

**Research Article**

## **Evaluation of Antimicrobial Effect of Hydroalcoholic Extract of Rosa Damascene, Cichorium intybus L and their Synergism on *Acinetobacter Baumannii* Strains Resistant to Different Antibiotics**

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**ABSTRACT**

**Introduction & Objective:** Although most microbial diseases are treated with antibiotics today, but the side effects of these drugs, also resistance to these drugs are a source of doubt about the prescription of these drugs. Antimicrobial herbs can be an appropriate complement to antibiotic therapy. Therefore, this study was aimed to determine the bactericidal effect of Hydroalcoholic extract of Rosa damascene, Cichorium intybus L and their mixture on *Acinetobacter baumannii* isolated from clinical specimens that is resistant to most antibiotics

**Materials and Methods:** In this experimental study, the Hydroalcoholic extract of Cichorium intybus L and Rosa damascene were obtained by maceration method and the mixture of them prepared with equal proportion of both extracts. Then strains isolated from clinical specimens of Shahrekord teaching hospitals were cultured. And after the diagnostic tests of *A. baumannii*, bacteria were prepared by standard culture methods, antibiogram test was performed and broth micro dilution method was used to determine the antimicrobial effect of their extracts. Then, based on the CLSI 2015 standard, with three replicates, for each sample, the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were obtained. SPSS software version 16 was used to analyze the data and the significance level of  $P \leq 0.05$  was used.

**Results:** Based on this work, MIC of Hydroalcoholic extract of Rosa damascene against *A. baumannii* strains that isolated from Trachea, blood, urine, wounds, respiratory and catheters secretions and standard strains was obtained, that they are respectively 16, 128, 128, 32, 64, 128 and 16  $\mu\text{g}/\text{cc}$  ; and for Cichorium intybus L are 512, 1024, 1024, 128, 512, 512 and 512  $\mu\text{g}/\text{cc}$  ; and for their mixture are 128, 128, 64, 64, 128, 32, 64  $\mu\text{g}/\text{cc}$ .

**Conclusion:** In this study the results show that *A. baumannii* which is resistant to different antibiotics is susceptible to hydroalcoholic extracts of these plants. The mixture of extracts has also synergistic effects, which means it can spoil bacterium in lower doses in comparison with extracts distinctly. So through more studies we can produce an appropriate supplementary for synthetic antibiotics which their bacterial resistance is growing every day.

**Keywords:** *Acinetobacter baumannii*, Cichorium intybus L, Rosa damascene, synergism

**INTRODUCTION**

Microorganisms play a very important role in causing disease in human. The abundant mortality resulting from these factors has always led human kind to seek ways to cope with

microorganisms (1). In the past, natural medicines, especially medicinal herbs, were the basis and, in some cases, the only remedy usage, while the raw materials used in the

pharmaceutical industry were used (2). In the early twentieth century, with the advancement of chemistry and the discovery of complex organic synthesis systems, the pharmaceutical industry developed and synthetic drugs replaced herbal medicines, but as the progress in the production of various chemical and antibiotic drugs the harmful effects of these drugs became known gradually and on the other hand, several bacteria with genetic alteration have become resistant to antibiotics that are still expanding (3-5). The problem of resistance to antibiotics, which occurs for various reasons, not only increases morbidity, but also cause of making new antibiotics every day (1). In this study, the increase in mortality from infectious diseases in the years 1980 to 1992 was reported at 39%; on the other hand, side effects of drugs are one of the biggest treatment problems and the drug side effects reported as the fourth major cause of death in the United States (7, 6).

Today, the antimicrobial compounds of medicinal plants are one of the most valuable sources of medicine, and it is attempted to use these plants and purify their effective compounds in the treatment of diseases. Antimicrobial compounds are not only used in the treatment of infectious diseases, but also at the same time reduce the number of side effects often associated with the use of antibiotics (1). One of the herbs that have been described as antimicrobial is Cichorium intybus L that Hydroalcoholic extract of Cichorium intybus L has an antibacterial effect, which is particularly effective on gram-positive bacteria such as *Staphylococcus aureus* (8). In traditional medicine, Cichorium intybus L is used as an analgesic, sudorific, antipyretic and etc.; all parts of this plant, especially leaves and root, can be used (9). Another useful herb is Rosa damascene which has been reported in the medical press for antibacterial, antioxidant, anti-contraction and seizure effects, which prevents cardiovascular diseases and protects the skin from extracts of this plant in recent years. (11, 12). *A. baumannii* is one of the most common pathogens in the intensive care unit around the world, and one of the cause of Nosocomial infection; it has been ignored since the 1970s when isolated from clinical specimens due to its

