An Evaluation of Antimicrobial Effects of *Zosima Absinthifolia* Extracts

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Abstract

For the purpose of this study, the antimicrobial effects of *Zosima absinthifolia* extract were tested on *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*). Dried seeds of *Zosima absinthifolia* plant (100 g) were powdered and soaked in a sufficient amount of water and the extraction process was done using a soxhlet apparatus. The obtained extract was then condensed in a rotary evaporator, transesterification process was applied and the resultant product was dehydrated completely.Using GC-GC/MS instruments, it was then analyzed. The extract contains essential ingredients including n-octanol, germacrene D, β-caryophyllene, α-pinene, limonene, octyl acetate, caryophyllene oxide and n-octanal. The extract was used to determine its antimicrobial effect. Culture medium was prepared and the test was performed by disk diffusion method. The preliminary results showed that the extract has antimicrobial effects on both bacteria. This suggests that the plant can be used in future as an effective substance in the treatment of infections caused by these bacteria.

Keywords: extract, GC/MS, *Escherichia coli*, *Staphylococcus aureus*, *Zosima absinthifolia*

Introduction

According to all-round progresses in the field of synthesis and application of synthetic medicines in treatment of various diseases, medicinal plants continue to be the primary and important source for global pharmaceutical industries (Collee et al., 1990; Jawetz and Adelbrg, 2001). With regard to therapeutic values, avoiding the side effects of synthetic drugs in modern medicines, as well as increasing request to herbal and natural products, great efforts have been made for presentation of them. This is while Iran is the world's second largest genetic resources of medicinal herbs (Igbal and Beg, 2001).World trade volume of medicinal plants is more than 43 billion dollars and Iran's contribution to this market is only about 60 million dollars, while this country has a privileged position in terms of various conditions of environment, geography and climate (Iran has 11 of the World's 13 climate zones), diversity of plant species richness, with a range of daily temperature that changes up to 50 ˚C and 300 days of sunshine per year and so on. Approximately, 500 million dollars of currency and 300 billion Tomans of Iran's public budget are yearly expensed to medicine supply while only 1 to 3% of medicines consumed in Iran are derived from medicinal plants. Development of processing of medicinal and aromatic plants requires sufficient knowledge of chemicals available in herbal essences and extracts (Jawetz and Adelbrg, 2001). With regard to the medicinal and nutritional values available in essence compounds,

informed use of resources which contain essence is also the other important objectives of such plants to prepare medicines and to understand the essence types in terms of chemical and antimicrobial structures (Collee et al., 1990).

Materials and Methods

For the purpose of this study, *Zosima absinthifolia* was prepared and the seeds were then collected. After separating the waste parts, it was dried in shade and then powdered with an electric mill. The product was soaked in an organic solvent as petroleum ether and n-hexane, and the extraction process was performed in a soxhlet apparatus. The solution was condensed in the rotary evaporator under reduced pressure and the transesterification process was conducted. The resulting mixture was dehydrated completely and analyzed using GC-GC/MSinstruments. The extract was then used to determine antimicrobial effects (Trujilano-Martin et al., 1999).

