Anti-Pseudomonal Effect of Argan Oil on Pseudomonas aeruginosa Recovered from Burn Patients in Hilla City, Iraq

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Abstract: The burn and wound infection is considered one of the major health problems in the world, and one of the most frequent and severe complications in patients who have sustained burn. *P. aeruginosa* is a common cause of wound infections, especially of thermal burns, this is because burns have large exposed areas of dead tissue free of any defenses and, therefore, are ideal sites for infection by bacteria from the environment or normal microbiota. To assess Antibacterial effect Different concentrations of both Argan Oil (which was fetched from Maraco Kingdom) and Hydrogen Peroxide (H2O2).
Bacterial isolates obtained from the Central Laboratory in Hilla City, Iraq. was preliminary diagnosed in this Lab. as *Pseudomonas aeruginosa*, the causative agents of skin and burns infections and the more resistant bacteria to antibiotics. Those isolates were reidentified in our Lab. for confirmation.
The test bacterial isolates were treated with; H2O2 (1.5%) alone, Argan oil alone and with physiological saline as a control (by using the wells in agar method). No effect on bacterial activities and vitality were observed in this experiment.
Argan oil and H2O2(1.5%) were combined together to form compounds in a rate of 1:1, 2:1 and 1:2 respectively. The results of this experiment revealed considerable effects on test organisms since the diameter of inhibition zones recorded as high as 23.82mm., 24.57mm. and 23.05mm. These effective of Argan oil Mixing with 1.5% H2O2 on *P. aeruginosa* burn isolates show remarkable results when compared to highly active antibiotics like Amikacin, Tobramycin, Ceftazidime, Aztreonam, Norfloxacin and Gentamycin.

Keywords: Argan oil, H2O2, Anti –Pseudomonal, *Pseudomonas aeruginosa*.

I. INTRODUCTION

Burns are one of the most common and devastating forms of trauma. Patients with serious thermal injury require immediate specialized care in order to minimize morbidity and mortality [1].Thermal destruction of the skin barrier and concomitant depression of local and systemic host cellular and humoralimmune responses are pivotal factors contributing to infectious complications in patients with severe burns. Although burn wound surfaces are sterile immediately following thermal injury, these wounds eventually become colonized with microorganisms[2].Bacteria rapidly colonize open skin wounds after burn injury. Microorganisms colonizing the burn wound originate from the patient's endogenous skin and gastrointestinal and respiratory flora. Microorganisms may also be transferred to a patient's skin surface via contact with contaminated external environmental surfaces, water, fomites, air, and the soiled hands of health care workers. Endogenous gram-negative bacteria from the patient's gastrointestinal flora also rapidly colonize the burn wound surface in the first few days after injury [3].

While *Staphylococcus aureus* remains a common cause of early burn wound infection, *Pseudomonas aeruginosa* from the patient's endogenous gastrointestinal flora and/or an environmental source is the most common cause of burn wound infections in many centers [4].Despite new techniques and solution for sterilization and disinfection but *P. aeruginosa* still isolated with high percentage [5].

Silver sulfadiazine has excellent broad-spectrum antibacterial coverage against Pseudomonas aeruginosa and other gram-negative enteric bacteria, although some resistance has recently been reported. Also Mafenide acetate (Sulfamylon) cream has a broad spectrum of activity against gram-negative bacteria, particularly *Pseudomonas aeruginosa* [6].

