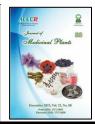


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#### **Research Article**

#### Antimicrobial and antioxidant activities of essential oils from seven species of Amaryllidaceae and Brassicaceae

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#### ABSTRACT

Background: Essential oils have been utilized for various purposes throughout history. These aromatic substances have become increasingly popular in alternative medicine, aromatherapy, and personal care products. Objective: In this study, essential oils from the aerial parts of four Allium species and three Brassicaceae members, namely Fortuynia garcinii, Draba verna, and Thlaspi arvense were evaluated for their antioxidant, antibacterial, and antifungal properties. Methods: The radical-scavenging properties were tested using DPPH assay. Antimicrobial activities were examined on nine standard pathogens: three Gram-positive bacteria including Staphylococcus aureus, Bacillus cereus, Streptococcus pyogenes, three Gram-negative bacteria including Escherichia coli, Pseudomonas aeruginosa, Salmonella enterica subsp. enterica and two fungi Aspergillus fumigatus, Fusarium oxysporum as well as the yeast Candida albicans. Results: The IC50 values of antioxidant assay ranged from 124.66 to 155.04 µg/ml. Allium zagricum showed the best antioxidant effects with IC<sub>50</sub> of 124.66 µg/ml compared to standard vitamin E  $(IC_{50} = 10.40 \mu g/ml)$ . Similarly, the MIC values of 25-400  $\mu g/ml$  with Fortuynia garcinii fruits-Zahedan were assessed as the best antimicrobial effects, while they were higher than the MIC values recorded for positive controls (0.06-16 for amikacin and 32-256 for clotrimazole). Conclusion: Essential oils extracted from Allium zagricum and Fortuynia garcinii can be prescribed for the treatment of oxidative stress-related and infectious diseases.

Abbreviations: FFH, Fortuynia garcinii Fruits-Zahedan; FFB, Fortuynia garcinii Fruits-Zabol; FLB, Fortuynia garcinii Leaves- Zabol; DVL, Draba verna L.; TAL, Thlaspi arvense L.; AIR, Allium iranshahrii; AAR, Allium alamutense Razyfard, Zarre; ACH, Allium chrysantherum; AZA, Allium zagricum; VIE, Vitamin E; AMK, Amikacin; CLT, Clotrimazole; DPPH, 2,2-Diphenyl-1-picrylhydrazyl; MIC, Minimum Inhibitory Concentration; MBC, Minimum Bactericidal Concentration; MFC, Minimum Fungicidal Concentration; PTCC, Persian Type Culture Collection; IBRC, Iranian Biological Resource Centre; UOZH, University of Zabol Herbarium; SD, Standard deviation; GC-MS, Gas Chromatography-Mass Spectrometry; EE, The essential oil extraction efficiency; DMSO, Dimethyl sulfoxide; ABTS, 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)

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#### 1. Introduction

For many centuries, herbal products were the only available option for the prevention and treatment of many diseases in humans and animals. Plant extracts and essential oils contain valuable chemical compounds that have shown promising potential in the fields of medicine, food, cosmetics, and agriculture [1]. Essential oils are intricate combinations of fragrant substances that occur naturally in various parts of aromatic plants. They are typically colorless or light yellow liquids, soluble in lipid and organic solvents, and with molecular weights less than 300 Da [2, 3].

Developing synthetic preservatives and fragrances in various industries is more expensive compared to natural, plant-based essential oils while the latter could be valueless and even unsafe for human applications. Some chemicals found in manufactured fragrances include endocrine-disrupting activities like and phthalates, and carcinogenic ingredients such as benzene derivatives [4, 5]. In addition, the increasing global tendency towards natural fragrances and additives, due to their safety and health benefits, has boosted the efforts to find natural options to replace synthetic fragrances and preservatives.

