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ORIGINAL ARTICLE

The Investigation of the Antibacterial effects of Ethanol extract of *Cichorium intybus L.* on Antibiotic-resistant *Staphylococcus aureus* strains

Abolfazl Jafari Sales[1*], Nader Nezamdoost Shadbad[2], Vahid Purabdollah Kaleybar[2]
*1 -Young Researchers and Elite Club, Ahar Branch, Islamic Azad University, Ahar, Iran
Email:a.jafari_1392@yahoo.com
Tel:+989147611841
2 – Department of Microbiology , Ahar Branch, Islamic Azad University, Ahar, Iran

ABSTRACT
Resistance to antibacterial agents and sensitivity reaction to such chemical compounds are the main reasons for investigators to develop new antibiotics from herbal sources. Therefore, this study is done to evaluate the antimicrobial effects of ethanol extract of Cichorium Intybus L.. plant on clinical resistant Staphylococcus aureus. After collecting plants and validate its scientific name by botanists of Agricultural Organization and after drying in shade, extract of Cichorium Intybus L. plant , was extracted by vacum distlllation method and different concentrations of the extracts were prepared. Then, the MIC and MBC of the extracts on Staphylococcus aureus strains that isolated from patients, was determined by broth dilution and agar well diffusion. .The results showed that the MIC and MBC of ethanol extract of Cichorium Intybus L. plant on Staphylococcus aureus were 6.5 and 13 mg/ml. Since some of concentrations of the extracts showed significant antibacterial activity on given Staphylococcus aureus, more studies on the plant in future, provides more information regarding probable substitution of that for current antibiotics.
Keywords*: Antibacterial effects, Extract, Staphylococcus aureus, Minimum Inhibition Concentration , Minimum Bactericidal Concentration*

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INTRODUCTION

Staphylococcus grows in most of culture media and is active from metabolic aspects. *Staphylococcus aureus* is turned into one of the important health problems in the world due to its potential pathogenesis power and increasing resistance to anti-microbial drugs. *Staphylococcus aureus* is resistant to penicillin by generating Beta-lactamase enzymes. Methicillin- resistant indicates the resistance to all Penicillinase and Cephalosporins – resistant penicillin[1, 2]. The organism has been found to be the most common bacterial agent recovered from blood stream infections, skin and soft tissue infections, pneumonia and hospital-acquired post-operative wound infections [3-6]. If untreated, staphylococcal infections may lead to bacteremic seedings of several organs, causing endocarditis, osteomyelitis and septic arthritis [7,8]. Changes in the pattern of antimicrobial susceptibility of *S. aureus* and other organisms have been reported worldwide, especially in developing countries [9-11], making antimicrobial agents increasingly less effective in treating bacterial infections

The need for new, effective and affordable drugs to treat microbial diseases in the developing world is one of the issues facing global health today. Available drugs for the treatment of these diseases are limited by factors ranging from microbe resistance to safety, compliance and cost. The use of medicinal plants for curative purposes is as old as mankind and can be traced from the beginning of civilization. The interaction between man and his environment is such that his food, shelter, the diseases that afflict him and the cure of such diseases are all within the environment. Thus, man uses his environment and the resources of nature to combat diseases that afflict him. Consequently over many centuries, people of various countries of the world have embarked on what is now known as traditional medicine [12].

Cichorium Intybus L. belongs to Compositae family and has anti-bacterial effect. Also it grows as wild with height 0.5 to 1.5m but if it is grown, it exceeds 2m. It has strong root with the length 0.5 to 1m, brown and yellow. The stem of this plant is thin, cylinder with low branches in the surrounding area of its top. The

blue flowers of *Cichorium Intybus L.* emerge in summer from July to September. This plant grows in relatively humid areas in most areas of Europe, Asia, North of Africa and northern America as wild. It is distributed widely in Iran in various regions and it is as annual, biannual and perennial in various regions [13]. Today, *Cichorium Intybus L.* extract has various effects as anti-toxicity of liver, *mast cell-mediated* immediate type allergic reactions and anti-diabetics [14-16]. This study attempted to evaluate the anti-bacterial effects of ethanol extract of *Cichorium Intybus L.* on antibiotic-resistant *Staphylococcus aureus*.

