INTERNATIONAL JOURNAL OF CURRENT MEDICAL AND PHARMACEUTICAL RESEARCH

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RESEARCH ARTICLE

# EVALUATION OF THE CHEMICAL COMPOSITION AND ANTIBACTERIAL EFFECTS OF THE VIOLA ODORATA LINN OIL'S

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**ARTICLE INFO**

***Article History:***

Received 19th September, 2016
Received in revised form 8th October, 2016
Accepted 4th November, 2016
Published online 28th December, 2016

***Key words:***

*Viola odorata Linn*, oil, Chemical composition, Antibacterial effects, Macro-dilution method, Agar disk diffusion method, Agar well diffusion method.

**ABSTRACT**

Increasing microbial resistance to chemical antibiotics and their probabilistic side effects cause popularity of medicinal plants, so there is an instantaneous and steady need for novel antimicrobial compounds from plants. As we know, there is no documented proof on antibacterial effects of *Viola odorataLinn* (VOL) oil in west of Iran. The aim of the study was to evaluate the chemical composition and antibacterial activities of the oil from VOL against *Escherchia coli O157:H7* and *Staphylococcus aureus*. Gaschromatography mass spectrometry was run to specify their chemical composition. As a screen test to detect antibacterial properties of the oil, agar disk and agar well diffusion methods were employed. Macro broth tube test was performed to determinate MIC. Presence of Pentane 2, 3, 4- Trimethyl, N- Hexadecanoic acid, 10- Undecyn-1-ol and Pentadecanoic acid were discovered in composition of the obtained oil of VOL Also, The MIC and MBC values were 0.031 g/ml for the oil in both of tested bacteria. Thus, the research represents the antibacterial effects of the medical herb on *E. coli* and *S. aureus*. We believe that the article provide support to the antibacterial properties of the oil. The results indicate the fact that the oil of the VOL can be useful as medicinal or preservatives composition.



## INTRODUCTION

Antibiotics provide the primary basis for the treatment of microbial (bacterial and fungal) infections. Since the detection of these antibiotics and their use as chemotherapeutic agents, there was a belief in the medical fraternity that this would cause to the presumptive eradication of infectious diseases. But overuse of antibiotics has become the main factor for the emergence and dissemination of multi-drug resistant strains of different groups of microorganisms[1]. The spread of drug resistant pathogens is one of the most serious threats to successful therapy of microbial diseases. Down the ages plants have evoked interest as sources of innate products. They have been screened for their potential uses as alternative remedies for the treatment of several infectious diseases[2-7]. Herbs and spices are invaluable resources useful in daily life as food additives, flavors, fragrances, pharmaceuticals, colors or directly in medicine. These plants contain medicinal properties which make them potent to cure or prevent diseases[8]. According to World Health Organization (WHO), more than 80% of world's population relies on traditional medicine for their healthcare needs. The uses of herbs in treatment of animal and human diseases have long been established. Most plant extracts have been shown to possess antimicrobial agents active against microorganisms in vitro. Some medicinal plants used in traditional Iranian medicine are effective in treating various ailments caused by bacterial and oxidative stress[9]. Studies have shown that the phenolic compounds play an important role in the antimicrobial properties of plants. These compounds spoil microorganisms through destroying the cell walls and proteins, interfering in the work of membrane enzymes and affecting DNA and RNA replication. Aromatic oils are used in many industries including food preservation, pharmacy and medicine[10-12]. They are expected to form new sources of antimicrobial drugs especially against bacteria[13]. The antibacterial effectiveness of aromatic oils has been

divided into a good, medium or bad[14, 15]. These oils can also produce some defense products (some secondary metabolites) against several natural enemies[16, 17].

VOL belongs to family *Violaceae*. It is commonly known as Sweet Violet, English Violet, Common Violet or Garden Violet and Gulbanafsa in Hindi. VOL is a native of Mediterranean countries and Asia Minor. From old ages, it has been grown in gardens and now it has spread to most of Europe. The plant is a perennial herb, spreading with stolons (above-ground shoots). Leaves are orbicular-reniform to broadly ovate. Flowers have dark violet or white color. The sweet, unmistakable scent of this flower has proved popular throughout the generations and has consequently been used in production of many cosmetic fragrances and perfumes[18]. It is used either alone or in mixture with other herbs for catarrhal and pulmonary troubles and for calculous affections[19]. VOL possess various important biological activities including uterotonic, insecticidal, anti-HIV, antimicrobial, antineurotensive, cytotoxic and haemolytic activities[20]. The study about antimicrobial activities of aqueous extract has been reported against certain opportunistic/secondary invaders and pathogenic bacteria of respiratory tract region[21-23]. Hence, exploring the scientific justifications behind its traditional use could support to find the probability to develop further new biotechnological applications.