The antioxidant, antimicrobial, and antiparasitic activities of essential oils have been well documented and are thought to come from the presence of phenolic functional groups [6]. The essential oils exert their antimicrobial activity through different mechanisms with more efficacy on Gram-positive bacteria than Gramnegative ones [7]. The researchers put a lot of effort into discovering plant-based antioxidant and antimicrobial components all around the world. The antimicrobial and antioxidant activities of a huge number of plants have been investigated worldwide, but there is still a long way to investigate many other valuable plant resources. Endemic plants that are found in limited phytogeographical, ecological, or political ranges are of critical importance. These invaluable plants are often unknown or inaccessible to many researchers. In addition, they are unique genetic pools of specific geographical regions and could be very important in different aspects. They may contain unique ingredients or characteristics but due to the small population size are prone to extinction, leading to the loss of all possible information and potentials.

The genus Allium L. (derived from the Greek word aleo meaning to avoid, due to the smell of garlic) was described by Carl Linnaeus in 1753 [8]. Allium (Amaryllidaceae) with around 1000 species is one of the largest genera in the monocotyledons distributed predominantly in the northern hemisphere [9]. Some of these species have been cultivated and used for centuries, not only as culinary ingredients but also for their medicinal properties. Allium is also the largest genus of petaloid monocots in Iran with around 153 species allocated to eight subgenera and 32 sections [10]. Members of this genus occupy many different habitats from sea shores, and the vicinity of salt lakes to montane and even subalpine areas [11]. A few Allium species such as A. cepa, A. sativum, and A. tenuissimum have been studied extensively for their chemical composition and antioxidant and antimicrobial properties [12-14]. However, there is no information regarding the biological properties of many endemic species of Allium from Iran. Four Allium species investigated in this study (A. alamutense Razyfard, A. chrysantherum Zarre & R.M.Fritsch, A. iranshahrii R.M.Fritsch, and A. zagricum R.M.Fritsch) are narrow endemics with very limited distribution ranges in West and North West of Iran while distribution range of A.

chrysantherum stretches from West of Iran to adjacent countries of Iraq, Turkey, Syria, and Jordan.

Fortuynia (Brassicaceae) contains only a single species, F. garcinii (Burm.f.) Shuttlew. which is endemic to Iran, Afghanistan, and western Pakistan (Flora iranica). the genus name and specific epithet refer to an Indonesian and a French botanist respectively. The plant is perennial, woody at base, glabrous, with simple, entire, ± fleshy leaves and biarticulated broadly winged Siliculae. Draba verna L. (Draba is Greek for bitter, referring to the taste of the plant and verna is Latin for springtime when the plant blooms) is a small annual member of the family Brassicaceae with basal leaves, bifid petals and leafless stems growing widely around the world. Thlaspi arvense L. is also a small glabrous annual plant in the family Brassicaceae native to the Mediterranean region but becoming widespread almost all around the world. The generic name comes from a Greek word for cress and arvense is derived from the Latin word "arvum" meaning ploughed, pertaining to the cultivated and disturbed lands where it grows [15].

The objective of this study was to gain a background information on antioxidant and antimicrobial properties of four species of the genus Allium namely A. Iranshahrii, A. Alamutense, A. zagricum and A. chrysantherum as well as three selected species from the Brassicaceae family including *Fortuynia* garcinii, Draba verna and Thlaspi arvense. Certainly, understanding the background knowledge and applicability of these unique plants can provide valuable insights into their potential medicinal, nutritional or agricultural benefits.

#### 2. Materials and Methods

#### 2.1. Plant Material Collection

The wild plant species were collected from their natural habitats during flowering stage. The scientific names, geographical localities, herbarium codes, and characteristics of the examined plant species are provided in Table 1. The plant species were identified using botanical resources by the third and fourth authors, at the Department of Biology, botanists University of Zabol and the curator of the IBRC herbarium, respectively. A herbarium sample of each species was prepared and restored either at the UOZH in Zabol, Iran or IBRC herbarium located in Karaj, Iran (for details see Table 1). A photograph of each studied plant species, its habitat and detailed characteristics has been presented in Fig. 1. The targeted parts of the sampled plants were shade dried for a week, of which 100 g were converted to powder using an electronic machine.