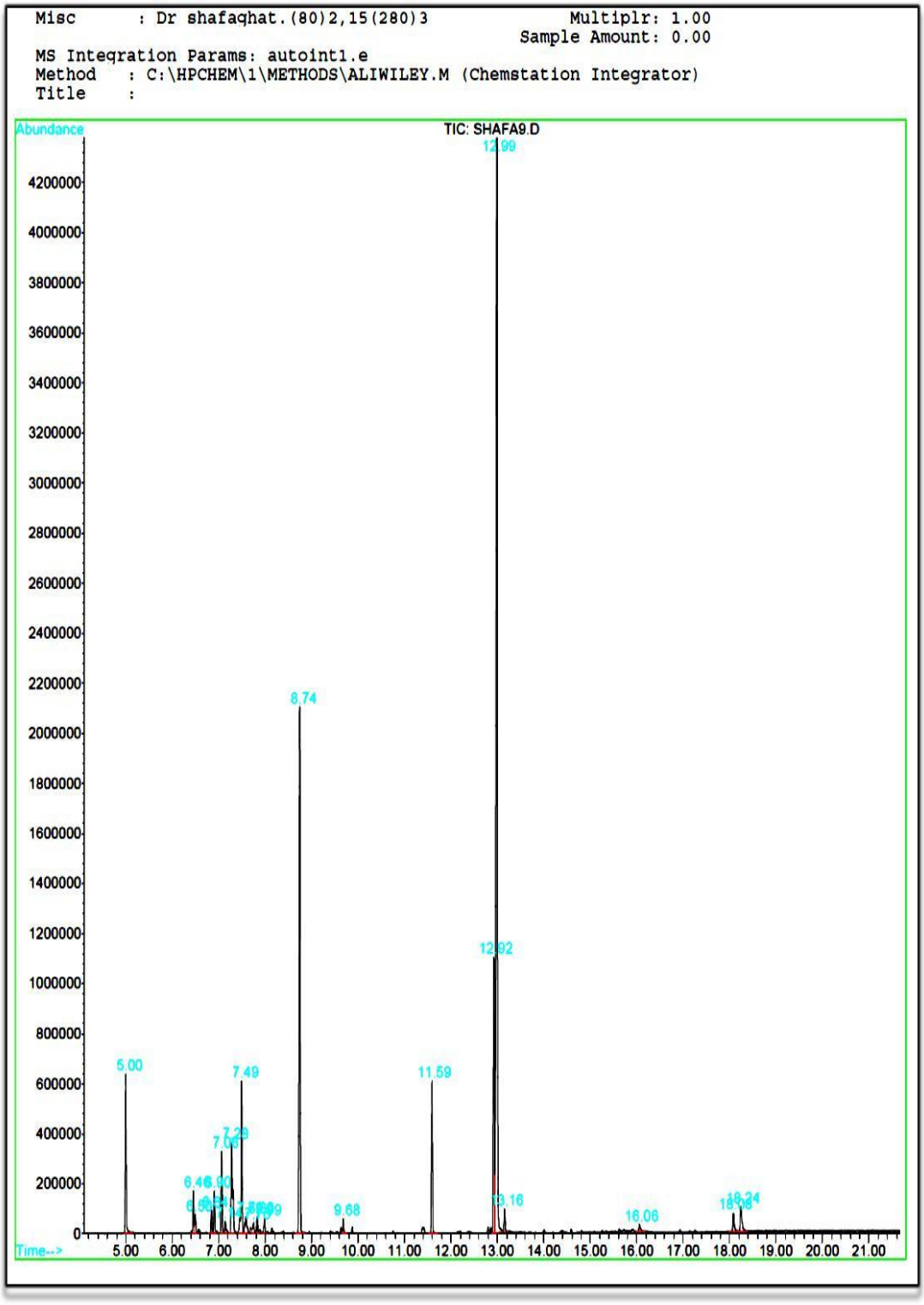
In this study, the antimicrobial effects of *Zosima absinthifolia* extracts on *Staphylococcus aureus* and *Escherichia coli* were evaluated in Mueller Hinton Broth and agar mediums andthe test was carried out three times. To evaluate the antimicrobial effects of hexane, methanol, and water extracts of *Zosima absinthifolia* plant on *S. aureus* and *E. coli*, Mueller Hinton agar and Eosin methylene blue mediums were prepared and they were then divided in plates with 4-5 mm thick and a well (approximately 5-mm in diameter) was drilled in each plate. *S. aureus* and *E. coli* bacteria were cultured by sterile swab on the culture mediums (Murray et al., 2005). Afterwards, methanol, hexane and water extracts were prepared at the ratios of 1:10,1:20, 1:40, 1:80, 1:160,1:320, 1:640, 1:1280, 1:2560, and 1:5120; and100 µL of the prepared solutions were poured into the wells. The plates containing the extracts and bacteria were incubated at 37 ˚C for 24 hours. Bacteria inhibition zones around each well on the plates were finally measured and the measurement was repeated three times (Mansouri Ghiasi et al., 2013; Trujilano-Martin et al., 1999).