The aim of this study was to screen the in vitro antibacterial activities of the plant oil against some bacteria including *E. coli* and *S. aureus.*

## MATERIALS AND METHODS

### *Plant sample collection*

In this empirical-experimental study, medicine plant collected from Kermanshah. The sample was cleaned from any strange, plants, dust, or any other contaminants.

### *Oil extraction of VOL with Hot Liquid Fats*

At one time widely used for extracting the oil from flowers other than tuberose and jasmine, extraction with hot liquid fats is now seldom employed commercially. It is cumbersome and the products obtained do not represent the true perfume of the flowers. VOL is immersed in a specially prepared fat. The mixture is heated to about 80°C for about half an hour and then allowed to cool for an hour. It is finally reheated and then strained or filtered to remove VOL. The proportion by weight of flowers to fat is about 1 to 4. New charges of VOL are introduced until the total weight of VOL immersed and macerated is about twice the weight of the fat solvent used. The perfume-saturated fat is sold and made oil from it in high temperature.

### *Gas chromatography mass spectrometry (GC/MS)*

To analyze oil of VOL by GC-MS, fused silica DB-5 column with 0.25 µm thickness film was used. The oven temperature was kept at 500°C for 5 minutes and then programmed from 50-2800°C for 40 minutes. Helium flow rate was maintained at 2 ml/min, with the split ratio of 1:3. Sample injection of 1µl and ionization voltage of MS-analysis was run by EI technique at 70ev. The volatile oil constituents were identified by matching their MS and retention index data with those of the standards spectra and by matching their fragmentation pattern in Mass Spectra[24]. NIST standard reference database (AMDIA version 2.70) was used to interpret the mass spectral data.

### *Source of microorganisms*

Two bacterial species namely *E. coli O157:H7* (ATCC No. 25922) and *S. aureus* (ATCC No. 25923) were procured from Iranian Research Organization for Science and Technology as lyophilized. Each bacterial strain was activated on Tryptic Soy broth, constant at 37°C for 18 h. Then 60 µl of the broth was transferred to Nutrient agar and incubated at 37°C for another 24 h; cell concentration was then adjusted to obtain final concentration of $10^8$ cfu/ml using Muller Hinton broth.

### *Culture media*

Mueller-Hinton Agar (Müller-Hinton agar is a microbiological growth medium that is commonly used for antibiotic susceptibility testing) was prepared according to the manufacturer's instruction, autoclaved and dispensed at 20 ml per plate in 12 x 12cm Petri dishes. Set plates were incubated overnight to ensure sterility before use.

### *Evaluation of antimicrobial activities*

Agar well and agar disk diffusion methods were used as screen tests to evaluate antibacterial properties of the oil from VOL based on standard protocol. The solution of the plant were yielded in 1g/ml from which six fold serial dilutions (v/v) were prepared. 60 µl of each dilution was poured on each disk and well in order. After a period of 24 hours incubation, the diameters of growth inhibition zones around the disks and wells were measured. DMSO was used as negative control whereas kanamycin and cephalexin were used as positive controls in case of *E. coli* and *S. aureus,* respectively. Minimum inhibitory concentration (MIC) means the lowest concentration of the probable antimicrobial agent which prevents growing of bacteria (regardless of killing the bacteria or stopping the growth of them). The lowest dilution which no gross microbial growth has been seen indicates MIC. Minimum bactericidal concentration (MBC) means the lowest concentration of the agent which causes death to test bacteria. The last can be revealed by pouring 60 µl of MIC tube and six dilutions before contents on agar plate. In this case, after incubation period, the lowest concentration which makes no growth indicates MBC. For determination of MIC value, macro broth dilution method was applied. Interpretation of the results was done due to national accepted letter[25].

### *Statistical Analysis*

Antibacterial effects were determined by One way variance analysis (ANOVA), using the SPSS 18 software package. Data were considered statistically significant at $p \leq 0.01$.

## RESULTS

### *Chemical composition*

GC-MS analysis in VOL revealed the presence of Pentane 2, 3,4- Trimethyl, N- Hexadecanoic acid, 10- Undecyn-1-l and Pentadecanoic acid.

