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Black Zira essential oil: Chemical compositions and antimicrobial activity against the growth of some pathogenic strain causing infection

Mohammad Noshad[a,*], Mohammad Hojjati[a], Behrooz Alizadeh Behbahani[b]

[a] Department of Food Science and Technology, Faculty of Animal Science and Food Technology, Ramin Agriculture and Natural Resources University of Khuzestan, Mollasani, Iran

[b] Department of Food Science and Technology, Ferdowsi University of Mashhad, Mashhad, Iran

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ABSTRACT

The aim of this study was to perform chemical compositions and phytochemical analysis of Black Zira essential oil and other goal of this research was to investigate the antimicrobial effects of Black Zira essential oil against *Enterobacter aerogenes*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Shigella flexneri*, *Staphylococcus epidermidis*, *Streptococcus pyogenes* and *Candida albicans*. Black Zira essential oil was extracted by hydrodistillation method using clevenger apparatus. Black Zira essential oil chemical composition was identified through gas chromatography/mass spectrometry. γ-terpinene with a percentage of 24.8% was the major compound of Black Zira essential oil. The antimicrobial effect Black Zira essential oil was evaluated by several qualitative and quantitative methods (disk diffusion, well diffusion, microdilution broth, agar dilution and minimum bactericidal/fungicidal concentration). Phytochemical analysis Black Zira essential oil were appraised based on qualitative methods. Antioxidant activity (2,2-diphenyl-1-picrylhydrazyl and β-carotene/linoleic acid inhibition) and total phenolic content (Folin-Ciocalteu) were examined. The results of phytochemical analysis of Black Zira essential oil showed the existence of phenolic, flavonoids, saponins, alkaloids and tannins. The total phenolic content and antioxidant activity (reported as IC_{50}) of Black Zira essential oil were equal to 120.50 ± 0.50 mg GAE/g and 11.55 ± 0.25 μg/ml, respectively. The MIC of the Black Zira essential oil ranged from 1 mg/ml to 8 mg/ml, while its MBC and MFC ranged from 1 mg/ml to 16 mg/ml. The results presented that the longest and the shortest inhibition zone diameter at the concentration of 8 mg/ml pertained to *C. albicans* and *E. aerogenes*, respectively.

1. Introduction

Pathogenic bacteria have developed several defense mechanisms on antimicrobial agents and resistance to old and novel manufactured drugs [1,2]. Antibiotic resistance is a serious and important phenomenon in contemporary medicine and has evolved as one of the concerns of pre-eminent public health in the 21st century, individually since it is linked to pathogenic microorganisms. Infection diseases must be critically controlled, in recent years, the occurrence of infectious diseases of bacterial and fungi is increasing, mainly in developing countries. A world health organization (WHO) account published in 2014 declared that this serious threat is not a prediction for the future anymore, whereas it is taking place right now all over the world and has the potential to impact all people, of any age, in any country. When bacteria change, antibiotics no longer work in people who require them to cure infections. This is now a principle threat to public health [3,4].

This is why numerous illnesses which had been controlled formerly, have appeared again and escaped from human control. The antibacterial activities of essential oils from different medicinal plants against microorganisms have been described by many researchers [5–7]. It is well-known that secondary metabolites are combined uniquely in specific plant species [8].

Black Zira, a member of Apiaceae family. This herb is a vital aromatic perennial plant that naturally grows in Iran. From the medicinal viewpoint, Black Zira is used as stimulant and carminative, and it also seems to be useful in treating diarrhea and dyspepsia [9–11]. The seed is normally employed to impart its flavor to rice in Persian cooking. In addition, it has been applied worldwide as stuffing [12].

The aim of this study was to identify the phytochemical analysis of Black Zira essential oil. The other goal of this research was to investigate the antimicrobial effects of Black Zira essential oil against the growth of some pathogenic strains causing infection.

* Corresponding author.
E-mail address: mo.noshad@gmail.com (M. Noshad).

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2. Materials and methods

2.1. Chemicals and reagents

Butylated hydroxyl toluene (BHT), 2,2-diphenyl-1-picrylhydrazyl (DPPH), β-carotene linoleic acid (β -CL), dimethyl sulfoxide (DMSO) and triphenyl tetrazolium chloride (TTC) were supplied from Sigma Chemical Co (Germany). Barium chloride ($BaCl_2$), Sulfuric acid (H_2So_4) and ethanol were prepared from Merck, Germany.

2.2. Microbial media

Mueller hinton agar (MHA), mueller hinton broth (MHB), sabouraud dextrose agar (SDA) and sabouraud dextrose broth (SDB), were supplied from Merck, Germany. Peptone bacteriological (BDH Chemicals Ltd., England), tryptone soya broth (East Anglia Chemicals, UK), sterile blank disc (Oxoid United Kingdom) and antibiotic disc (Oxoid United Kingdom) were supplied.

2.3. Collection of Black Zira, preparation of the essential oil and extraction yield

Black Zira was collected from Kerman, Iran. Black Zira powdered by a lab grinder. The Black Zira essential oil (50 g) was extraction using water-distillation method with clevenger apparatus. Then, Black Zira essential oil was collected in vials which had already been weighed by a 0.0001 balance and stored at 4 °C until testing. The extraction yield Black Zira essential oil was conducted according to the method of Alizadeh Behbahani and Imani Fooladi., (2018) [13].

