Effect of Essential oil and Extracts of Artemisia Herba – Alba Plant Against Some E Pathogenic Bacteria

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ABSTRACT

Artemisia Herba – Alba is the one of the most important genus of Asteraceae family. It has numerous values because of medicine properties. Artemisia Herba – Alba has numerous application in traditional medicine from ancient times. In this research, antimicrobial effects of leaf aqueous, alcoholic extracts and essential oil of Artemisia Herba – Alba against Staphylococcus aureus PTCC 1431 and Escherichia coli PTCC 1399 were studied, using agar disc diffusion method. Methanol and ethanol extracts obtained from leaves of A. Herba – Alba exhibited antimicrobial activity against test microorganisms. In addition Artemisia herba-alba essential oil was inhibitory effects against all studied pathogenic bacteria. The results showed that essential oil of Artemisia herba-alba had the most inhibition diameter zone in Staphylococcus aureus than E.coli (25-17mm successively). Therefore in this research we showed that the positive gram bacteria are more sensitive than negative gram bacteria. our results indicate the possibility of using essential oil in the treatment of bacterial infections, and the results of this study was encouraging, despite the need for clinical studies to determine of the real effectiveness and potential toxic effects in vivo.

Key words: Affect, Artemisia plant, Gram Negative Bacteria, alcoholic Extracts, Essential oils.
Introduction

*Artemisia herba alba* Asso (chih), belonging to the family "Asteraceae"; This is among the most medicinal plants used by the local population because of their medicinal properties, as well as a flavoring in tea and coffee (Bezza et al., 2010). The plant known as the “wormwood” is characteristic of the steppes of the Middle East and North Africa. It is extensively used to treat stomach disorders, hepatic, in addition to a wide variety of ailments and against certain forms of poisoning, also as antitumor agent, antispasmodic, antiseptic, antigen toxic, ant diabetic, antibacterial and antioxidant (Bezza et al., 2010, Goudjilet et al., 2015). Essential oils (EO) have been used for centuries in traditional medicine for treatment against various diseases. (Al-Shuneigat et al., 2014). EO’s are composed of a mixture of secondary metabolites that often possess antimicrobial properties and thus can play an important role for the plant defense (Al-Shuneigat et al., 2015). Gram positive bacteria such as *Staphylococcus aureus* is mainly responsible for post-operative wound infections, toxic shock syndrome, endocarditis, osteomyelitis and food poisoning (Benayache et al., 2001). Gram negative bacterium such as *Escherichia coli* is present in human intestine and causes lower urinary tract infection, coleocystis or septicaemia (Benhassaini et al., 2003; Benjilali et al., 1986). This Study aims To assess the antimicrobial effect of *Artemisia Herba alba* plant extracts and essential oil against isolates *E. coli* and *S. aureus*.

Materials and Methods

Plant material and extraction

Fresh leaves of *Artemisia Herba alba* were collected from Al-Jabal Al-Akhdar (is an upland region which lies at the northeast part of Libya. (Alhassi 2005). Plant leaves were cleaned with deionized water and dried at shade for a week. After drying, the leaves were powdered. For aqueous extraction, 20 g of powder was added to 150 ml of distilled water and boiled on slow heat for 2 hours. Then it was filtered through Whatman No.1 filter paper, and 20 g of Powdered leaves were extracted with methanol and ethanol by the aid of a Soxhelet apparatus. Finally, the obtained solution was passed through Whatman No.1 filter paper and allowed to evaporate in oven at 45 ºC.
Aquatic extract dissolved in distilled water, while alcoholic extract dissolved in 10% Dimethyl sulfoxide (To prepared 200mg/ml as a standard concentration).

**Tested microorganisms**

The standard strains used in this study were *Staphylococcus aureus* PTCC 1431, and *Escherichia coli* PTCC 1399. The strains were obtained from ibznahr laboratory (700 district) in Al-marj city. The microorganisms were grown overnight at 37 °C for 24 h in Mueller-Hinton agar. Ciprofloxacin (0.3%w/v) was used as a standard antibiotic for in vitro antimicrobial activity.

**Antibiotic activity assay**

The filter paper discs (antibiotic) were placed on the surface of a Mueller-Hinton agar that has been inoculated with test microorganisms. During incubation, the antibiotics diffuse outward from the discs creating a concentration gradient. After 24-48 hours, the zone diameter of inhibition is measured.