Results

As the results revealed, the compounds of GC/mass from hexan extract include Geraniol, Neryl acetate, β-Caryophyllen, E-β-Farnesen, (-)-β Acoradiene,β-Sesquiphellandre, Aromadendrene, β-Bisabolen, C, Carotol, Juniper camphor, Hexadecanoic acid, methyl ester, 9,12-Octadeadienoic acid, methyl ester or ω-3, 9,12,15-Octadecenoic acid, methyleste, 6-Octadecanoic acid methyl ester, Octadecanoic acid methyl ester, Bis (2-ethylhexyl) phthalate, and Eicosane. Most of the compounds are as Carotol, Hexadecanoic acid, methyl ester, ω-3, 9,12-Octadeadienoic acid, methyl ester or 9,12,15-Octadecenoic acid, 6-Octadecanoic acid methyl ester. As the findings showed, the antimicrobial effects of methanol and water extracts from hexane extracts were better on *Staphylococcu saureus* and the antimicrobial effects of hexane extracts from methanol and water extracts were better on *Escherichia coli*. The results obtained in this study have been presented in the following Table, Graph, and Figures.

Table 1. *Zosimiaabsinthifolia* Compounds

Compound	Rt (min)	(%)
Geraniol	5.0	**4.7**
Neryl acetate	6.5	**0.6**
β-Caryophyllene	6.9	**1.3**
E-β-Farnesene	7.2	**4.6**
(-)-β-Acoradiene	7.5	**5.1**
β-Sesquiphellandren	7.6	**1.5**
Aromadendrene	7.7	**0.4**
β-Bisabolene	7.8	**0.5**
Carotol	8.7	**16.9**
Juniper camphor	9.7	**0.5**
Hexadecanoic acid, methyl ester	11.6	**4.5**
9,12-Octadecadienoic acid, methyl ester	12.7	**8.8**
9,12,15-Octadecatrienoic acid, methyl ester(linolenic acid) or ω- 3	12.8	**23.2**
6-Octadecenoic acid, methyl ester	13.0	**20.2**
Octadecanoic acid, methyl ester	13.2	**0.8**
Bis(2-ethylhexyl) phthalate	16.0	**0.4**
Eicosane	18.1	**0.8**
Total	--	**94.8%**



Graph 1. GC –Chromatoghraphy of *Zosimiaabsinthifolia*

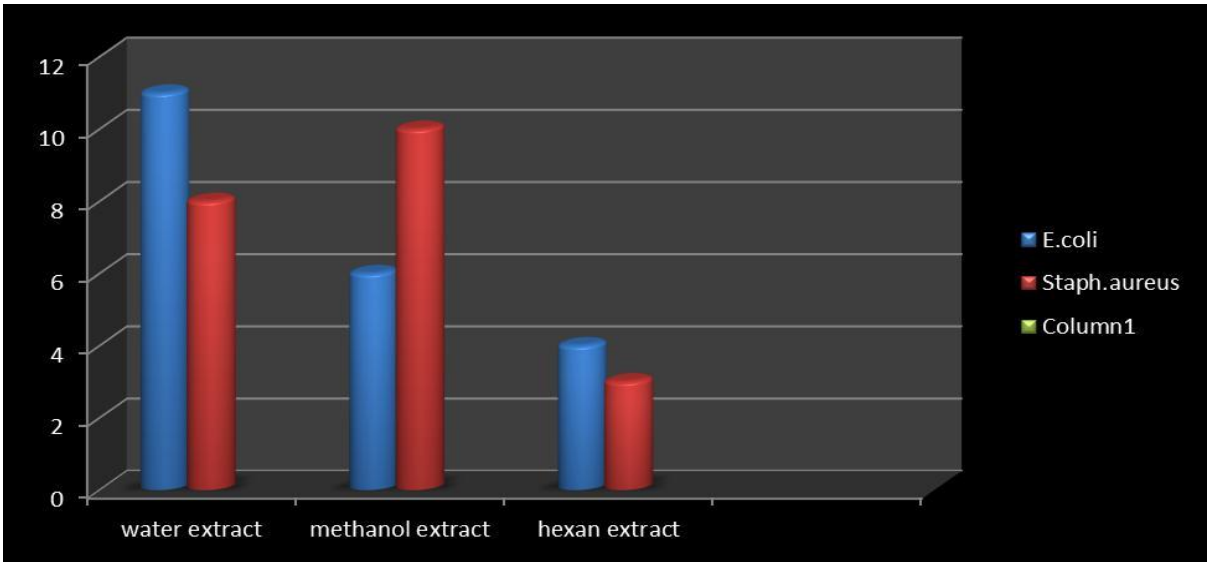


Figure 1.Inhibition of growth in extracts of Zosimia absinthifolia (Diffusion Method)