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Argan oil is a plant oil produced from the kernels of the Argan tree (Arganiaspinosa L.) that is endemic to Morocco. It is used for nutritive and cosmetic properties. The tree is extremely well adapted to drought and other environmentally harsh conditions of southwestern Morocco. The genus Argania once covered North Africa and is now endangered and under protection of UNESCO [7]. The argan tree grows wild in semi-arid soil, its deep root system helping to protect against soil erosion and the northern advance of the Sahara. This biosphere reserve, the Arganeraie Biosphere Reserve, covers a vast intramontane plain of more than 2,560,000 hectares, bordered by the High Atlas and Little Atlas Mountains and the Atlantic in the west. Argan oil remains one of the rarest oils in the world due to the small and very specific growing areas. The fruits of the Argan tree are nut-sizes and may be round, oval or conical in shape. The fruits are covered by a thick peel which covers the fleshy pulp. The pulp surrounds a hard-shelled nut which represents approximately 25% of the weight of the fresh fruit. Contained within the nut are one to three Argan oil–rich kernels. Argan oil is extracted from the kernels, with yields varying from 30% to 55% depending on the extraction method used [8]. Argan oil consist of 42.8% oleic, 36.8% linoleic,12% palmitic, 6% stearic and <0.5% Linolenic[9]. Argan oil has a relative density at 20 °C (68 °F) ranging from 0.906 to 0.919 [10]. Argan oil contains tocopherols (vitamin E), phenols, carotenes, squalene, and fatty acids, (80% unsaturated fatty acids). The main natural phenols in Argan oil are caffeic acid, oleuropein, vanillicacid, tyrosol, catechol, resorcinol, (-)-epicatechin and (+)-catechin. Depending on the extraction method, Argan oil may be more resistant to oxidation than olive oil [11].

Virgin Argan oil of edible or beauty grade is composed of 99-percent acylglycerides (primarily triglycerides). Unsaponifiable matter, which represents the remaining one percent, is composed of carotenes, tocopherols, triterpene alcohols, sterols, and xanthophylls [12].Fatty acids that compose acylglycerides are principally oleic and linoleic acid, 43-49 percent and 29-36 percent, respectively. Palmitic and stearic acid are saturated fatty acids found at a concentrations of 11-15 percent and 4-7 percent, respectively [13].Some Argan oil pharmacological properties are likely to result from its high unsaturated fatty acid content. Oleic acid, a monounsaturated fatty acid, has numerous therapeutic effects[14] that contribute to the important properties of Argan oil. Because a linoleic acid deficiency can induce poor wound healing [15]. The high linoleic acid content of Argan oil may contribute to its traditional indication as a cure for skin inflammation. However, several other oils are rich in oleic and/or linoleic acid but do not possess the same therapeutic effects. Many of argan oil's specific health benefits are attributed to its composition of unsaponifiable matter (although it constitutes a small amount of the oil) and high tocopherol content [16].The tocopherol content of Argan oil is 620 mg/kg, compared to 320 mg/kg in olive oil. Tocopherols are molecules with strong antioxidant and free radical scavenging properties. gamma-Tocopherol, the most efficient free radical scavenger of all tocopherols composes 69 percent of argan oil total tocopherol content [17].Because tocopherols and sterols can act synergistically, the specific combination of molecules found in the unsaponifiable matter is theorized to contribute to the therapeutic aspects of Argan oil [18].

Many studies on organ oil show their activity as anti-sebum[19], antiproliferativeAactivity[20,21,22], antioxidant and hypocholesterolemic effect[23] and antidiabetic activity[24]. According to my search, the antibacterial (anti-pseudomonal) activity of organ oil not documented until yet and so the current study aimed to study this effect of Argan oil.

II. MATERIAL AND METHODS

A. *Bacterial isolates*

Thirty five identified *pseudomonas aeruginosa* isolates, recovered from burned patients, were received from main lab of Hilla Teaching Hospital-Iraq during a period from October 2013 to February 2014. All isolates were confirmed using VITEK 2 compact system VITEK 2 GN card.

B. *Antibiotics and Argan Oil Susceptibility Test*

Antibiotics Susceptibility Test performed according to guidelines of CLSI 2012[25]. Also the anti-pseudomonal effect of Argan oil was tested using well diffusion techniques. The *p. aeruginosa* suspension was standardized with 0.5 McFarland and the streaked on Muller Hinton agar plate and the well made using cork borer. Fifty microliter of Argan oil only, 1.5% H2O2 only and mixture of Argan oil:1.5% H2O2 (1:1, 1:2 and 2:1) were applicated in the well and incubated at 37˚C overnight. The diameter of inhibition zone (mm) was recorded.

