Improvement of the Ethanol activity by using leaves extract of Artemisia herb alba against Pseudomonas aeuroginosa

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Abstract. The current study objective to improve the influence of ethanol as antibacterial via mixing it with Artemisia herba-alba leaf extract by using a good diffusion method. Ethanol is well known as antimicrobial so that has been used as a hand sanitizer and disinfectant for a long time. Nowadays microbial resistance to disinfectants become a major problem in health care centers, Pseudomonas aeruginosa one of these microbes that tend to create biofilm in low concentrations of ethanol and considered a healthcare-associated pathogen causing nosocomial infections. Seven concentrations of ethanol 70, 60, 50, 40, 20 and 10% were used and mixed with Artemisia herba-alba leaf extract at 50, 25, 12.5, 6.25, 3.12, 1.56 and 0.78 mg/ml in all concentration of ethanol. The results showed that the clear zone (C.Z.) of ethanol alone was 10, 10, 8 and 7 mm by using 70, 60, 50 and 40% respectively, well the low concentrations don’t show any antibacterial effect. Additionally, the antibacterial of A. Herba-alba extracts also calculated, showing high C.Z. was 33, 29 and 27 mm at 50, 25 and 12.5 mg/ml, respectively. All the doses of mixture ethanol and A. Herba-alba extract showed a higher effect comparing to previous results, where 50 mg extract mixed with 70% and 60% ethanol showed C.Z. 39 mm also the low concentration of ethanol showed antibacterial effect up to 33 mm, Even 0.78 mg of extract show inducing in the effect of ethanol against P. Aeruginosa. In conclusion, mixing A. Herba-alba leaf extract can improve the antibacterial effect of ethanol.

Key words: Ethanol, Pseudomonas aeruginosa, nosocomial infections, Artemisia herba-alba.

1. Introduction
Pseudomonas aeruginosa is the most concern infection healthcare-associated pathogen which can cause nosocomial infections [1]. This bacteria is known as an acute worry in hospitals because it can create multi-resistance to several antibiotic classes also due to its capacity to acquire practical resistance to all effective antibiotics [2]. Nowadays, it is associated with infections in intensive care units and in urology patients, including bacteraemia, urinary tract infections and surgical site infections, but it considers as the predominant agents of lower respiratory tract infections [3]. P. aeruginosa contains several mechanisms of action for developing resistance to both antibiotics and antiseptics, these mechanisms gain this bacteria the ability to spread and difficult to treat. It has structural changes in the outer membrane that leads to an absence of outside membrane porins, as well as the presence of ESBLs β-lactamase, efflux system pumps and plasmid resistance, furthermore, it owns the genes of resistance to disinfectants, including the qacEΔ1, which represent a mutation of the qac gene, which acts as a multidrug transfer gene. The qacEΔ1 gene located in the integron class 1, permits the gene to transport between the chromosome and the plasmid creating resistant P. aeruginosa to most disinfectants that commonly used in hospitals [4].
Ethanol is the most popular anti-bactericidal agent, and it is used in the disinfection of the skin, medical apparatus. The use of ethanol, consider more reasonable because of its low toxicity, is almost harmless to the human body, also ethanol has the characteristics of quickly absorbing water this causing dehydration of viruses and bacteria, leading to dehydration of the protein and freeze, causing quick death of viruses and bacteria [5].

