JOURNAL OF CURRENT RESEARCH IN SCIENCE (ISSN 2322-5009)
CODEN (USA): JCRSDJ
2014, Vol. 2, No. 6, pp: 641-645
Available at www.jcrs010.com

ORIGINAL ARTICLE

EVALUATION OF ANTIBACTERIAL ACTIVITY OF ETHANOL EXTRACT OF *LAVANDULA STOECHAS L.* PLANT ON ANTIBIOTIC-RESISTANT STRAINS OF *STAPHYLOCOCCUS AUREUS*

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ABSTRACT: Introduction and aim: Infections caused by antibiotic-resistant strains of *Staphylococcus aureus* are mainly hospital-based prevalence is increasing in many countries; Hence efforts taken to find new compounds as an alternative to antibiotics. Therefore, this study is done to evaluate the antimicrobial effects of ethanol extract of *lavandula stoechas L..* plant on clinical resistant *Staphylococcus aureus.*Material and methods: After collecting plants and validate its scientific name by botanists of Agricultural Organization and after drying in shade, extract of *lavandula stoechas 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 *lavandula stoechas L.* plant on *Staphylococcus aureus* were 6.5 and 13 mg/ml. The results of this study show that can be taken *lavandula stoechas L.* plant in herbals groups with antibacterial properties and after evaluating their effects in vivo condition and to identify the active ingredients, as an alternative to synthetic drugs that commonly used to *treat infections are used.*

***Key words*:** Antibacterial effects, Extract, *Staphylococcus aureus*

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 (Nimmo et al., 2006; Orrt and Land, 2006). Despite their antibiotic treatment, the infections of these bacteria are with considerable complications. Thus, preventing the infections of this bacterium and finding the causes of its diffusion in hospitals are necessary items. *Staphylococcus aureus* causes wide range of diseases including Endocarditis, Osteomyelitis, Pneumonia and Toxic shock syndrome. Based on the over mentioned items and increasing resistance of *Staphylococcus* to other antibiotics such as Erythromycin, Tetracycline and intermediate vancomycin/resistance or vancomycin-resistant (VISA or VRSA), it is caused considerable efforts are made to find new anti-microbial medicines (Orrt and Land, 2006).

Indeed, using herbal medicines is the oldest strategy of human being for diseases treatment and there was a close relationship between human being and plant in all human civilizations. However, most of the plant species are not identified yet and we have a long way to discover the new and valuable plant resources. Thus, we can consider plants as a source of potential chemical materials that only a part of it is used. These useful potential chemical materials can be applied not only as a medicine but also as a unique model as a starting point for building medicine analogs and also it is used as an interesting tool for better understanding of biological phenomena (Skaltsa et al., 1999; Skaltsa et al., 2003; Digrak etal., 2001).

One of the important therapy challenges is coping up with infectious diseases due to their high prevalence. After the identification of penicillin in decade 40s and its wide application in treatment, new antibiotics are presented for infection treatment every day. The result is developing the clinical application of natural and Synthetic antibiotics to treat clinical infections. Unduly application of these anti-microbial drugs leads to the increase of medicine resistance against different antibiotics in most of the bacteria (Weinstine, 2001). This is one of the reasons of

increasing application of plants as low-risk natural, available and cheap materials compared to synthetic antibiotics in treatment of bacterial infections. Also, these herbal medicines are highly common among people (Mosaddegh et al., 2002; WHO, 2002; Switzerland, 1998). All these reasons have increased the global studies and introduction of the anti-bacterial effects of various plants in recent years (Marjorie, 1999). *Lavandula stoechas L.* is a perennial with 0.5 height with opposite, narrow, long green leaves covered by white hair. Mint family species are prevalent all over the world and they are also found in Mediterranean regions, Africa and India (Morita, 2006). The researches of 1995 in Spain showed that aerial parts of *Lavandula* have high anti-microbial impact compared to other parts of plant (Ozcan, 2003). The leaf of this plant is including diterpenes , great amount of *cyclic alcohols*, *flavonoids* and organic acids as *Carnosic acid* and *Saponin* and *Saponin* has effective anti-bacterial impact. *Lavandula* has good smell and bitter taste and is applied in aromatherapy and is one of the complementary treatments to treat people suffering from different brain anomalies (Kim et al., 2007).