III. STATISTICAL ANALYSIS

A. *Result*

The data gathered from this study revealed presence of multidrug resistance (MDR) among burn *P. aeruginosa* isolates. The isolates show high level of resistance (97.14%, 97.14%, 94.28%, 85.71% and 82.85%) for Ampicillin, Carbenicillin, Co-trimoxazole, Tetracyclin and Cefuroxime respectively. Low level of resistance displayed for Amikacin (0.00%), Tobramycin (5.71%), Ceftazidime (5.71%),

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Aztreonam (11.42%), Norfloxacin (17.14%) and Gentamycin (34.28%) figure (1). These results were in accordance with Al-Dahmoshi, 2013[5] who found that the overall MAR index of the burn *P. aeruginosa* isolates was (0.4). Our results were in agreements with those presented by other studies in which the resistance to co-trimoxazole (94.7% - 90.9%), tetracyclin (81.8%), Carbenicillin (98.4%) [26, 27].

Anti-pseudomonal effect of Argan oil was studies extensively, Argan oil alone, 1.5% H2O2, mixture of Argan oil: 1.5%H2O2 in different ratio (1:1, 1:2 and 2:1). The results displayed no effect of Argan oil alone (Mean of inhibition zone=2.11mm) and medium effect of 1.5%H2O2 alone (Mean of inhibition zone=12.54mm) and high effect of mixture of Argan oil: 1.5%H2O2 (1:1 Mean of inhibition zone=23.82mm), (1:2 Mean of inhibition zone=23.05mm) and (2:1 Mean of inhibition zone=24.57mm) figure (2). All *P. aeruginosa* isolates show high level of sensitivity toward Argan oil mixtures in different ratio and show non-significant differences in the mean of inhibition zone and sensitivity percentage among different Argan oil mixtures ratio. Also the results revealed high anti-pseudomonal effect of Argan oil mixture when compared with investigated antibiotics figure (3 and 4).

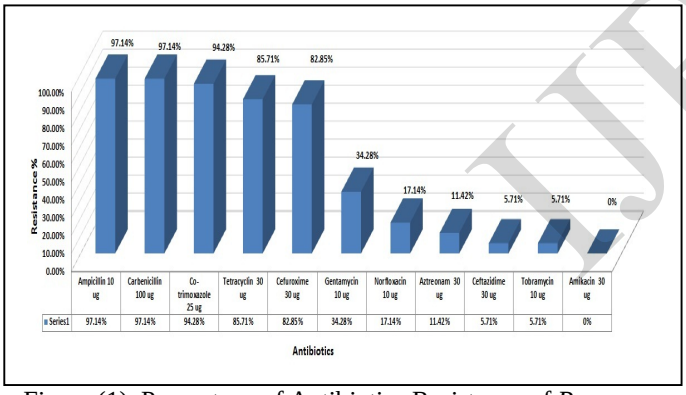


Figure (1): Percentage of Antibiotics Resistance of *P. aeruginosa* Isolates

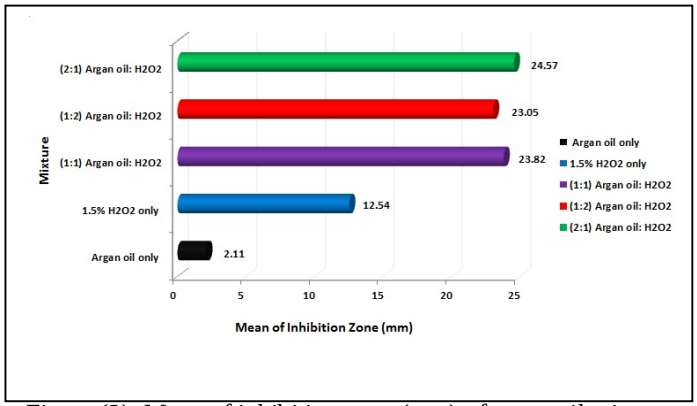


Figure (2): Mean of inhibition zone (mm) of argan oil mixture on *P. aeruginosa* isolates.