Medicinal plants have been discovered for decades and used in traditional medicine practices for centuries extends to prehistoric times [6]. The medicinal plants can include varied types of herbs, trees, and shrubs, also several parts of the plants can be used, including root, leaf, flower, fruit, and bark are to cure diseases. On the other hand, lethal diseases such as cancer can be controlled by consuming certain amounts of medical plants every day [7]. Using medicinal plants as a remedy for different diseases is the oldest way of applying medical plants in health care known to humankind. However, since every plant contains multi-phytochemicals, the effects of a whole plant using as medicine are uncertain, so that there are continuous researches to discover more potentials in its components [8]. The species Artemisia herba-alba is an important medical plant belong to Artemisia genus its leave are hairy, silvery, small, and deeply bi-pennated with a leaner strip [9]. A. herba-alba widely used in folk medicine, this plant commonly used for the treatment of gastric disturbances, such as diarrhea, abdominal cramps, and for healing external wounds. The other names of this plant are desert wormwood, also in Arabic culture, is known as ‘shih’ [11]. For a long time, this plant has been used by the natives of many cultures for the preparations of traditional medicines to cure diabetes and hypertension [11]. Moreover, its components (phytochemicals) have emerged as a potential source of natural antioxidants [12]. The preparation of herbal tea from this species possesses antibacterial, analgesic, and anti-spasmodic properties. Additionally, this plant is utilized as a fodder plant for livestock in certain parts of the world. The study aims to evaluate the effect of A. herba-alba leaf extract on enhancing the ethanol activity as an antiseptic against P. aeruginosa.

2. Material and Method:

2.1. Collection and preparation of Artemisia herba-alba leave:
Samples of A. herba alba were collected from Samawah desert in March during the flowering period and the plant identification was performed by the agriculture college of Al-Muthanna University. The healthy leaves were separated from the stems and washed by tap water to remove dust and dirt, then dried in the shade, away from sunlight at room temperature, with constant stirring to prevent rotting, then dried leaves were crushed into small pieces (about 1 to 2 cm), they were kept in glass bottles with a tight lid away from light, heat, and moisture until use.

2.2. Plant leave extraction:
For obtaining a crude extract, small pieces of A. herba-alba leaves were crusade using mortar and pestle into powdered form. From the powdered plant, 150 g was soaked in 600 ml of ethanol for 72 hrs. then filtered with Whatman filter paper No. 1. After that, the filtrates were dried using a rotary evaporator at a low temperature of 35°C until reaching the small amount of the solvent, then remaining solvent was evaporated by a dry oven at 40°C to complete dryness. The obtained crude extract was kept in a refrigerator and away from light for further use (13).

2.3. Preparation of Alcohol and Plant Extract Concentrations:
To evaluate the ethanol effect as antibacterial, seven concentrations were prepared by dilution ethanol 99% by distill water to obtain 70%, 60%, 50%, 40%, 30%, 20% and 10% from ethanol. In addition to estimating the antibacterial effect of the crude extract of A. herba-alba leaves against P. aeruginosa several concentrations of crude extract were prepared by dissolving 50, 25, 12.5, 6.25, 3.12, 1.56 and 0.78 mg in one ml of DEMSO.
Moreover, for evaluation of the combined effect of extraction and ethanol against \( P. \text{aeruginosa} \), serial concentrations of crude extract mixed with different concentrations of ethanol. The concentrations of ethanolic leaf extracts were prepared as 50, 25, 12.5, 6.25, 3.12, 1.56 and 0.78 mg in one ml of ethanol in all concentrations. Additionally, 100 µl of DEMSO was used as a control.

2.4. Preparation of test bacteria:
The test organism *Pseudomonas auroginosa* was collected from Alhussein hospital, then isolated and diagnosed in the microbiology laboratory at the College of Science- Al-Muthanna University. Then these bacteria were grown on nutrient agar at 37°C for 24 hrs. After overnight, bacterial culture was taken to prepare the inoculums and adjust to 0.5 McFarland standard in 0.9% autoclaved normal saline.

