Antimicrobial Effect of Essential Oil of *Ziziphora tenuior* on Water by Heterotrophic Plate Counts Method in Kerman (Southeast of Iran)

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Abstract—The increasing demands of consumers for using less chemical preservatives has led to increasing number of researches to produce natural compounds with antimicrobial properties. Fresh aerial parts of wild *Ziziphora tenuior* were collected in Eastern Iran. *Z.tenuior* samples were collected after initial preparation isolated from the aboveground plant parts and plant samples preparation by water distillation using Clevenger apparatus after determining the optimal conditions for making essential oil was obtained. Essential oil with an efficiency of 1/1 percent (g/g) was obtained and by GC-MS to determine the composition of the essential oils was analyzed and indicates 21 compounds. Key combinations were as: pulegone (82.6 %) and limonene (6.8%) which was extracted. the studied essential oil would exhibit good antimicrobial properties, this essential oil in 0.08 ml concentration could eliminate microorganisms in water. but this was not evaluated and further investigation should be carried out for use of this essential oil in various industries.

Index Terms—*Ziziphora tenuior*, antimicrobial, essential oil, GC-MS, heterotrophic plate counts method.

I. INTRODUCTION

The increasing demands of consumers for using less chemical preservatives has led to increasing number of researches to produce natural compounds with antimicrobial properties [1]. Using essential oils of plants, known as natural compounds, is expanding since these compounds have significant antimicrobial effects on a wide range of microorganisms [2]. Lamiaceae family of plants has been used as spice in foods and as medicine for digestion and combatting viral diseases [3]. This tribe includes 46 genera and 410 species and sub species in Iran. *Ziziphora tenuira* belongs to *lamiaceae* tribe, *lamiales* order and *asteridae* subclass [4].

This plant is an herbaceous, annual, 5 to 15 cm high with a short stem. Its leaves are narrow and pointed with short internodes. Its flowers are small, pale purple or violet. Geographical distribution is in Iran, Turkey, Russia,

Turkmenistan, Afghanistan, Pakistan, Caucasus and Siberia [5]. A substance, which is mainly seen in the essential oils from *lamiaceae* family including *Z.tenuira*, is pulgone. Pulgone owns antibacterial and antifungal properties [6]. The substance can also prevent the growth of *candida albicans* and *salmonella typhimurium*. Meanwhile, their effect on *candida albicans* is twice Nastatine [7].

Due to the existence of compounds such as pulgone, the essential oil compounds of *Ziziphora* species have been repeatedly studied in varying vegetative and weather conditions around the world. The results of the analysis of gas chromatography in a research revealed the existence of 22 different compounds in the essential oil of this herb. 5 of these compounds formed more than 73% of the essential oil and pulgone was the most [8]. Gozed *et al.* examined the antibacterial activity of *Z.tenuior* subsp, native to Turkey. The study by Gozed was conducted in laboratory conditions and on 8 species of bacteria. It showed that the essential oil of this species considerably prevented the growth of microorganisms [9]. Javidnia et al studied the composition and antimicrobial activities of the essential oil of a population of *Z.tenuior* in Iran. The essential oil compounds of these species were studied using GC and GC-MS and the results demonstrated that pulgone was the main compound in the essential oil of the species [10].

Plants and their essential oils are potentially useful sources of antimicrobial compounds. Numerous studies have been published on the antimicrobial activities of plant compounds against many different types of microbes [11]-[14]. Also essential oils are known to possess antimicrobial activity, which has been evaluated mainly in liquid medium. Systematic evaluation of the vapour activity was first reported by Maruzzella *et al.* and Kienholz in 1959, using the inverted Petri dish technique [15]-[17].

Regarding the importance of *Z.tenuior* species in terms of medical, health and food purposes, the presented study was carried out with the aim of investigating the effect of warm and dry climate (weather conditions of Kahnooj, Iran) on the quality and the essential oil compounds of *Z.tenuior* and study and examine antimicrobial effects of essential oils of aerial parts of *Z.tenuior* by HPC method.

