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Original Article

Chemical Composition, Antimicrobial and Antioxidant Potential of Essential Oil of *Ziziphus spina-christi* var. *aucheri* Grown Wild in Iran

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Abstract

Nowadays, medicinal plants are considered as a valuable source of natural compounds used in the development of antimicrobial and antioxidant drugs. The objectives of this study were to evaluate chemical composition, antimicrobial and antioxidant activities of *Ziziphus spina-christi* var. *aucheri* (Boiss.) Qaiser & Nazim. essential oil. Essential oil was obtained by hydro-distillation using Clevenger type apparatus during approximately 3 hours and analyzed using gas chromatography/mass spectrometry (GC/MS). Eleven components were identified in *Z. spina-christi*var. *aucheri* essential oil that represented 92.14% of the oil. The main components of the oil were Carotol (42.20%), hexadecanoic acid (13.75%), linoleic acid (11.76%), vetivenic acid (9.56%) and (-)(+)valeranone (7.06%). Antioxidant activity of the essential oil was performed using 1,1-diphenyl–2–picrylhydrazyl (DPPH) and ribosomal degradation assay (HRS). Antimicrobial activity was investigated by micro broth dilution method. The antioxidant activity of *Z. aucheri* was IC50 = 53.91 ± 2.431 and showed only by DPPH method. The oil had antimicrobial activity in low concentrations against *Aspergillusniger, Penicillium digitatum* and *Klebsiella pneumonia.*

Keywords: *Ziziphus aucheri Boiss*, GC/ MS, DPPH, HRS, Antioxidant, Serial dilution method.

Introduction

Over the past few years, searching for alternative methods of disease control either human or plants has been developing. Using synthetic products like antibiotics for controlling animal infections or pesticides for managing plant diseases is the first and seems to be the best choice. But their interruption on natural biological control, antibiotic resistance of microorganisms, and pesticide residues in agriculture products is still challenges for the scientists. So the use of natural products such as flavor, antimicrobial and antioxidant products is developing [1].

Plants produce various secondary metabolites and most of these products have been reported as defensive products against pathogens. Some products in plants have been found in specific plant organs during special phenological period of the plant [2].

The genus *Ziziphus* belongs to Rhamnaceae family which contains 900 species in the world, originated from tropical Asia, Africa and America. Five species are distributed in Iranian flora, namely *Z. spina-christi, Ziziphus nummularia* (Burm.f.) Wight &Arn. , *Ziziphu sjujuba* Mill., *Ziziphus mauritiana* Lam. and *Ziziphus oxyphylla* Edgew. Boissier reported *Ziziphus aucheri* Boiss. from Bushehr. However, Re change mentioned it as a synonym of *Z. spina-christi*. Following that, Qaiser and Nazimuddin reported it as a variety of *Z. spina-Christi* and has named *Z. spina-christi*var*aucheri* [3].

The *Ziziphus* species (Rhamnacese) are commonly used in folklore medicine for the curing of various diseases [4]. In addition, *Z. Spina-christi*has been reported to have activity against bacterial and

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fungal pathogens that are normally quite resistant to modern medications [5]. Also, pharmacological studies have demonstrated that *Z. spina-christi* were known to possess hypoglycemic, hypotensive, antimicrobial, hepatic protective, antioxidant, antitumor and immune stimulatory activities [6].

Z. spina-christi var. *aucheri* occurs more in east of Nudo-Sindian that is drier than west in Iran. In *Z. spina-christi* var. *aucheri* the epidermis with deep cellular wall ad axially, wide cuticle on each sides and simple papilla with high frequently axially is noticed.

Z. spina-christi var. *aucheri* has very nutritious fruits and is usually eaten fresh [7]. Forasmuch as, so far no research has been done on the constituents and pharmacological action of this medicinal plants, the study was focused on evaluation of antioxidant and antimicrobial activities of *Z. spina-christi* var. *aucheri* essential oil.

Material and methods

Plant Material

Aerial parts of *Ziziphus spina-christi* var. *aucheri* (specimen number in the herbarium of Islamic Azad University, Shiraz Branch is P920403) were collected from various regions of Bushehr provinces of Iran. The harvest plants were then washed with distilled water to remove dirt and soil particles. The plants were dried at room temperature (25 °C) for 2 weeks. Then, air-dried plants ground and powdered with mixer for essential oil extraction.

Essential Oil Extraction

Essential oil was obtained from dried parts (100 g) from *Ziziphus spina-christi* var. *aucheri* that were subjected to water-distillation using a Clevenger-type apparatus (w/w) for 3 h. Then the oils dried by anhydrous sodium sulfate. The isolated oils were stored in tightly closed vials at 4°C until analysis [8].