2.5. Antibacterial activity bioassay
To evaluate the antibacterial activity of *A. herba-alba* leaves extracts against the human pathogenic *P. auroginosa*, an agar well diffusion procedure was used. For this method, preparing the Muller Hinton agar medium was done and sterilized via autoclave at 121°C for 15 min, after reaching appropriate temperature poured into Petri dishes and leaves aside to solidify under the hood conditions. Mueller Hinton agar plates were swabbed with a suspension of 0.1 ml *P. auroginosa* prepared as mentioned earlier, using a sterile cotton swab. The 7 mm diameter wells were bored using a cork borer in this media. The wells were filled with 50 µl of ethanol, serial plant extract concentrations, and the combination of both (ethanol and plant extract) then allowed to stand for 1 hr. at room temperature for spreading into the medium before incubation at 37°C for 24 hours. The clear zone (C.Z.) size was measured by the transparent ruler in mm (14).

3. Results and Discussion:
In this study, *in vitro* antibacterial activity of leaves, crude extracts of *A. herba-alba* was determined on the basis of the clear zone (C.Z), by using the same method, the inhibitory activity of ethanol was measured at different concentrations against *P. auroginosa*, then the combination of different concentrations of the ethanol and crude extract were mixed and the biological activity of this new mixture was measured.

| Alcohol Conc. | 70% | 60% | 50% | 40% | 30% | 20% | 10% | DEMSO |
|---------------|-----|-----|-----|-----|-----|-----|-----|-------|
| C. Z.         | 10  | 10  | 8   | 7   | 0   | 0   | 0   | 0     |

**Table 1:** The results of antibacterial activity caused by different concentrations of ethanol against *P. auroginosa*

| Conc. of extract | 50 mg/ml | 25 mg/ml | 12.5 mg/ml | 6.25 mg/ml | 3.12 mg/ml | 1.56 mg/ml | 0.78 mg/ml |
|-----------------|----------|----------|------------|------------|------------|------------|------------|
| C.Z.            | 33       | 29       | 24         | 19         | 13         | 9          | 7          |

**Table 2:** The results of antibacterial activity of *A. herba-alba* crude leaves extract dissolved in DEMSO
Table 3: Antibacterial Effect of Different Concentration of Artemisia Herba-Alba Leaves in Combination with Different Concentrations of Ethanol showed as a clear zone (C.Z) in mm.

| Ethanol % | Plant extract (mg) | 50 | 25 | 12.5 | 6.25 | 3.12 | 1.56 | 0.78 |
|-----------|-------------------|----|----|------|------|------|------|------|
| 70        |                   | 39 | 35 | 29   | 25   | 19   | 16   | 14   |
| 60        |                   | 39 | 35 | 27   | 24   | 19   | 14   | 14   |
| 50        |                   | 37 | 34 | 27   | 24   | 17   | 12   | 12   |
| 40        |                   | 35 | 33 | 26   | 22   | 16   | 12   | 11   |
| 30        |                   | 34 | 34 | 24   | 21   | 15   | 11   | 11   |
| 20        |                   | 33 | 31 | 24   | 21   | 15   | 10   | 9    |
| 10        |                   | 33 | 31 | 24   | 20   | 15   | 10   | 9    |

The C.Z. of alcohol against P. auroginosa was shown in Table (1) where 70% and 60% of ethanol give 10 mm, whereas the other concentration of ethanol has shown no effects against the bacteria growth. In Table (2) the C.Z. of A. herba-alba leaves extract were revealed, all concentrations of the leaf extract have an antibacterial effect, the high concentrations 50 and 25 mg/ml of extract giving 33 and 29 mm C.Z. respectively, against P. auroginosa. Now to evaluate the combined effect of the A. herba-alba leaves crude extract and ethanol together against P. auroginosa, different concentration of extract (50, 25, 12.5, 6.25, 3.12, 1.56 and 0.78 mg) were mixed with different concentrations of ethanol (70, 60, 50, 40, 30, 20 and 10 %) and the C.Z. of each combination was recorded. As shown in Table (3) the combination of 50 mg of a leaf extract with ethanol in different concentrations giving high C.Z. value, where it has given 39, 39 and 37 mm respectively, which higher than C.Z. resulting from using ethanol alone. Even the low concentrations of ethanol 20 and 10 % showing antibacterial effect at 33 mm as shown in Table (3). Furthermore, mixing 25 mg of a leaf extract with ethanol at different concentration has enhanced the antibacterial activity showing 35, 35 and 34 mm by using 70, 60 and 60%, respectively. Additionally, the combination of 12.5 mg from the plant extract with several ethanol extracts raised the antibacterial effect of each ethanol and plant extract itself, where the C.Z. reaches 29 and 27mm. In the same way, the low concentration of plant extracts (6.25, 3.12, 1.56 and 0.78 mg) enhances the effect of ethanol.