II. MATERIALS AND METHODS

In this study, after collecting and essential oils of plants, the constituents of the essential oil were determined and antimicrobials effects was studied by Heterotrophic Plate Counts method.

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A. Plant Material

Fresh aerial parts of wild *Ziziphora tenuior* were collected in Southeast of Iran (Coordinates: 27°56'N57°52'E) in April 2014. Identification of the species was confirmed in Islamic Azad University, Kahnooj Branch. Samples were ground to a suitable size and stored in plastic container before analysis. The essential oils were extracted by hydro distillation by Clevenger-type apparatus of dried plant material for 3h (40 g of sample in 350mL of distilled water). The oil was separated from water by using n-hexane and then oils were dried by anhydrous sodium sulfate and stored in sealed glass vials at 4-5 °C prior to analysis.

B. Gas Chromatography-Mass Spectrometry

Analysis of the essentials oils was performed using a Shimadzu GC-17A equipped with a Shimadzu GCMSQP 5050A mass selective detector and a HP-5 MS capillary column (30m×0.25mm, film thickness 0.25 µm). For GC/MS detection, an electron ionization system with ionization energy of 70 eV was used. Helium at a flow rate of 0.8 mL/min was used as carrier gas. Injector and MS transfer line temperatures were set at 250 and 270 °C, respectively. Oven program temperatures were the same as for the GC analysis. Diluted samples (1/10 acetone, v/v) of 0.2 µL were injected automatically in the split mode. The components were identified by comparing their relative retention times and mass spectra with those of standards (for the main components), Wiley library data of the GC/MS system, Kovats Index and literature data. All the tests were performed in triplicate.

C. Antimicrobial effect by Heterotrophic Plate Counts (HPC) Method

The antimicrobial activity was carried out by using the HPC method. For this purpose, the field cultivation t R₂A and nutrient agar to be sterilized in autoclave in 60 °C, after 4 hours cold it to the 20 °C. Then 10 ml of sample of water with 0.02, 0.04, 0.06, 0.08, 0.1, and 0.2 ml of essential oil increase to the field cultivation in pottery dishes. Rested sample in the autoclave in 35 ± 0.5 °C for 48 ± 3 hours. We counted the colony of microorganisms under the microscope and identified minimal inhibition concentration for essential oil of *Ziziphora tenuior* in water for microorganisms [18], [19].

III. RESULTS AND DISCUSSION

Ziziphora tenuior samples were collected after initial preparation isolated from the aboveground plant parts and plant samples preparation by water distillation using Clevenger apparatus after determining the optimal conditions for making essential oil was obtained. Essential oil with an efficiency of 1/1 percent (g/g) was obtained and by GC-MS to determine the composition of the essential oils was analyzed. GC chromatogram of the essential oil of *Z. tenuior* showed in Figure I and the analysis results of the essential oil of *Z. tenuior* can be seen in Table I.

Results from the plant oils, indicates 21 effective combinations of *Z. tenuior* essential oils that have a considerable amount of the phenolic compounds that this indicates that there is anti-microbial or anti-fungal nature of

the essential oils of *Z. tenuior*.

Fig. 1. GC Chromatogram of the Essential Oil of *Ziziphora tenuior*.

Major compounds in the oils of this plant include: Pulegone with 69.9% and Limonene with 4.8%.

TABLE I: CHEMICAL COMPOSITION OF HYDRODISTILLATION ESSENTIAL OIL OF *ZIZIPHORA TENUIOR*

No.	Compounds	RI	%
1	Tricyclene	925	0.7
2	α -thujene	930	0.5
3	α -pinene	937	3.4
4	Sabinene	968	1.5
5	β -pinene	977	1.8
6	Myrcene	993	2.2
7	Limonene	1021	4.8
8	Isopulegone	1066	1.2
9	Terpinolene	1081	3.1
10	Pulegone	1213	69.9
11	2-cyclohexen	1248	4.1
12	α -copaene	1334	0.2
13	β -bourbonene	1388	0.3
14	Benzenepropanoic acid	1392	1.2
15	trans-caryophyllene	1398	0.1
16	α -humulene	1417	0.1
17	β -cubebene	1431	1.4
18	Germacrene D	1492	1.8
19	γ -cadinene	1516	0.9
20	Caryophyllene oxide	1578	0.5
21	Hexadecanoic acid	1682	0.3

