	+	++

*- ve : gram negative , + : positive result , - : negative result

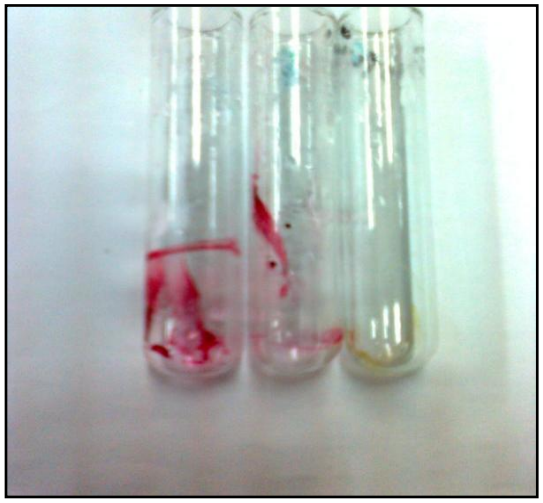

Figure 1: Adherence of *P. aeruginosa* on the smooth surface

Table 3: The result of antibiotics sensitivity of *P. aeruginosa* isolates

Antibiotic	Result	Diameter of inhibition zone (cm)
Tetracyclin	R	-
Amikacin	S	3
Gentamycin	S	2.5
Ciprofloxacin	S	4
Amoxicillin	R	-
Piperacillin	S	2
Cephaloxine	R	-
Ceftriaxone	R	-

R : Resist S : Sensitive

Table 4: The result of minimal inhibitory concentrations (MICs) of antibiotics

Antibiotic	MIC (µg/ ml)	Sub MIC (µg/ ml)
Amikacin	3.8	1.9
Ciprofloxacin	0.244	0.122

Table 5: The result of antibacterial activity of *Aloe vera* gel, Sesame Oil and Camphor Oil

Test	Concentration inhibit bacteria (%)
Aloe vera gel	> 75
Sesame Oil	20
Camphor Oil	10

Table 6: The effect of combinations of Sub MICs of antibiotics with Sub MICs of A*loe vera* gel, Sesame Oil and Camphor Oil on the growth of the *P.aeroginosa*

Combination of Sub MICs	Result
Ciprofloxacin(0.122 µg/ ml)+ *Aloe vera* gel(75%)	No growth
Ciprofloxacin(0.122 µg/ ml)+ Sesame Oil(15%)	No growth
Ciprofloxacin(0.122 µg/ ml)+ Camphor Oil(5%)	No growth
Amikacin(1.9 µg/ ml)+ *Aloe vera* gel(75%)	No growth
Amikacin(1.9 µg/ ml)+ Sesame Oil(15%)	No growth
Amikacin(1.9 µg/ ml)+ Camphor Oil(5%)	No growth

Table 7: The effect of combinations of Sub Sub MICs of antibiotics with Sub Sub MICs of A*loe vera* gel, Sesame Oil and Camphor Oil on the growth of the *P.aeroginosa*

Combination of Sub Sub MICs	Result
Ciprofloxacin(0.061 µg/ml)+ *Aloe vera* gel(37.5%)	No growth
Ciprofloxacin(0.061 µg/ml)+ Sesame Oil(7.5%)	growth
Ciprofloxacin(0.061 µg/ml)+ Camphor Oil(2.5%)	No growth
Amikacin(0.95 µg/ ml)+ *Aloe vera* gel(37.5%)	No growth
Amikacin(0.95 µg/ ml)+ Sesame Oil(7.5%)	growth
Amikacin(0.95 µg/ ml)+ Camphor Oil(2.5%)	No growth

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