MATERIALS AND METHODS

Providing bacteria strains and their antibiotic resistance

30 applied samples in this study are selected among the clinical samples of Razi Hospital of Marand town and are cultured on specific Mannitol salt agar and blood agar. Pure isolates obtained on artificial culture media are identified by Catalase and other biochemical tests and finally by performing coagolase test by tube and slide methods and agglutination evaluation, *Staphylococcus aureus* species are identified and then the pure isolate by Corbei-baer method are antibiogramed on Mueller–Hinton agar culture media and their sensitivity to antibiotics is also evaluated. The applied antibiotics include trimethoprim/ sulfamethoxazole (SXT10+10 µg), ampicillin (AM 10µg), ceftazidime (SAZ 30µg), cefoxitin (CF 5 µg), amikacin (AN 30 µg), penicillin (P 10 µg), ceftriaxone (CRO 30 µg), Erythromycin (E 15 µg) and , Tetracycline (TE 30 µg) made in Padtan Teb company. After 24h incubation at temperature 37^{0}C the diameter of the zone of inhibition is measured and sensitivity and resistance of isolates are determined and the results are compared with standard table.

Sampling and providing herbal extract

The herbal samples are collected from natural fields around Marand town in East Azerbaijan Province, Eishabad village in two stages at the end of May and early June. The samples are cleaned after collection and transference. Then, they are dried in a great space away from sun. After the complete drying and separating the aerial parts, stem and leaves from the roots, they are prepared for being powdered. After powdering the samples, 100g of herbal powder are mixed as 1:8 with distilled water and ethanol 80%. The mixtures are kept for 48h at lab temperature and are mixed by a glass rod each hour. The mentioned mixtures are filtered by four-layer sterile gas and funnel. To separate the impurities in extract, it is centrifuged at 2500 rpm for 20 m at temperature 4 °C

Then, the filtered extract is transferred to distilled system in vacuum to remove the solvents and finally, a strong extract is obtained. The extract is divided by microbial filters 0.45 micron sterile and in micro tubes 1.5mL sterile and is kept at temperature -80 °C [17-18].

Determining the antimicrobial effect of extract by well diffusion in agar method

In this method, at first by tested bacteria, a suspension equivalent standard turbidity 0.5 McFarland standards is provided and by sterile swap, each of samples is diffused for Mueller–Hinton agar culture media. Immediately, on the above culture media, some wells with the diameter 5mm is created and 90 microliter of various dilutions are inoculated inside the well and are kept for 24 hours in incubator at temperature 37^{0}C and finally, the bacteria non-growth zones are measured [19-20].

Determining MIC and MBC of *Cichorium* extract by Macrodilution on the experiment bacteria:

To determine MIC (Minimum Inhibition Concentration) and MBC (Minimum Bactericidal Concentration) of extracts, Macrodilution method is applied. To do this, at first the concentrations 418 to 0.8mg/mL are provided in Mueller–Hinton broth. Then, by adding 1mL microbial suspension equivalent to standard turbidity 0.5 McFarland standard, the final concentrations of extract is regulated as 209 to 0.4mg/mL. The tubes are incubated in incubator at temperature 37^{0}C for 24 hours. The turbidity of tubes is investigate daily and is sub cultured on Mueller–Hinton media. This is done three times and beside test tubes to determine MIC, positive control including bacteria is done in the media without extract to compare the turbidity of test tubes. The first tube of the low concentration of extract without turbidity of bacteria growth is computed as MIC concentration and the first tube of low concentrations of extract in which added bacteria is removed is computed as MBC concentration (19-20). The results of the experiments are analyzed by SPSS (version 18) software and the samples are plotted by Excel software. ANOVA one-way test (one-way variance analysis) and LSD are used for classification and comparison of means. Here the level $p<0.05$ is significant.

RESULTS

3-1 Reaction of bacteria to antibiotic

The results of the experiment showed that each bacteria isolate has similar behavior under the presence of antibiotic. However, there are differences among the isolates in terms of resistance or sensitivity to

antibiotic. In addition, some differences are observed in antibiotics inhibition mechanism in which penicillin and erythromycin antibiotics had the lowest antibiotic impact (Figure 1).