low levels of pathogenicity (13). *Acinetobacter* are gram-negative coccobacilli, negative oxidase, non- motile, and aerobic. Different species of *Acinetobacter* are widely distributed in nature and can be separated from water, soil, human skin, food and sewage. They are more stable in wet conditions, but they survive in dry conditions for several days to several weeks (14). Due to its clinically significant acidity, in particular in recent years, and its ability to achieve drug resistance, this bacterium is one of the most threatening microorganisms that is highly resistant to the treatment with antimicrobial drugs. These bacteria are responsible for various bacterial infections such as bacteremia, urinary tract infection and secondary meningitis, but their main role is the development of hospital pneumonia, in particular pneumonia caused by the upper respiratory tract of patients admitted to specialized care units. *Acinetobacter* are resistant to most antibiotics that have been reported so far (15). Therefore, this study was conducted with regard to drug resistance in *Acinetobacter* as well as attempts to find new drugs that do not have common antibiotic problems with the aim of determining the antimicrobial effect of Hydroalcoholic extract of Rosa damascene, Cichorium intybus L and their mixture on *A. baumannii* isolated from clinical specimens that is resistant to most antibiotics.

## MATERIAL AND METHODS

In this experimental study, the standard strain of *A. baumannii* with ATCC 19606 were provided from the Iranian Research Organization for Science and Technology; and clinical strains of *A. baumannii* isolated from different clinical specimens including Trachea, blood, urine, wound, Respiratory and catheter secretions from different parts of Shahrekord teaching hospitals and after differential diagnostic tests such as Gram staining, oxidase, catalase and etc.; purification and culture were done according to the manufacturer's instructions. To ensure the purity of the strains, the colonies' characteristics were matched to the national standard, and then the bacterial suspension was prepared in accordance with 0.5 McFarland ( $1.5 \times 10^8$  CFU/ml) (16). In order to investigate the

susceptibility of isolated strains to common antibiotics, a suspension based on 0.5 McFarland was prepared from each of the isolates of *A. baumannii* and cultured on a Mueller Hinton agar. Then, the antibiotic discs of cefalotin, ceftriaxone, Cephalexin, cefixime, ceftazidime, amoxicillin, nalidixic acid, cefazolin, Trimethoprim/sulfamethoxazole, gentamicin and ciprofloxacin were placed on the medium at certain intervals, and the plates were incubated at 37 ° C for 72 hours in standard condition (17). Then, the Rosa damascene and Cichorium intybus L were prepared from Shahrekord attributes and extracted by maceration method at the Medicinal Plants Research Center of Shahrekord University of Medical Sciences. The extraction was carried out in such a way that at first 100 grams of Rosa damascene and Cichorium intybus L in combination with 70% alcohol in a volume of 1 liter were stored for 72 hours in a dark environment. After filtration with 0.5 Whatman paper, the liquid was placed in a rotary evaporator (distillation under vacuum conditions) and after that incubated at 37 ° for 72 h. after incubation, finally the dry extract was obtained. Stock was prepared with Normal saline and the extract and mixture of them was prepared from the equal ratio of both extracts at similar concentrations (18). In the next step, the antibacterial properties of the extracts on bacteria were investigated by broth micro dilution method, so that the extract of Rosa damascene, Cichorium intybus L and their synergism mixed on bacteria in sterile 96-well plate according to the CLSI standard with three repetitions for each sample. Hydroalcoholic extract of Rosa damascene, Cichorium intybus L and their mixture at concentrations of 2 µg/cc, 4 µg/cc, 8 µg/cc, 16 µg/cc, 32 µg/cc, 64 µg/cc, 128 µg/cc, 256 µg/cc, 512 µg/cc, 1024 µg/cc, 2048 µg/cc were used for the test. after adding 100 µl of Mueller Hinton broth to all wells, the

**Table 1:** MIC and MBC of Hydroalcoholic extract of mixture of Rosa damascene and Cichorium intybus L with equal proportion of both extracts on *A. baumannii*

Microorganism	MBC (µg/cc)	MIC (µg/cc)
standard strain of <i>A. baumannii</i>	256	64
<i>A. baumannii</i> Isolated from Trachea	512	128
<i>A. baumannii</i> Isolated from Blood	512	128
<i>A. baumannii</i> Isolated from urine	256	64

dilutions of the extracts were added to the Muller Hinton Broth and then 100 µl of bacterial suspension ( $1.5 \times 10^8$  CFU/ml), were added to the wells. The first well only contained a bacterial suspension and Mueller Hinton broth (positive control) and the second well contained a Mueller Hinton broth and extract and solvent extract (negative control). Afterwards, all the samples were incubated at 37 ° C for 24 hours, and then optical densities of the samples were read at 650 nm wavelength with an ELISA reader (State Fax 2100, USA) (19). To determine the minimum inhibitory concentration (MIC), the lowest concentration that did not have any turbidity was considered MIC. And to determine the MBC All wells without turbidity cultured on Mueller-Hinton agar separately and then incubated for 24 hours at 37 ° C and the lowest concentration of the extract that the bacteria are not grown as MBC reported (20). At the end of this work, SPSS software version 16 was used to analyze the data.