2.4. Determination of phytochemical screening and chemical compositions of Black Zira essential oil

Phytochemical screening Black Zira essential oil (phenolic (Ferric chloride), flavonoids (Shinoda test), saponins (Froth test), alkaloids (Mayer and bosshardt) and tannins (Ferric chloride test)) were appraised based on qualitative methods and according to the research that was explained by Alizadeh Behbahani and Imani Fooladi., (2018) [14] and Njoku and Obi., (2009) [15]. Identification of the chemical compositions of Black Zira essential oil was performed by injecting its into a gas chromatograph and coupled to a mass spectrometer [14].

2.5. Determination of the Black Zira essential oil total phenolic content (TPC)

For this purpose, the method of Folin-Ciocalteu was performed by determination of TPC. The result was reported as mg of gallic acid/g of the dried Black Zira essential oil [16,17].

2.6. Determination of the Black Zira essential oil antioxidant activity (DPPH free radical-scavenging assay and ß-Carotene-linoleic acid assay)

For this purpose, the method of MubarakAli et al. (2018) [18] was used for evaluation of inhibitory activity of Black Zira essential oil on DPPH free radical-scavenging. The ß-Carotene-linoleic acid assay (β -CL). The study that was described by Alizadeh Behbahani et al. (2017) [19]. The average inhibition percent was calculated as the following equation:

$$\%\text{Inhibition} = [(AA_{120} - AC_{120})/(AC_0 - AC_{120})] \times 100 \quad (1)$$

where, AA_{120}, AC_{120}, and AC_0 are the absorbance for the antioxidant activity of sample after 120 min, control sample after 120 min, and control sample at the beginning of experiment ($t = 0$), respectively.

2.7. Preparation of the microbial strains

Microbial strains of *Enterobacter aerogenes* ATCC 13048, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, *Shigella flexneri* ATCC 12022, *Staphylococcus epidermidis* ATCC 12228, *Streptococcus pyogenes* ATCC 19615, and *Candida albicans* PTCC 5027 were used in this research. All microbial strains were supplied from the department of food science and technology at Ferdowsi University of Mashhad (FUM), Mashhad, Iran. The microbial suspensions turbidity was made based on 0.5 McFarland (1.5×10^8 CFU/ml) [20].

2.8. Antimicrobial activity Black Zira essential oil

In this paper, several qualitative and quantitative methods (five methods) were used to examine the antimicrobial activity of the Black Zira essential oil. These methods are summarized as follows:

2.8.1. Disk diffusion agar (DDA)

For this purpose, the Black Zira essential oil concentrations of 1, 2, 4 and 8 mg/ml were prepared in a proper solvent (DMSO). The Black Zira essential oil were sterilized by a PVDF 0.22 µm syringe filter. Then, the blank discs (Oxoid United Kingdom) were immersed in the Black Zira essential oil for 15 min so that they would be overall smeared. Next step, microbial suspension equivalent to 1.5×10^8 CFU/ml (equivalent to 0.5 McFarland standard) were readied. The petri-dishes were incubated at 35 °C for 24 h and 25 °C for 72 h for bacteria and fungi, respectively. The antimicrobial effect was determined in term of inhibition zone diameters (IZD) (mm) [21]. The results were tested by measuring the diameters of the halos formed around the disks and the results get from antibiotics were contrasted with the clinical and laboratory standards institute (CLSI) tables [22].

2.8.2. Well diffusion agar (WDA)

For this purpose, this test was performed using a suspension with a McFarland standard turbidity of 0.5. The Black Zira essential oil concentrations of 1, 2, 4 and 8 mg/ml were prepared in a suitable solvent (DMSO). The Black Zira essential oil were sterilized by a 0.22 µm syringe filter. Well of 6 mm in diameter were created on the plates (8 mm thick) of MHA and SDA. The petri-dishes were incubated at 35 °C for 24 h and 25 °C for 72 h for bacteria and fungi, respectively. The antimicrobial effect was determined in term of IZD (mm) [19,23].

2.8.3. Determination of minimum inhibitory concentration (micro dilution broth and triphenyl tetrazolium chloride indicator)

The minimum inhibitory concentration (MIC) was determined according to microdilution broth method and according to the research that was explained by Bottari et al. (2017) [24] and Quatrin et al. (2017) [25]. The Black Zira essential oil concentrations of 1, 2, 4, 8, 16, 32, 64 and 128 mg/ml were prepared. The 96-well plate were incubated at 35 °C for 24 h and 25 °C for 72 h for bacteria and fungi, respectively. In order to estimate, the first concentration in which microbial growth did not fall out and red color was not seen, recorded for MIC.

2.8.4. Determination of minimum inhibitory concentration (agar dilution method)

For this purpose, the method of Wiegand et al. (2008) [26] was performed by determination of MIC.

2.8.5. Minimum bactericidal/fungicidal concentration (MBC/MFC)

The MBC/MFC was determined according to the research that was explained by Alizadeh Behbahani et al. (2018) [27] with slight modifications. All the plate which showed no visible growth were on MHA and SDA media, next, incubated at 35 °C for 24 h and 25 °C for 72 h for bacteria and fungi, respectively.