**Plant extracts activity assay**

Paper Disk Diffusion Assay: The test microorganisms were spread on MHA medium by with the 24h cultures of bacteria growth in nutrient broth. After solidification the filter paper discs (5mm in diameter) inoculated with the test microorganisms and then impregnating with 20μl of plant extract (concentration 200 mg/ml)and essential oil. The plates were subsequently incubated at 37o C for 24 Hrs. After incubation the growth inhibition rings were quantified by measuring the diameter of the zone of inhibition in mm (Kumara., *et al* 2009). The experiments were performed in duplicate and the mean values were observed. Statistical analyses were performed using SPSS software.

**Results and Discussion**

Natural products are considered an important source of new antibacterial agents. Medicinal plants continue to be used world-wide for the treatment of various diseases and have a great potential for providing novel drug leads with novel mechanism of action (Vatľák et al., 2014). Table 1 showed the antimicrobial activity of leaf methanol, ethanol, and aqueous extracts and essential oil of *Artemisia Herba – Alba*.
by paper Disk Diffusion method against selected pathogenic bacteria. Methanol and ethanol extracts obtained from leaves of Artemisia Herba – Alba exhibited antimicrobial activity against studied microorganisms.

Table 1. Effect of Artemisia Herba – Alba extracts, essential oil, and antibacterial drug on studied bacteria.

<table>
<thead>
<tr>
<th></th>
<th>Inhibition Zone (mm)</th>
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<tbody>
<tr>
<td></td>
<td>E. coli PTCC 1399</td>
</tr>
<tr>
<td>Methanol extract</td>
<td>11.5±0.7</td>
</tr>
<tr>
<td>Ethanol extract</td>
<td>12±0.0</td>
</tr>
<tr>
<td>Aqueous extract</td>
<td>6±0.1</td>
</tr>
<tr>
<td>Essential oil</td>
<td>17±1.5</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>18±0.5</td>
</tr>
</tbody>
</table>

In our experiment, Artemisia herba-alba (leaves) (extracted by ethanol) was showed the highest effect against S. aureus with a zone of inhibition = 15 mm. While, antibacterial activity of aqueous extract of Artemisia herba-alba was found against E. coli and showed low antimicrobial activity with a zone of inhibition = 6 mm. Also, essential oil of Artemisia Herba-alba with a zone of inhibition 25 mm were recorded against S. aureus, whereas E. coli was 17 mm (figures 1 and 2). It was noted that alcoholic extract has greater effect in the inhibition from aqueous extract, which may be due to the fact that alcohol is the best solvent for the active compounds extracted from the plant when compared with distilled water used in the case of aqueous extracts (Al-Saimary et al., 2006). The essential oil of A. herba-alba gave the best antibacterial activity against S. aureus. Artemisia spp. have been widely used for a variety of medicinal purposes for many years. Many studies confirm positive role of Artemisia herba-alba extract in inhibitory pathogenic bacteria. Artemisinin, one of the bioactive compounds, with antimalarial activity has been successfully isolated from Artemisia herba-alba. Other than antimalarial activity, artemisinin was found to be a good antibacterial, antifungal, antileishmanial, and antitumor agent. The antibacterial properties of artemisinin had been tested on a wide range of bacteria, such as Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, and Mycobacterium intracellular (Appalasamy et al., 2014). A. herba-alba essential oil produced inhibitory effects against all isolates and susceptibility varied considerably. Positive gram bacteria in comparison of negative gram bacteria were more sensitive
for essential oil of A. herba-alba in disc diffusion method (figure 3), the positive gram bacteria, *Staphylococcus aureus* showed that inhibitor diameter (25mm) of higher than negative gram bacteria, *E.coli* (17mm) (figure 4 and 5). Therefore in this research we showed that the positive gram bacteria are more sensitive than negative gram bacteria. It might be due to membrane of polysaccharide of cell walls. These bacteria show less sensitive to antibacterial effects of essence. Among gram positive bacteria direct connection of essence hydrophobic combination perform with two layer phospholipid. This impact takes place whether increasing ions penetrating or leaking cell crucial combination, and occur on inability system of bacterial enzyme (Sandri et al., 2007).

Figure 1. Comparison of antibacterial effects of *Artemisia herba-alba* and antibiotics on *S aureas*.

Figure 2. Comparison of antibacterial effects of *Artemisia herba- alba* and antibiotics on *E coli*.
Figure 3. Comparison of effect of essential oil on *E. coli* and *S. aureas* bacteria.

Figure 4. Inhibition diameter of bacteria *Staphylococcus aureus* (essential oil) in disc diffusion method.

Figure 5. Inhibition diameter of bacteria *Escherichia coli* (essential oil) in disc diffusion method.
Conclusion

According to increasing limitation on using chemical material of antimicrobial due to spreaders of drug resistance, it is appeared volatile oils are better antimicrobial resource in keeping nutritive material and human illnesses control. The usage of essential oil of plant can be used for infectious substance in medical science, and food industry.

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