#### 2.2. Essential Oil Extraction

10 g of each powdered material was hydrodistilled for 4 h with 300 ml of distilled water, using a micro-clevenger type apparatus. The volatile fractions were extracted with 10 ml diethyl ether. The extract was dried with 1 g anhydrous magnesium sulfate. Finally, the resulting solution was condensed under a nitrogen stream.

**Table 1.** The scientific names, geographical distribution, localities, herbarium codes, and characteristics of the examined plant species

| No | Species   | Family                                     | Herbarium<br>code | Characteristics  | Locality   | Distribution range                           | Used<br>part     |
|----|---|--|-------------------|--|--|--|------------------|
| 1  | Allium<br>iranshahrii<br>R.M.Fritsch                        | Amaryllidaceae                             | 1824              | Bulbous; Leaves 1-<br>2, laminae broadly<br>ovate to elliptic,<br>upper side with<br>deep furrows; tepals<br>whitish to rose with<br>a broad green to<br>brown median vein,<br>anthers carmine | W Azerbaijan: Piranshahr, Silveh, Mashkan, Chighidarreh mt. 2835m.                             | NW Iran                                      | Aerial<br>parts  |
| 2  | Allium<br>alamutense<br>Razyfard,<br>Zarre &<br>R.M.Fritsch | Amaryllidaceae                             | 1616              | Bulbous; scape 2-5<br>cm long; leaves 2,<br>laminae broadly<br>canaliculate;<br>Flowers ± broadly<br>funnel-shaped   | Zanjan:<br>Zanjan<br>towards<br>Gilvan, 5 km<br>before<br>Khanchaei<br>Rahdarkhaneh<br>. 2435m | NNW Iran                                     | Aerial<br>parts  |
| 3  | Allium<br>chrysantheru<br>m Boiss. &<br>Reut.               | Amaryllidaceae                             | 1630              | Bulbous; scape 50-<br>80 cm long; tepals<br>yellow, narrowly<br>linear-lanceolate,<br>all flower parts of<br>the innermost<br>flowers often purple<br>flushed to dark<br>purple                | Ilam: Ilam<br>towards<br>Eyvan,<br>Chovar, Reno<br>forest park,<br>1464m                       | W Iran to N<br>Iraq, S Turkey<br>and Jordan. | Aerial<br>parts  |
| 4  | Allium<br>zagricum<br>R.M.Fritsch                           | Amaryllidaceae                             | 1673              | Bulbous; leaf<br>laminae up to 8 cm<br>broad, leaves glossy<br>yellowish-green;<br>tepals lanceolate-<br>triangular, pale lilac  | Lorestan: N<br>Khorramabad,<br>Qalemorghi,<br>Taf Mts.<br>2285m                                | W Iran<br>(Zagros<br>Mountains)              | Aerial<br>parts  |
| 5  | Fortuynia<br>garcinii<br>(Burm.f.)<br>Shuttlew.             | Brassicaceae                               | UOZH 1505         | Perennial with,<br>entire, ± fleshy<br>leaves and, winged<br>Siliculae   | Sistan and<br>Baluchestan,<br>Zabol, 25 km<br>from Zabol<br>towards<br>Nehbandan<br>478m       | Iran,<br>Afghanistan<br>and Pakistan         | Leaves<br>Fruits |
| 6  | Fortuynia<br>garcinii<br>(Burm.f.)<br>Shuttlew.             | garcinii<br>(Burm.f.) Brassicaceae UOZH 15 |                   | Perennial with, entire, ± fleshy leaves and, winged Siliculae  Siliculae  Sistan and Baluchestan, Zahedan 25 km from Zahedan towards Khash   |  | Iran,<br>Afghanistan<br>and Pakistan         | Fruits           |
| 7  | Draba verna<br>L.   | Brassicaceae                               | UOZH 1507         | Small therophyte<br>with basal leaves,<br>bifid petals and<br>leafless stems   | Hamedan,<br>Asadabad,<br>Shamsabad<br>village 1673m  | Native to<br>Eurasia                         | Whole plant      |
| 8  | Thlaspi<br>arvense L.                                       | Brassicaceae                               | UOZH 1508         | Small glabrous<br>therophyte with<br>narrow and toothed<br>stem leaves   | Hamedan,<br>Asadabad,<br>Shamsabad<br>village 1673m  | Cosmopolitan                                 | Whole plant      |