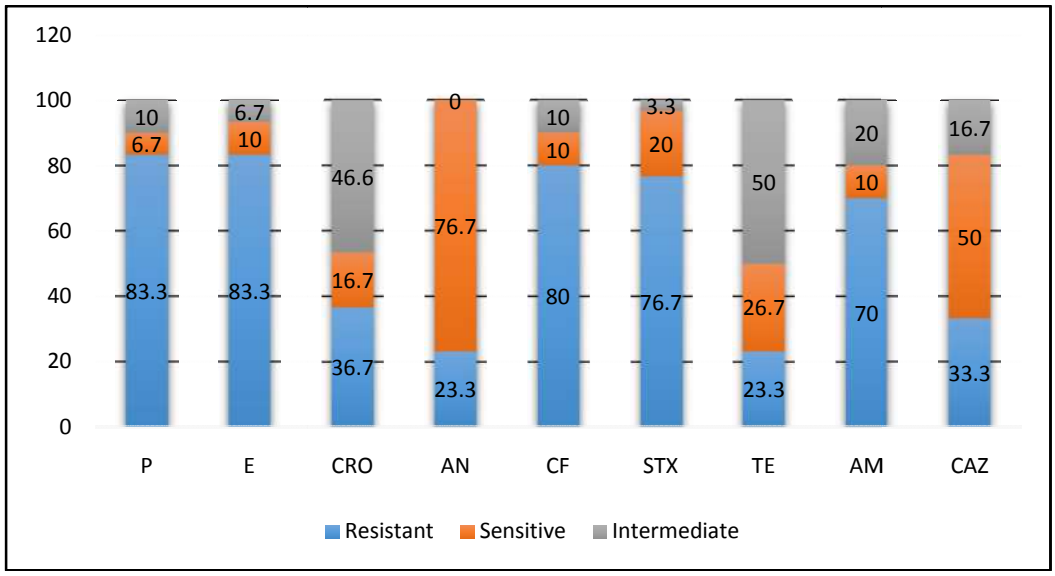

Figure 1: Sensitivity model of isolates of *Staphylococcus aureus* against antibiotic (%)

Anti-microbial features of extracts

Ethanol extract *Cichorium Intybus L.* had inhibiting impact on the studied bacteria. The results of anti-microbial effects of *Cichorium Intybus L.* are shown in Table 1. The results of MIC and MBC test are isolated by macrodilution isolate *Staphylococcus aureus* obtained from patients are shown in Table 2.

Table 1- The antibacterial impact of *Cichorium Intybus L.* on antibiotic- resistant *Staphylococcus aureus* (mean ±SD)

Plant	**Concentrations (microliter) (non-growth zone diameter in mm)**							
	1.6	3.2	6.5	13	26.1	52.2	104.5	209
Cichorium Intybus L.	0	0	0.84±8.16	0.4±10	0.4±12.5	0.61±14.7	0.84±16.16	0.82±19

Table 2- The results of MIC and MBC by macrodilution of *Cichorium Intybus L.* extract on antibiotic-resistant *Staphylococcus aureus*

Plant	**Concentrations of ethanol extract mg/ml**	
	MIC	MBC
(*Cichorium Intybus L.*)	6.5	13

DISCUSSION AND CONCLUSION

Based on the increase of bacteria resistance to different types of antibiotics, various efforts is made to achieve and apply the existing composition in the plants and their use in various diseases treatment. Since thousands of years, plants have played important role in keeping health and improving life quality of people. Herbal plants have useful features as anti-bacterial, anti-parasite, anti-fungus and anti-oxidant features [11].

According to a study conducted in India by Rani and Khullar regarding the investigation of the anti-bacterial effects of alcohol and aqueous extract of 54 herbal medicines including *Cichorium Intybus L*, all extracts had anti-bacterial effect [21]. Ghaderi et al.,[22] in a study showed that alcohol extract of *Cichorium Intybus* L has anti-bacterial impact on *Staphylococcus aureus* but this effect is less than that of *gentamicin* and Cephalexin.

Totally, the results of the investigations under laboratory conditions showed that the extracted extract of herbal medicine *Cichorium Intybus L.* has anti-microbial activity against antibiotic-resistant *Staphylococcus aureus* and it can be used as a good alternative to produce new herbal medicine after more investigations in lab animals with the least side effects against the above bacteria.

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