The genetic mutations that occur in P. auroginosa made it resistant to many antibiotics and disinfectants, especially in hospitals and other health centers. Therefore, new types of disinfectants must be developed. The process of developing the antimicrobial activity of detergents and disinfectants on a continuous basis will lead to preventing the occurrence of resistance (15). In this study, ethanol was chosen for improvement in its effects because it considers as the main disinfectant used in medical centers so that developing its activity against P. auroginosa is under consideration. Ethanol is known to be affected by bacteria by disrupting the plasma the membrane structure and the permeability of membrane of bacteria causing decreased the integrity of contents of bacterial cells and leading to destroy the cells (16). When ethanol using alone as antibacterial its activity decreased gradually because ethanol evaporated under normal conditions, which allowed the bacteria to grow again easily. According to the classification of the Centers for Disease Control and Prevention, ethanol is most available and used in the health care services in addition to ethyl alcohol and 2-propanol, basically because it’s not expensive when compared with other types of antiseptics (17). This plant A. herba-alba has been reported to have several phytochemicals like apigenin, β- sitosterol, Taurin, Erivanin, Isoerivanin, Herbalbin and Cycloartenol that known to possess multibiological activity (18).
The antibacterial activity of *A. herba-alba* was observed in a number of the previous study, where the conclusion of other studies (19) points out that the essential oil of *A. herba-alba* can be origin for natural antibacterial agents with novel pharmacological agents that have potential applications, where a significant antibacterial effect was observed with important zones of inhibition against *Klebsiella oxytoca* (31.3 mm) by disc diffusion method and against *Acinetobacter baumannii* (47.6 mm) by micro atmosphere method. Also, the study by (20) showed that the sesquiterpene lactones extract from the extract of the *A. herba-alba* has shown antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus faecalis* and *P. aeruginosa*. So that, *A. herba-alba* considered a promising plant to discovering novel drugs for facing up the growing problem of multiresistant bacteria in hospitals and health care centers. Ethanol used as a disinfectant for a long time for several reasons, but there are multi reported that argue ethanol can stimulate biofilm formation by activating transcription of the alginate genes by prolonged exposure to ethanol (21) or exposure to the low concentration of ethanol, according to (22) that can be stimulated *P. auroginosa* biofilm formation, but in our study findings even low concentration of ethanol (30%, 20%, and 10%) can inhibit the growth of *P. auroginosa* by mixing it with *A. herba-alba* leave extract. Furthermore, the improvement of the ethanol effect as a disinfectant is under investigation in many studies one of these was to demonstrate the enhancing of ethanol activity against MRSAs by mixing it with different concentrations of *Pulicaria undulata* leaves and stem extracts showing promising results (23). We suggest the mechanism by which the plant extract improved the ethanol effect of disrupting the plasma membrane of bacteria that facilitate the entry of plant extract into the bacterial cell causing the elimination of the cells since ethanol evaporated in a little time but the plant extract can stay and destroy the bacterial cells.

4. Conclusion

According to the result obtained from the current study, ethanol activity can be improved by mixing it with serial crude leaf extract of *A. herba-alba* plant, these combinations can increase the activity of ethanol to eliminate *P. auroginosa* that cause a serious problem in hospitals. Further works required to certain our results by using a different combinations of solvents and plant extracts against another microorganism.

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