Hajhashemi et al., conducted different tests and proved the impact of water alcohol and polyphenol extracts and essential oil of this plant as an anti-inflammatory matter (Hajhashemi et al., 2003). Also, Danish researchers supported the inhibiting effect of *Lavandula* extract on acetylcholinestrase enzyme (Adsersen et al., 2006).

MATERIALS AND METHODS

2-1 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°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.

2-2 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 (Sokmen et al., 2004; Sattari et al., 2006).

2-3 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 standard 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°C and finally, the bacteria non-growth zones are measured (Igbal and Beg, 2001; Kim et al., 2007; Sattari et al., 2006).

2-4- Determining MIC and MBC of *lavandula* 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 ℃ 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 (Igbal and Beg, 2001; Kim et al., 2007; Sattari et al., 2006). 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

Anti-bacteria activity of some antibiotics is evaluated against the isolates obtained in laboratory conditions. 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 (Table 1).

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

	P	**E**	**CRO**	**AN**	**CF**	**STX**	**TE**	**AM**	**CAZ**
R	83.3	83.3	36.7	23.3	80	76.7	23.3	70	**33.3**
S	6.7	10	16.7	76.7	10	20	26.7	10	**50**
I	10	6.7	46.6	0	10	3.3	50	20	**16.7**

R: Resistant / S: Sensitive / I: Intermediate

3-2 Anti-microbial features of extracts

The variety of geographical and climate conditions in Iran caused that there are various source of plant species in Iran. Some plants have medicine features as anti-bacteria activities. The studies showed the following results regarding the anti-microbial effects of plant extract:

Ethanol extract of *lavandula stoechas L* had inhibiting impact on the studied bacteria. The results of anti-microbial effects of *lavandula stoechas L* are shown in Table 2. The results of MIC and MBC test are isolated by macrodilution isolate *Staphylococcus aureus* obtained from patients are shown in Table 3.

Table 2- The antibacterial impact of *lavandula stoechas L* on antibiotic- resistant *Staphylococcus aureus* (mean ±SD)

Plant	**Concentrations (microliter) (non-growth zone diameter in mm)**							
	Staphylococcus aureus							
	1.6	3.2	6.5	13	26.1	52.2	104.5	**209**
) *lavandula stoechas L* .(	0	0	0.84±10.16	0.4±12	0.4±14.5	0.61±16.7	0.84±18.16	**0.82±21**

Table 3- The results of MIC and MBC by macrodilution of *lavandula stoechas L.* extract on antibiotic- resistant *Staphylococcus aureus*

Plant	**Concentrations of ethanol extract mg/ml**	
	MIC	**MBC**
lavandula stoechas L .	6.5	**13**

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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 (Zargari, 1995). Gram positive bacteria are more sensitive than gram negative bacteria against *lavandula stoechas L* extract. This is due to the difference in the structure of gram positive and gram negative bacteria as gram positive bacteria has more mucopeptides in their cellular wall but gram negative bacteria only have one thin layer of mucopeptide. Thus, gram negative bacteria are more resistant (Ghalem and Mohamed, 2008; Tassou and Nychas, 1995).

Haghighati et al., (2006) found that *lavandula stoechas L* extract has antibacterial effect on , Actinobacillus actinomycetem, Candida albicans and Streptococcus mutans (Haghighati et al., 2006). Panis et al., evaluated the impact of oil extract of four plants of mint family on *Staphylococcus* aureus (Panizze et al., 1993).

Totally, the results of the investigations under laboratory conditions showed that the extracted extract of herbal medicine *lavandula stoechas 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.

Acknowledgment

My gratitude goes to My teacher, Dr. Parviz Malekzade, Mr Morteza Hanifezade and Ms. Rana Yusefzade who encouraged us in all stages of the study and helped us. Also, I am thankful of the chief of Razi hospital of Marand town and the authorities of microbiology lab of Azad University of Ahar and Marand to present lab facilities in this study.

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