### *Agar disk diffusion test*

About VOL, the most sensitive bacterium was *S. aureus* by developing the halo around which in 11 mm in diameter in dilution 0.125 g/ml. There was no inhibition zone in *E. coli* due to dilution 0.007 g/ml whereas *S. aureus* showed sensitivity in this amount. No inhibition zone was observed due to DMSO. Growth inhibition zones due to different dilutions are listed in figure1.

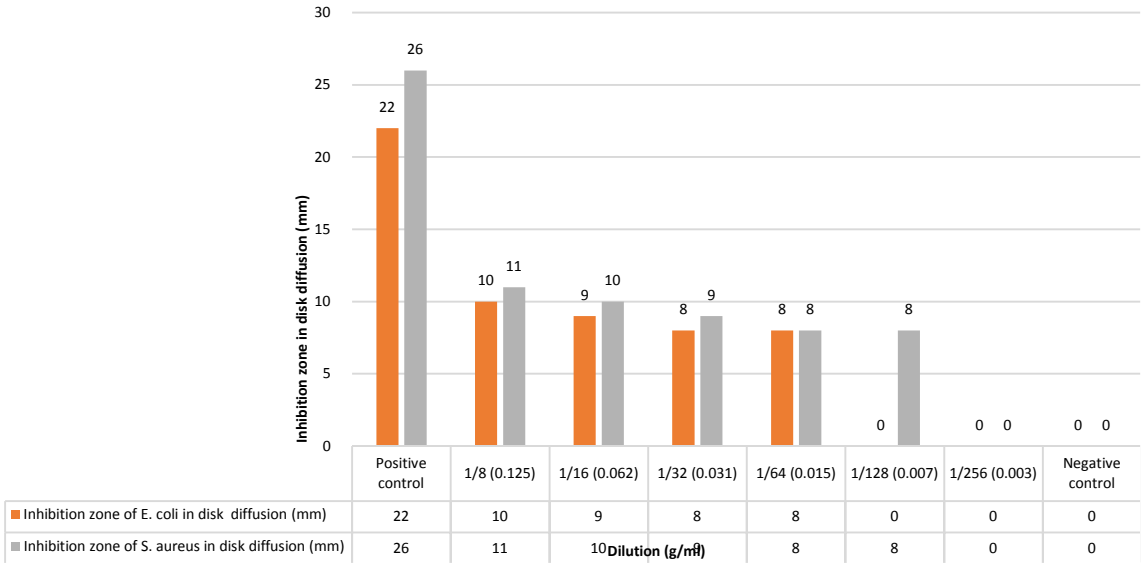


**Figure1** The diameters of growth inhibition zones in agar disk diffusion test in different dilutions of the oil from VOL.

### *Agar well diffusion test*

In regard to oil of VOL, both of tested bacteria showed sensitivity in dilution 0.031, 0.062, and 0.125g/ml concentrations. The zone was no growth inhibition in 0.003 and 0.007g/ml concentrations. The data are discoverable in figure2.

## DISCUSSION

The development of resistance in bacteria is one of the mechanisms of natural adaptation to the presence of an antimicrobial agent that inhibits susceptible organisms and selects the resistant ones.

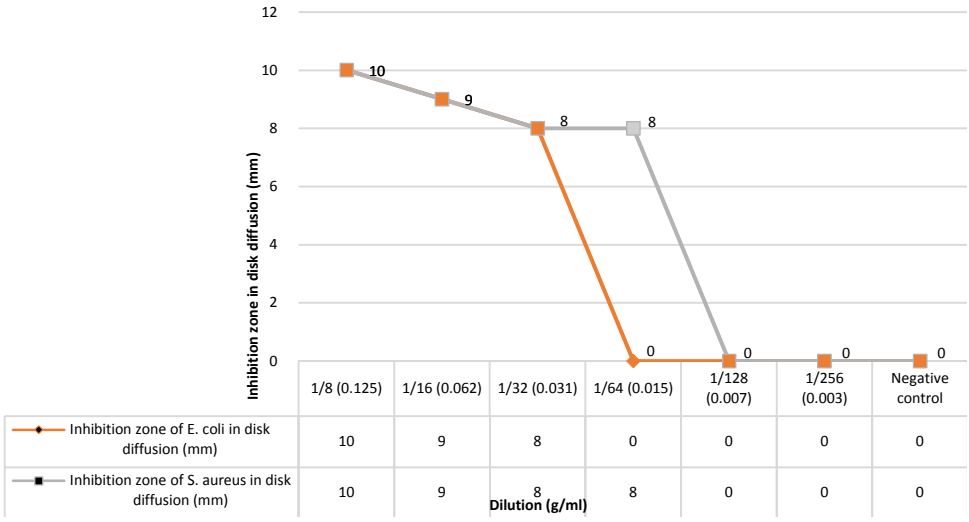


**Figure2** The diameters of growth inhibition zones in agar well diffusion test in different dilutions of the oil from VOL.