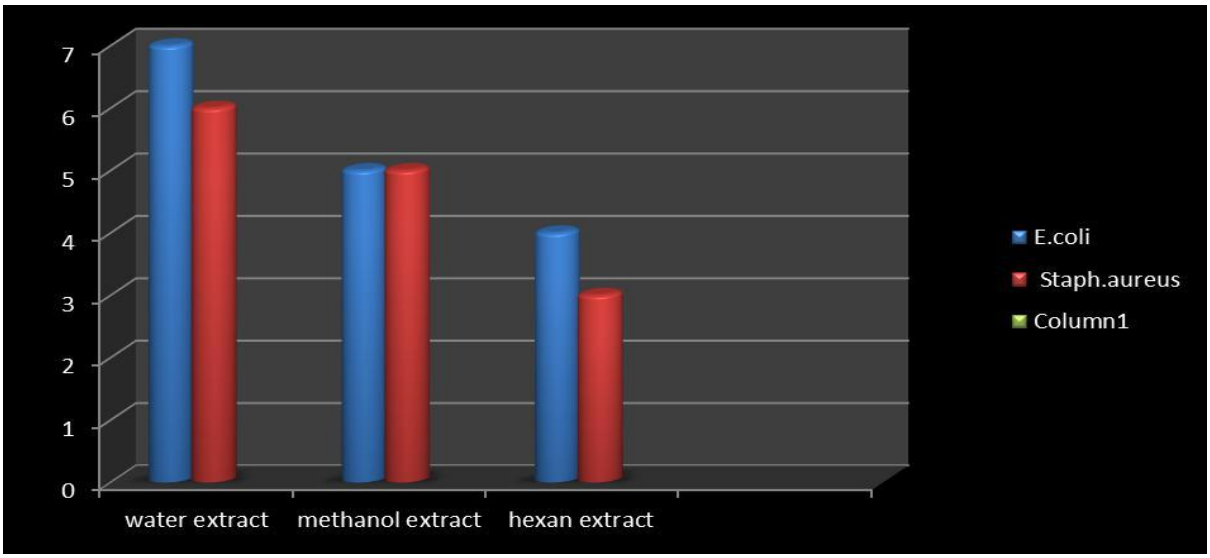


Figure 2. Inhibition of growth in extracts of *Zosimia absinthifolia* (Disk Method)

Discussion

Due to a broad dispersion of medicinal plants as well as climatic conditions, geographical location and suitable growth conditions for these plants, Iran is considered as one of the world's richest regions. Studies on medicinal plants, in terms of their antibacterial effects, have prepared a suitable condition so that their results can be applied to replace chemical drugs with medicines derived from plants or other natural sources to control and treat bacterial infections. This can reduce the consumption of synthetic medicines and the complications arising from them.

The aim of this study therefore was to introduce an effective antibacterial agent on some infectious bacteria. The idea that some plants are capable of healing goes back to many years ago. Some of these natural products contain substances commonly known as antimicrobial agents (Brantner and Grein, 1994; Collee et al., 1990). Ancient Egyptians were the world's first people who wonderfully used medicinal plants. China oldest medical book that contains description of more than one hundred plant species is attributed to one of Chinese emperors called Shino who lived about 2,800 years before Christ. Knowledge of medicinal herbs reached to urban communities from inside of monastery gardens in the Middle Ages. In fact, Western countries have started herbal therapy from the early medieval period (Nkri and Mirelman, 1999). The oldest Iranian scientists who had been pioneer in the field of herbal therapy were Muhammad ibn Zakariya al-Razi and ibn-Sina, known in the west as Avicenna. Reasons for extraction of effective herbal ingredients and purification and application of them in pharmaceutical formulations can be summarized as follows:

- Lack of long-term maintenance of plants
- Lack of rapid access to plant resources
- Lack of specific criteria for dosage and administration to patients(Paudel Chhetri et al., 2008)