**Fig. 1.** Photographs of the studied plant species and their habitat and some morphological details. A) *Allium chrysantherum*, with a close up of the inflorescence; B) *Allium alamutense*; C) *Allium iranshahrii*; D) *Allium zagricum*; E) *Draba verna*; F) *Thlaspi arvense*, with details of fruiting inflorescence; G) *Fortuynia garcinia*, showing also details of fruits and the sampling for this study

#### 2.3. Antimicrobial Activity

Gram-positive Six and Gram-negative bacteria, two fungal strains and a yeast were tested for their susceptibility to the essential oils extracted from four species of Allium and three species of Brassicaceae. The standard strains of Pseudomonas aeruginosa (ATCC 10145), Salmonella enterica subsp. enterica (ATCC 19585), Escherichia coli (ATCC 25922), Staphylococcus (NCTC aureus 7445), Streptococcus pyogenes (ATCC 12204), Bacillus cereus (ATCC 14579), Aspergillus fumigatus (PTCC 5009), Fusarium oxysporum (CBS 620.87) and Candida albicans (ATCC 10231) were used in this in vitro antimicrobial assay. All microbial strains were brought from the PTCC, Karaj, Iran. The MIC, MBC and MFC values of the essential oils were quantified in sterile 96well plates by employing microdilution method [16]. The stock solutions of each essential oil were prepared using DMSO as solvent at initial concentrations of 80000, 40000, 20000, 10000, 8000, 4000, 2000, 1000, 500, 250, 125, and 62.5 µg/ml. 20 µl of each stock solution was respectively added to the 1<sup>st</sup> to 12<sup>th</sup> wells in each row of the 96-well plate. Then, 100 µl of microbial suspensions and 80 µl of liquid medium were added to all wells. The final concentrations of 8000, 4000, 2000, 1000, 800, 400, 200, 100, 50, 25, 12.5, 6.25 μg/ml of each sample were obtained in each row. The 96-well plates were incubated in 37 °C for 24 h. Amikacin and clotrimazole were applied as positive controls in this study. DMSO was also used as a negative control. While its final concentration was 10% (v/v) in each well, no inhibitory activity was observed with it. Finally, the wells with the least concentration that showed no visual turbidity were identified as the MIC. The MBC and MFC were determined by reculturing the contents of the transparent wells in MIC phase with the lowest concentration where all microorganisms were disappeared.

## 2.4. Antioxidant Capacity and IC<sub>50</sub> Determination

The antioxidant capacity of essential oils was estimated according to their capacity to scavenge DPPH stable free radicals, as outlined in our previous publications [17, 18]. methanolic solutions of 50, 100, 200, and 400 μg/ml were created using each essential oil. Then, 3 ml of a fresh DPPH solution (0.004 % w/v) was added to 1 ml of each methanolic solution. The mixture was allowed to rest for 30 minutes in dark at ambient temperature to complete the reaction and discolor DPPH. Finally, a UV spectrophotometer (UV-2100 RAY Leigh) was used to determine the absorbance of each solution at 517 nm. The absorbance of the DPPH solution was read as blank. The equation I  $\% = [(A0 - As)/(A0)] \times 100$ was employed to detect the DPPH scavenging effect in which I % is inhibition percentage, A0 is the absorbance of the blank and As is the absorbance of the test sample. Finally, the antioxidant activity was reported as IC<sub>50</sub> values (μg/ml), indicating the required concentration of essential oil to exert 50 % inhibition. A similar procedure was followed for vitamin E which was used as a positive control in this study.

#### 2.5. Statistical Analyses

The tests were performed three times to ensure the validity of the results, with the reported values representing the mean of these three independent trials  $\pm$  SD. No SD was observed in susceptibility antimicrobial tests. Graphs were constructed using EXCEL software, and the data were statistically analyzed using version 16 of the SPSS software.