### *MIC and MBC ascertaining*

In oil of VOL, MIC and MBC were 0.031 g/ml for both of tested bacteria (figure3).

**Figure 3** MIC and MBC of the oil of VOL.

| | The oil of VOL. | |
|---|---|---|
| **Microorganism** | ***E. Coli*** | ***S. aureus*** |
| **MIC** | 1/32 (0.031) | 1/32 (0.031) |
| **MBC** | 1/32 (0.031) | 1/32 (0.031) |

As the Figures shows, the oil of VOL have prevented the growth of *E. coli* and *S. aureus*. Also in many of samples, by increasing the concentrations of the oil of VOL the inhibition zone increased. The results determined that in tested bacteria, there was a significant difference in terms of sensitivity to the oil. In other words, the most sensitivity was observed in *S. aureus*.

The problem of antibiotic resistance, which has limited the use of cheap and old antibiotics, has necessitated the need for a continued search for new antimicrobial compounds. Plants as a source of medicinal compounds have continued to play a dominant role in the maintenance of human health since ancient times. According to the World Health Organization plant extracts or their active constituents are used as folk medicine in traditional therapies of 80% of the world's population. Over 50% of all modern clinical drugs are of natural product origin[26]. Medicinal plants may have the ability to treat bacterial resistance to many types of antibiotics[27-31]. The search for such compounds which can be combined with antibiotics in the treatment of drug resistant infections may be an alternative to overcoming the problem of resistance in bacteria. Oils of medicinal plants stand out as veritable sources of potential resistance modifying agents. Oils are rich in a wide variety of secondary metabolites, such as tannins,

terpenoids, alkaloids, and flavonoids, which have been found *in vitro* to have antimicrobial properties[32, 33].

VOL is a species of the genus *Viola*, native to Europe and Asia, but has also been introduced to North America and Australia[18]. In herbal medicine, VOL has been used for a variety of respiratory ailments, insomnia and skin disorders[20]. Concerning the method of oil and preventing from using high temperature to decrease the rate of destruction of effective herbal compound. GC-MS analysis in oil of VOL revealed the presence of Pentane 2, 3, 4-Trimethyl (41.85%), N-Hexadecanoic acid (29.35%), 10-Undecyn-1-ol (16.79%) and Pentadecanoic acid (9.14%). Pentane 2, 3, 4-Trimethyl is a branched alkane. It is one of the isomers of octane. The results of GC/MS indicated that oil of VOL was rich of Pentane 2, 3, 4-Trimethyl, which may be correlated with its antibacterial activities. But, in other studies showed N- Hexadecanoic acid, pentadecanoic acid and 10-Undecyn-1-ol have antimicrobial activities[34, 35].

The results demonstrated that VOL with concentration about 0.031 g/ml has prevented *E. coli* and *S. aureus*. Thus, the research suggests the antibacterial effects of the medical herb on Gram-negative and Gram-positive pathogenic bacteria. Khatibi *et al*. documented the considerable antimicrobial activities of aqueous extract of VOL (aerial parts) against *E. coli* and *S. aureus*[22].Khan *et al*. reported that aqueous extract of VOL(flowers) showed strong antibacterial action against *E. coli* and *S. aureus*[21]. Ramezani *et al*.[23] and Pranting *et al*.[36] assessed the effects of cold and warm environmental temperature on antibacterial activities of aqueous extracts of different parts of VOL against three bacteria e.g. *S. aureus*, *E. coli* and *P. aeruginosa* and concluded its maximum effects on *S. aureus* and minimum effects on *P. aeruginosa*.

VOL is an aromatic medicinal plant with antibacterial activities toward *E. coli O157:H7 (ATCC No. 25922)* and *S. aureus (ATCC No. 25923)*. The growth of both of bacteria were inhibited by the oil tested, these results indicate that oil of VOL has its own chemical composition, which may be correlated with its antibacterial activities. It can be used as antibacterial supplement in the developing countries towards the development of new therapeutic agent. Additional *in vivo* studies and clinical trials would be needed to justify. Also, further evaluation is necessary on potential of it as an antibacterial agent in topical or oral applications.

**Acknowledgment**

We, the authors wish to thank Medical Sciences University of Kermanshah, Iran for the financial support of this work.

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