#### FINDINGS

Base on the result of this study; MIC of Hydroalcoholic extract of Rosa damascene against *A. baumannii* strains that isolated from Trachea, blood, urine, wounds, respiratory and catheters secretions and standard strains was obtained, which they are respectively 16, 128, 128, 32, 64, 128 and 16 µg/cc; and for Cichorium intybus L are 512, 1024, 1024, 128, 512, 512 and 512 µg/cc; and for their mixture are 128, 128, 64, 64, 128, 32, 64 µg/cc. And also the amount of MBC of Hydroalcoholic extract of Rosa damascene on *A. baumannii* strains that isolated from Trachea, blood, urine, wounds, respiratory and catheters secretions and standard strains was obtained, that they are respectively 64, 512, 512, 128, 256, 512 and 64 µg/cc ; and for Cichorium intybus L are 2048, 2048, 2048, 512, 1024, 2048 and 2048 µg/cc ; and for their mixture are 512, 512, 256, 256, 512, 128 and 256 µg/cc. (Table 1-3).

<i>A. baumannii</i> Isolated from wound	256	64
<i>A. baumannii</i> Isolated from respiratory secretions	512	128
<i>A. baumannii</i> Isolated from catheter secretions	128	32

**Table 2:** MIC and MBC of hydroalcoholic extract of Cichorium intybus L on *A. baumannii*

Microorganism	MBC (µg/cc)	MIC (µg/cc)
standard strain of <i>A. baumannii</i>	2048	512
<i>A. baumannii</i> Isolated from Trachea	2048	512
<i>A. baumannii</i> Isolated from Blood	2048	1024
<i>A. baumannii</i> Isolated from urine	2048	1024
<i>A. baumannii</i> Isolated from wound	512	128
<i>A. baumannii</i> Isolated from respiratory secretions	1024	512
<i>A. baumannii</i> Isolated from catheter secretions	2048	512

**Table 3:** MIC and MBC of Hydroalcoholic Extract of Rosa damascene on *A. baumannii*

Microorganism	MBC (µg/cc)	MIC (µg/cc)
standard strain of <i>A. baumannii</i>	64	16
<i>A. baumannii</i> Isolated from Trachea	64	16
<i>A. baumannii</i> Isolated from Blood	512	128
<i>A. baumannii</i> Isolated from urine	512	128
<i>A. baumannii</i> Isolated from wound	128	32
<i>A. baumannii</i> Isolated from respiratory secretions	256	64
<i>A. baumannii</i> Isolated from catheter secretions	512	128

During the investigation of susceptibility of isolated strains to cefalotin, ceftriaxone, Cephalexin, cefixime, ceftazidime, amoxicillin, nalidixic acid, cefazolin, Trimethoprim/sulfamethoxazole, gentamicin and ciprofloxacin, the results showed that all the *A. baumannii* that isolated from Trachea, blood, Urine, wound, respiratory and catheters secretions are resistant to the antibiotics described.

## DISCUSSION

Antibiotics are valuable drugs for the treatment of many infectious diseases, however, the use of these drugs can lead to more severe microbial contamination. Antibiotic resistance patterns among hospital pathogen bacteria may vary significantly from one country to another in different regions of a country. Previous studies have shown that the first line treatment for infections caused by *A. baumannii*, including: amikacin, carbapenem (imipenem, meropenem and doripenem), ceftazidim and quinolones (21). In addition, most pathogens are present in some new antibiotics such as broad-spectrum cephalosporins (Like cefotaxime and ceftazidime) have almost completely resisted. Prior to this, across the globe, imipenem was the

most active drug against infection caused by *A. baumannii*, but recently there has been evidence of the Imipenem resistant bacteria (22).