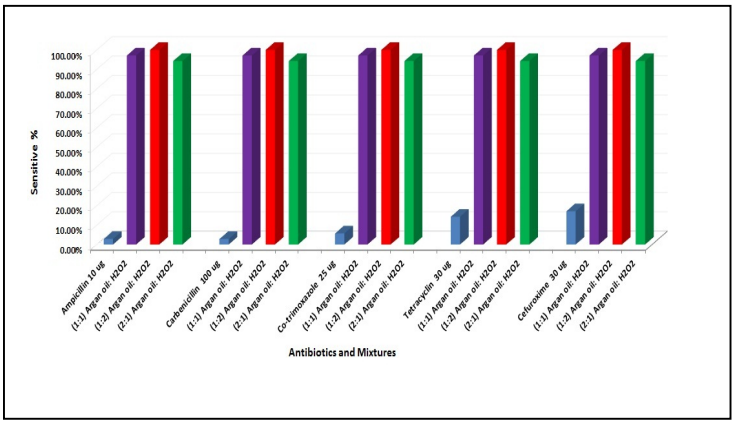


Figure (3): Sensitivity percentage of Argan oil mixture on P. aeruginosa isolates in compares with low level sensitivity

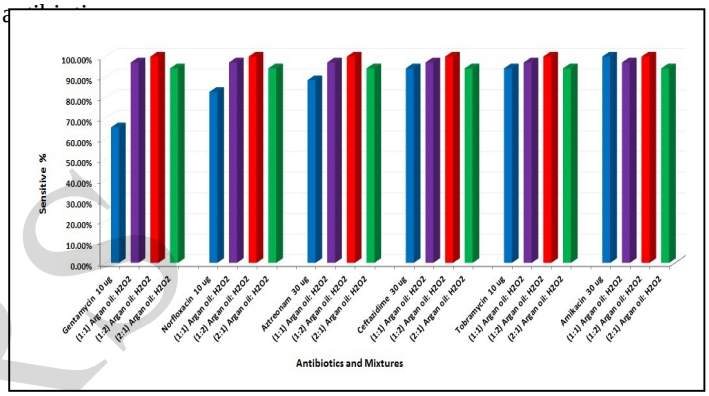


Figure (4): Sensitivity percentage of Argan oil mixture on *P. aeruginosa* isolates in compares with high level sensitivity antibiotics

B. Discussion

The burn and wound infection is considered one of the major health problems in the world, and one of the most frequent and severe complications in patients who have sustained burn[28]. *P. aeruginosa* is a common cause of wound infections, especially of thermal burns, this is because burns have large exposed areas of dead tissue free of any defenses and, therefore, are ideal sites for infection by bacteria from the environment or normal microbiota. The antibiotics susceptibility profile of *P. aeruginosa* isolates revealed high resistance for more than four antibiotics of different groups used in this study means multidrug resistance (MDR). Such high antimicrobial resistance is probably promoted due to selective pressure exerted on bacteria due to numerous reasons like non adherence to hospital antibiotic policy, and excessive and indiscriminate use of broad-spectrum antibiotics [27]. Also increasing numbers of the immuno compromised patients in our population due to different diseases and contaminations of the environment. The inordinate accessibility of antibiotics in shops and open markets from different origins of low

quality and ineffective for treatment as well as consumption of drugs without proper medical prescription- a common practice in resource poor countries [5].In addition, misuse of antibiotics and relaxation in general hygienic measures are associated with increasing infections with these bacteria [29].

Multi-drug resistant *P. aeruginosa* selection in burn centers can be enabled through transmission from person to person as well as widespread applications of anti-pseudomonal antibiotics. Burn wound can be contaminated by microorganisms that migrate from the gastrointestinal, urinary and respiratory tract [30].

The effect of Argan oil on *P. aeruginosa* burn isolates show remarkable results when compared to highly active antibiotics like Amikacin, Tobramycin, Ceftazidime, Aztreonam, Norfloxacin and Gentamycin. This activity clearly showed when mixed with 1.5%H2O2 in different ratio. The results show non-significant differences between the ratios (all ration give approximately similar results). In activity of Argan oil alone may be due to the masking of the active group or inactivity of the one of the important anti-pseudomonal components of Argan oil. Mixing with 1.5%H2O2 (safe concentration of H2O2) may also leads to convert of the one of the important anti-pseudomonal components of Argan oil from inactive form to active one. This study settles the activity of Argan oil as anti-pseudomonal and can be used externally as cream to treat the wound and burn infections.

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