Essential Oil Analysis

Essential oil was analyzed by Hewlett – Packard GC/MS (model 6890 series II) operating at 70eV ionization energy, equipped with a HP–5 capillary column phenyl methyl siloxane (30m 0.25mm, 0.25 µm film thickness) with helium as the carrier gas and a split ratio of 1:20. The retention indices for all the components were determined according to the Van Den Dool method, using n–alkanes as standard [9]. The compounds were identified by comparison of retention indices (RRI–HP–5) with those reported in the literature and by comparison of their mass spectra with the Wiley and Mass finder 3 libraries or with the published mass spectra [10].

Microorganisms and Their Growth Conditions

Microbial strains obtained from Persian Type Culture Collection (PTCC) in Iranian Research Organization for Science and Technology that including *Escherichia coli* (PTCC 1154), *Staphylococcus aureus* (PTCC 1189), *Pseudomonas aeruginosa* (PTCC 1571), *Staphylococcus epidermidis* (PTCC 1435), *Bacillus cereus* (PTCC 1816), *Klebsiella pneumonia* (PTCC 1290), *Aspergillusniger* (PTCC 5154) *and Penicillium digitatum* (PTCC 5204). All bacterial and fungi strains were cultivated in Nutrient Agar and Potato Dextrose Agar for 48hrs at 37ºC (bacteria) and 25 ºC for 3 days (fungi) respectively following refrigeration storage at 4°C until required for sensitivity testing [11].

Antimicrobial Activity

In vitro antimicrobial activity of the essential oil of *Z. spina-christi* var. *aucheri* was evaluated by micro broth dilution with determination of minimum inhibitory concentration and minimum bactericidal concentration. The tested bacteria and fungi were cultured on Nutrient broth medium (NB) and Sabouraud dextrose broth (SDB) respectively for period of time required. Then the suspensions were prepared with concentration of 10^8 CFU/mL bacteria and 10^6 CFU/mL fungi purified on NA and SDB medium [12].

Determination of Minimum Inhibition Concentration (MIC)

Serial dilution method is used to decide the minimum inhibitory concentrations (MICs) of antimicrobial activity of *Z. spina-christi* var. *aucheri* and is the reference method for antimicrobial susceptibility. In this method the capability of microorganisms to produce the visible growth on microplate wells of broth containing dilutions of the antimicrobial agent are tested. Briefly, serial two-fold dilutions of *Z. spina-christi* var. *aucheri* (10% W/V) were prepared in 96-well micro-titer plate (from 1:2 to 1:8192) containing acation-adjusted Mueller-Hinton broth (Merck, Darmstadt, Germany). Control micro-titer plates containing medium and 80% ethanol at the same

dilutions were also made. Microbial suspensions were adjusted to the 0.5 McFarland standards (approximately 1×10^8 CFU/mL for bacteria and 1×10^6 CFU/mL for fungi).The solutions of the wells were mixed then it was incubated at 37 °C for 24 h. The lowest concentration of an antimicrobial agent in order to inhibit the visible growth of a microorganism is called as the MIC. The experiment was carried out in triplicate. For each test enrofloxacin and gentamycin were used as the control antimicrobial agents [12].

Determination of Minimum Bactericidal and Fungicidal Concentration (MBC) and (MFC)

The minimum bactericidal/ fungicidal concentration (MBC/MFC) evaluated by subculturing5µL of solution from each well on to a NA for bacteria and SDB for fungi. Then the solution was incubated at 37 °C for 24h.Least concentration of essential oil showing no visible growth on subculture was taken as MBC/MFC [13].

Antioxidative Assay

DPPH Free Radical Scavenging Activity

The radical scavenging capacities of each of the essential oil, in different concentrations were estimated. In this method the decolorization of methanol solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) has been used to examine the hydrogen atom donating ability of the plant extractives. Different aliquots of the sample were dissolved in methanol, incubated with a methanolic solution of DPPH (100µL) in 96-well microplates. After that the mixture has been shaken at room temperature for 30 min. And the absorbance was taken at 517 nm against a blank by using a spectrophotometer (UV-1800) (Milton Roy Company Spectronic 2OD). All reactions were carried out in triplicate. Gallicacid was used as positive controls. The percentage inhibition (I%) for each concentration was calculated by using absorbance (A) values according to the following formula: $I\% = [(A_{DPPH} - A_P)/A_{DDPH}] \times 100$ [14].

Deoxyribose Degradation Assay

Hydroxyl free radical scavenging activity (HRS) determined by the assay of malondialdehydechromogen formation of 2-deoxy-D-ribose degradation [15]. The assay mixture contained in a final volume1.0 mL: 100 µL of 28mM 2-deoxy-D-ribose dissolved in phosphate buffer, pH7.4, 500 µL of essential oil in various concentration in buffer, 200µL of mixture of 1.04 mM EDTA and 200 µM $FeCL_3$ (1:1 v/v), 100µl of 1.0 mM H_2O_2,and 100 µL 1.0 mM ascorbic acid. After incubation of test sample at 37°C for 1h the extent of free radical damage imposed on the substrate deoxyribose was measured using thiobarbituric acid (TBA) test. Percentage inhibition of deoxyribose degradation was calculated. Gallic acid was used as standard.