Pulegone is a naturally occurring organic compound obtained from the essential oils of a variety of plants such as *Nepeta cataria*, *Lamiaceae*, and *pennyroyal* [20], [21]. It is classified as a monoterpene. Pulegone is a clear colorless oily liquid and has a pleasant odor similar to pennyroyal, peppermint and camphor. It is used in flavoring agents, in perfumery, and in aromatherapy. It was reported that the chemical is toxic to rats if a large quantity is consumed [22]. Pulegone is also an insecticide the most powerful of three insecticides naturally occurring in many mint species [23].

Limonene is a colourless liquid hydrocarbon classified as a cyclic terpene. The more common d-isomer possesses a strong smell of oranges [24]. It is used in chemical synthesis as a precursor to carvone and as a renewables-based solvent in cleaning products. Limonene is a relatively stable terpene and can be distilled without decomposition, although at elevated temperatures it cracks to form isoprene [25]. It oxidizes easily in moist air to produce carveol, carvone, and limonene oxide [26]. With sulfur, it undergoes dehydrogenation to p-cymene [27]. Limonene is common in cosmetic products. As the main odor constituent of d-limonene is used in food manufacturing and some medicines, e.g. as a flavoring to mask the bitter taste of alkaloids, and as a fragrance in perfumery, aftershave lotions, bath products and other such products that include fragrance; it is also used as botanical insecticide, the d enantiomer is most active as an insecticide. It is added to cleaning products such as hand cleansers to give a lemon-orange fragrance and because of its ability to dissolve oils. In contrast, l-limonene has a piney, turpentine-like odor. In natural and alternative medicine, d-limonene is marketed to relieve gastro esophageal reflux disease and heartburn [28].

In other hand these components have been reported to have antimicrobial effects on bacteria and fungi and any microorganisms in water.

Fig. 2. Results of antimicrobial effect of essential oil of *Ziziphora tenuior* by heterotrophic plate counts method.

According to (Fig. 2) this essential oil in 0.08 ml concentration could eliminate microorganism in water.

IV. CONCLUSIONS

The chemical composition of the essential oil of *Ziziphora tenuior* growing southeast of Iran was investigated. The data obtained in this study showed that the essential oil of aerial

part of *Z. tenuior* has variability of hydrocarbons and can be a good source of them.

Z. tenuior is one of herbs which several studies in various fields have been done on it, especially in Iran. One such study was conducted by P. Babakhanlou et al. that the phytochemical studies on the one species of *Z. tenuior* around Tehran that used organs were aerial parts of the plant and using the water and steam distillation method of Kasier and Lang was done essential oils making that the extracted essential oils with yield about 80% relative to the dry weight of plant and key combinations were as: pulegone (82.6 %), limonene (6.8%), cineol (1.9 %) which was extracted [29]. In same research Karimi et al. has a study on *Z. tenuior* and GC-MS analysis of hydro distilled essential oil derived showed twenty five known compounds in. Pulegone, iso menthone, limonene, and 8-hydroxy- δ -4(5)-p-menthen-3-one were major compounds. In aromatic water of *Z. tenuior* showed hypo cholesterol emic and overt hypo tri glyceride emic effects [30].

Compounds identified in essential oils of the aerial parts of *Z. tenuior* are close to the research conducted by Babakhanlou et al. However, there are significant differences in the amount of compounds that can be caused by climatic differences, environmental factors and harvest time.

Due to the extent of the inhibitory effect of the oil on micro-organisms being attributed to the presence of aromatic nuclei containing a polar functional group, it can be awaited that the studied essential oil would exhibit good antimicrobial properties, this study showed that the antimicrobial effect of essential oil of aerial parts of *Z. tenuior* shows high level of phenolic compounds present in the oil is fully compatible and these compounds and their effects on micro-organisms in tap water can be used in the future to clean municipal water but for use of this essential oil in various industries.

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