#### 3. Results

Volatile oils of of all seven species were extracted using steam distillation. The essential oil extraction efficiency was calculated according to the following formula:

 $EE (\%) = [m_o (g) / m_p (g)] \times 100$ 

Where  $m_o$  and  $m_p$  are the mass of the obtained essential oil and the plant organ. The oil extraction yields were reported in Table 2.

All plants were evaluated for their possible antioxidant and antimicrobial properties. For this purpose, hydrogen-donating ability of essential oils were investigated against DPPH free radicals, as shown in the Fig. 2. Moderate antioxidant effects were observed with them. *Allium* species were more successful in inhibiting radicals than *Fortuynia*, *Draba* and *Thlaspi* species. The IC<sub>50</sub> values of *Allium* species were in the limited range of 124.66-128.73 μg/ml. In *F. garcinii* samples, Zabol fruit had better effects than its Zahedanian counterpart. DVL and TAL had intermediate antioxidant effects. The IC<sub>50</sub> values of esential oils were higer than those of vitamin E as standard (10.40 μg/ml).

Table 2. The essential oil extraction efficiency of samples

| Oils   | FFH   | FFB   | FLB   | DVL   | TAL   | AIR   | AAR   | ACH   | AZA   |
|--------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| EE (%) | 0.040 | 0.745 | 0.208 | 0.559 | 0.526 | 0.161 | 0.040 | 0.140 | 0.035 |

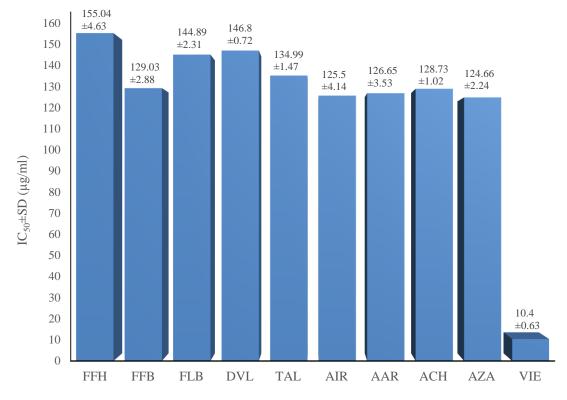


Fig. 2. DPPH free radical scavenging activity of essential oils

Antimicrobial potential of all essential oils was assessed against nine pathogenic bacterial and fungal strains from different genera. The MIC, MBC and MFC values of effective samples were recorded in the range of 25-8000 µg/ml. As shown in Table 3, no inhibitory activity was observed at the highest selective concentration (8 mg/ml) against *B. cereus* and *C. albicans*. Essential oil of FLB was the only effective specie on *S. pyogenes*. All three samples of *Fortuynia* 

could inhibit the growth of *S. aureus* strain. AZA and DVL affected only *P. aeruginosa* and *A. fumigatus*, respectively. No antibacterial activity was observed with TAL against tested pathogens. AMK and CLT as positive controls present better inhibitory effects than essential oils with MICs of 0.06-16 and 32-256 μg/ml, respectively. Major essential oils (6 out of 9) could inhibit the growth of *S. aureus* and *A. fumigatus* strains with MICs of 400-8000 and 200-8000 μg/ml, respectively.