Results and Discussion

Essential Oil Components

The chemical composition of the essential oil of *Z. spina-christi* var. *aucheri* are shown in Table 1. Eleven components were identified in *Z. spina-christi* var. *aucheri* essential oil that represented 92.14% of the oil. The main components of the oil were Carotol (42.20%), Hexadecanoic acid (13.75%), Linoleic acid (11.76%), Vetivenic acid (9.56%) and Valeranone (7.06%). So far no research has been done on the constituents of *Z. spina-christi*var. *aucheri.*

Antimicrobial activity

The number of multi-drug resistant microbial strains and the appearance of strains with reduced susceptibility to antibiotics are continuously increasing. In addition, in developing countries, synthetic drugs are not only expensive and inadequate for the treatment of diseases but also often with adulterations and side effects [15]. Thus, investigations on the antimicrobial activity of plant essential oil against different pathogens have been performed worldwide. Our results have importance because they provide information about this subject.

The MIC, MBC and MFC values of the essential oil against all microorganisms tested are reported in Table 2. *Aspergillus niger*, *Penicillium digitatum* and *Klebsiella pneumonia* revealed the highest sensitivity to essential oils, while *Pseudomonas aeroginosa*, *Staphylococous aureus* and *Bacillus cereus* didn't reveal any sensitivity. So that, the growth of *Aspergillus niger*, *Penicillium digitatum* and *Klebsiella pneumonia* were inhibited by *Z. spina-christi* var. *aucheri* in concentrations of 32 µg/mL, 128 µg/mL and 512 µg/mL respectively. Also, 99.9% of *Aspergillus niger* not growth in concentrations of 64 µg/mL of *Z. spina-christi* var. *aucheri* essential oil. Some researchers reported

that there is a relationship between the chemical structures of the most abundant compounds in the tested oil, the proportions in which they are present and to inter-actions between them and the antimicrobial activity [16]. However, the synergistic or antagonistic effect of one compound in minor percentage in the mixture has to be considered [17].

In this study the antimicrobial activity can relate to presence of two compounds such as, menthol and carotol. Because, Menthol is a major essential oil constituent of a very limited number of aromatic plants, known to exhibit various biological properties such as antimicrobial, anticancer and anti-inflammatory activities. This compound is also used as insect repellents or fumigants [18]. Also, carotol is sesquiterpene alcohol. Additionally, previous studies have shown that carotol may be involved in allelopathic interactions expressing activity as an antifungal, herbicidal and insecticidal agent [19].

Antioxidant Activity

Given the evidence of problems that can be caused by the consumption of synthetic antioxidants, research has emerged with the goal of finding natural products with antioxidant potential, which are an alternative to substitute the synthetic compounds or even promote an association between them, order to reduce their amount in food. In this research, the abilities of the essential oil to donate hydrogen atoms or electrons were measured spectrophotometrically. The essential oil of *Z. spina-christi* var. *aucheri* reduced DPPH to the yellow-colored product, diphenylpicryl hydrazine, and decreases the absorbance at 517 nm, possessed antioxidant activity Table 3. The antioxidant activity of *Z. spina-christi* var. *aucheri* was IC50 = 53.91 ± 2.431. It can relate to presence of linoleic acid. Since, linoleic acid has the detoxification and anticancer properties [4] which it was ingredient of the major components of *Z. spina-christi* var. *aucheri* essential oil. The hydroxyl (OH) scavenging activity of *Ziziphus spina-christi* var. *aucheri* was quantified by measuring the effect on 2-deoxyribose degradation. The OH radical scavenging activity was negative at all the concentrations. Emami *et al* (2007) also didn't see antioxidant activity in some concentration and different parts of *Juniperus communis* and *J. oblonga* by ribosomal degradation assay method, this may be due to a pro-oxidative effect or to the ability to produce thiobatbituric acid reactive substance. Also the antioxidant activity of natural product component can be done with different ways [14]. For recognizing exact antioxidant activity of plant it is better to test several methods. Assaying for site specific action recommended.

Conclusion

The present study concluded that the good antimicrobial and antioxidant activity of *Z. spina-christi*var. *aucheri* essential oil were against *Aspergillus niger*, *Penicillium digitatum* and *Klebsiella pneumonia* respectively. Because of that no research has so far been done to determine chemical composition, antimicrobial and antioxidant activity of *Z. spina-christi*var. *aucheri* essential oil and extract, further work need to be done on *Z. spina-christi*var. *aucheri* essential oil and extract to isolate active component and treatment diseases. The result of antioxidant activity which was demonstrated by DPPH scavenging assay, suggest the use of this essential oil in low concentrations for preserving food product.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

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