The highest resistance to imipenem among Acinetobacter species have been observed in *A. baumannii*. The emergence of *A. baumannii* resistant to imipenem is a global problem that threatens to continue the successful treatment of infections caused by this bacterium. In a study conducted in Shahrekord in 2015, the antibiotic resistance of *A. baumannii* strains isolated from Shahrekord teaching hospitals against the antibiotic ceftazidime (94%), cefotaxime (93%), cefepime (91%), gentamicin (85%), ciprofloxacin (89%), norfloxacin (87%), imipenem (86%), meropenem (73%), Tobramycin (67%) And amikacin (66%) and the most susceptibility was in relation to antibiotics of colistin (76%) and ampicillin-sulbactam (70%), respectively. Also, 93% of the isolates had multiple antibiotic resistance (23). In the present study, the resistance of strains isolated from antibiotics cefalotin, Cephalexin, ceftriaxone, cefixime, ceftazidime, ciprofloxacin, nalidixic acid, cefazolin, gentamicin, Trimethoprim/sulfamethoxazole and amoxicillin close to 100%, which indicates an increase in antibiotic resistance of *A. baumannii*.

Due to the rise in antibiotic resistance, scientists have conducted several studies on different parts of the herbs to discover new Antibacterial compounds (24). In a study by Bahador et al. In 2015, the antimicrobial effect of alcoholic essential oil of Satureja khuzestanica On the *A. baumannii*, isolated from clinical specimens (blood, urine, wound, sputum, cerebrospinal fluid), MIC was 0.3 (ml / mg) (25) and in Current work MIC of Hydroalcoholic Extract of Cichorium intybus L on *A. baumannii* Isolated from wound was determined 128 ( $\mu\text{g} / \text{cc}$ ) and showed at least 512 ( $\mu\text{g} / \text{cc}$ ) for the Trachea, blood, urine, wound, respiratory and catheter secretions, as well as the antimicrobial effect of mixture of hydroalcoholic extract of Cichorium intybus L and Rosa damascene are More effective than this effect for the alcoholic essence Satureja khuzestanica. Also, the present study proved the synergistic property of the mixture of hydroalcoholic extract of Rosa damascene and Cichorium intybus L.

Ozak et al. evaluated the effect of alcoholic extract of Rosa damascene over 15 types of bacteria (*enterococcus faecalis*, *Bacillus cereus*, *Mycobacterium Smegmatis*, *Escherichia coli*, *proteus vulgaris*, *yersinia enterocolitica*, *enterobacter aerogenes*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Salmonella enteritidis*, *Pseudomonas fluorescens*, *Aeromonas hydrophila* and *staphylococcus aureus*) showed The extracts of this plant Only effect on *E. coli* (26). In the present study, hydroalcoholic extract of the Rosa damascene inhibited the growth of isolated and standard strain. In the study of Rani and Khullar the antibacterial effects of alcoholic and Aqueous extracts of 54 medicinal plants such as Cichorium intybus L were determined, it was found that Cichorium intybus L extract has moderate antibacterial effects on resistant against *Salmonella typhi*. The results of this study are in line with current work (27).

In this study, it was also shown that the Extracts had different effects on bacteria. As in this study, other researchers have pointed to the different susceptibility of bacterial species to the tea extract (28). The difference between the sensitivity of different bacteria to antimicrobial substances is probably due to the different

structure of microorganisms. Different concentrations of the extracts are effective in Growth of bacteria, probably the reason for the stronger antimicrobial effect of the hydroalcoholic extract of Rosa damascene than Cichorium intybus L, is more effective antimicrobial components like camphorol and etc. in this plant. Also, this study, like some other studies, showed increased antimicrobial activity in the mixture of extracts (29).

Considering the findings of this study and comparing it with other studies, it can be concluded that Rosa damascene and Cichorium intybus L extracts have a significant effect on controlling and inhibiting the growth of *A. baumannii* that isolated from clinical specimens invitro. Obviously, the use of these compounds as antibiotic supplements requires more research about it. It is also worthwhile studying the mechanisms of the effects on bacteria and the physiological responses of bacteria in contact with these materials.

## CONCLUSION

The results of this study showed that *A. baumannii* that isolated from clinical specimens of different parts of Hajar and Kashani hospitals in Shahrekord have been resistant to a wide range of antibiotics and Hydroalcoholic extract of Rosa damascene and Cichorium intybus L not only have inhibitory growth On *A. baumannii* that isolated from clinical specimens, but also they could kill these bacteria in higher concentration completely. It should be noted that the inhibitory effect of extracts on the standard *A. baumannii* strain was higher than those isolated from the clinical specimen, which confirms the increased resistance of isolated bacteria from the clinic. The synergistic effect of these extracts in the mixture is also evident, so that the mixture of these extracts is more antibacterial and more stable.

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