Plants having antimicrobial effects inhibit bacterial growth with different mechanisms and even different from antibiotics. This reminds the needs for more comprehensive researches in the field of medicinal plants. This issue justifies increasing articles published in the field of antimicrobial effects of plants (Brantner and Grein, 1994). A lot of researches have been conducted in recent years in the field of antimicrobial effects of various plants and it has found that some of them have the same effects as chemical medicines or even far more effect than them. Due to potent pathogenic ability and increasing resistance against antimicrobial drugs, *Staphylococcus aureus* and *Escherichia coli* have become one of the major health problems in the world. Preventing the incidence of infections caused by the bacteria and stemming the centers of their release in hospitals is of great importance (Ahsam,1999; Breithaupt, 2003). Mass consumption of broad-spectrum antibiotics such as third-generation cephalosporins, macrolides, and fluoroquinolones are the factors that stimulate and produce antibiotic-resistant strains to *S. aureus*. The bacterium can cause a broad range of illnesses including endocarditis, osteomyelitis, pneumonia, toxic shock syndrome and abscess. *E. coli* as a commensal microorganism is a member of normal flora which causes infection in an opportunistic way. Infections caused by this bacterium can implicate almost all parts of the body through spread inside the organism in susceptible patients. *Escherichia coli* strains have specific virulence factors to create some diseases, such as urinary tract infections (UTIs) and gastroenteritis. To treat infections caused by *E. coli*, ciprofloxacin and imipenem antibiotics are prescribed. This bacterium, as mentioned earlier, has a wide range of virulence factors. Due to increased bacterial resistance to a variety of antibiotics, efforts to achieve a greater awareness of effective use of available compounds in plants and their applications in the treatment of various diseases have performed (Trujilano-Martin et al., 1999).

Concerning what has been explained before; the antimicrobial effects of hexane, methanol, and water extracts of *Zosima absinthifolia* plant on *S. aureus* and *E. coli* were therefore investigated in the present study. In addition to the above-mentioned drug resistance, per capita consumption of fish, which is an important source of omega-3, is now 5 kg in Iran. This statistic is negligible in comparison with global per capita fish

consumption. After feeding with milk, what is important for children is to be fed with foods containing omega-3 fatty acids in their diet which can be much effective preventing them from many diseases. The importance of this valuable substance has also been found in adults and some of its properties are now under consideration. Given the importance of omega-3 fatty acids, it is recognized that the substance plays a vital role in human health at all ages. In addition to fish, some plants also have rich sources of omega-3 but a handful of them (such as soya and linseed) have identified. However, access to fish due to its high cost and seasonality is not easily and repeatedly possible for all people. However, omega-3 substances can be economically affordable by discovering of new herbal sources. Also, due to the indiscriminate use of antibiotics and resistance of infectious agents, it seems essential that medicines and alternative substances should be introduced to eliminate the problem that is growing day by day (Stryer and Etal, 2007). Studies on *Zosima absinthifolia* plant has recently started in Iran. In a study, Shafaghat (2010) investigated the chemical compositions of volatile oil isolated from above-ground organs of the plant. He collected *Zosima absinthifolia*plants from Khalkhal area, Ardabil Province, Iran on summer 2005studyingtheessential oil derived from above-ground organs of the plant. The valuable oil was extracted by hydrodistillation method and it was analyzed and identified with GC and GC/MS instruments. The results obtained was reported as follows: out of 21 identified compounds which contained 96% of essential oil, octyl acetate (24.69%), beta-caryophyllene (22.24%) and z-β-ocimene (8.9%) compounds were the major components forming the essential oil. The overall findings showed that terpenes were a little more than monoterpenes (Orhanand Orhan, 2006).

A research was also performed by Amiri (2007) evaluating the quantitative and qualitative changes of essential oil in *Zosima absinthifolia* in different stages of growth. He prepared the above-ground organs of the plant from Aleshtar Mountains located in the north of Lorestan Province, Iran. After drying in the shade, hydrodistillation method was used to extract the essence from it. Efficiencies of the essence in pre-flowering, flowering and fruiting periods were 0.42%, 0.65% and 0.8% (w/w), respectively. The extracted essence from the plant was analyzed by GC and GC-MS instruments. Totally, 47, 38 and 37 compounds were identified in the essential oil obtained from the plant in pre-flowering, flowering and fruiting periods, respectively. Some major components available in the essential oil extracted from *Zosima absinthifolia* during different stages of growth included n-octanol, germacrene D, β-caryophyllene, α-pinene, limonene, octyl acetate, caryophyllene oxide and n-octanal (Mohsen nezhad et al., 2009; Nkri and Mirelman, 1999; Perez et al., 1990).

Conclusion

The results of the study showed that the antibacterial effect of the essential oil was considerable on bacteria. This effect suggests that the plant can be used in the future as one of effective medicines in the treatment of infections caused by bacteria.

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