Table 3. Antimicrobial properties of essential oils and standards

|              |                  | Essential oils |      |      |      |     |      |      |      |      | Standards |     |
|--------------|------------------|----------------|------|------|------|-----|------|------|------|------|-----------|-----|
| Strains      |                  | FFH            | FFB  | FLB  | DVL  | TAL | AIR  | AAR  | ACH  | AZA  | AMK       | CLT |
| С            | MICa             | 400            | 2000 | 2000 | ND   | ND  | 2000 | 8000 | 2000 | ND   | 0.5       | -   |
| S. aureus    | MBCa             | 800            | 2000 | 2000 | ND   | ND  | 2000 | 8000 | 2000 | ND   | 1         | -   |
| B. cereus    | MIC              | ND             | ND   | ND   | ND   | ND  | ND   | ND   | ND   | ND   | 0.5       | -   |
| D. cereus    | MBC              | ND             | ND   | ND   | ND   | ND  | ND   | ND   | ND   | ND   | 0.5       | -   |
| S. pyogenes  | MIC              | ND             | ND   | 4000 | ND   | ND  | ND   | ND   | ND   | ND   | 1         | -   |
| s. pyogenes  | MBC              | ND             | ND   | 4000 | ND   | ND  | ND   | ND   | ND   | ND   | 4         | -   |
| E. coli      | MIC              | 100            | 4000 | ND   | ND   | ND  | 4000 | ND   | ND   | ND   | 16        | -   |
| E. con       | MBC              | 100            | 4000 | ND   | ND   | ND  | 4000 | ND   | ND   | ND   | 16        | -   |
| Р.           | MIC              | 25             | ND   | 4000 | ND   | ND  | 4000 | ND   | 4000 | 8000 | 0.06      | -   |
| aeruginosa   | MBC              | 25             | ND   | 4000 | ND   | ND  | 4000 | ND   | 4000 | 8000 | 0.06      | -   |
| S. enterica  | MIC              | ND             | ND   | ND   | ND   | ND  | 2000 | ND   | 2000 | ND   | 0.5       |     |
| 5. emerica   | MBC              | ND             | ND   | ND   | ND   | ND  | 2000 | ND   | 4000 | ND   | 1         |     |
| A. fumigatus | MIC              | 200            | ND   | 2000 | 2000 | ND  | 1000 | 8000 | 2000 | ND   | -         | 32  |
| A. jumiguius | MFC              | 200            | ND   | 2000 | 2000 | ND  | 2000 | 8000 | 2000 | ND   | -         | 32  |
| F orvenorum  | MIC              | 200            | ND   | 4000 | ND   | ND  | 2000 | ND   | 4000 | ND   | -         | 256 |
| F. oxysporum | MFC <sup>a</sup> | 200            | ND   | 4000 | ND   | ND  | 2000 | ND   | 4000 | ND   | -         | 512 |
| C. albicans  | MIC              | ND             | ND   | ND   | ND   | ND  | ND   | ND   | ND   | ND   | -         | 256 |
| c. awicans   | MFC              | ND             | ND   | ND   | ND   | ND  | ND   | ND   | ND   | ND   | -         | 512 |

#### 4. Discussion

The primary treatment of diseases was mainly based on the use of medicinal plants, which is still thriving despite the development of synthetic drugs. For this purpose, the plant itself is consumed directly or prescribed in the forms of extract and essential oil. These extracts and essential oils can be obtained from aerial and underground organs of plant such as flowers, fruits, leaves, peels, seeds, roots and bark. In this

study, antioxidant and antimicrobial potentials of essential oils of three Brassicaceae members and four Allium species were assessed against DPPH and a variety of microbial pathogens. No biological investigation was observed on F. garcinii despite its use in the treatment of spasm sedative, menstruation additive, and migraine in traditional medicine [19]. The water and ethyl acetate extracts of D. tortuosa, a plant closely related to F. garcinii and native to Pakistan, exhibited significantly greater antioxidant activity than that of F. garcinii. Moreover, its were found to possess antibacterial properties effective against a wide range of bacteria and fungi [20]. Antimicrobial and antioxidant data from D. verna, T. arvense, A. iranshahrii, A. chrysantherum, A. zagricum and A. alamutense was also not found for comparison. While none of the investigated Allium species in this study have been subjected to antioxidant and antimicrobial examinations, several other species have been extensively studied for their biological activities. A. rubellum which is phylogenetically related to A. iranshahi [21] showed significant free radical scavenging activity in ABTS and DPPH assays and good antibacterial activity against E. coli and S. aureus using disc diffusion method [22]. This is in congruence with the results of this paper. The Allium species that are phylogenetically close to alamutensis. zagricum, A. chrysanthemum have not been studied yet, however, the antimicrobial effect of the widely used Allium species such as A. sativum, A. cepa, and A. stipitatum has been demonstrated against various bacteria and fungi, showing wide spectra of bacteriocide and fungicide properties [23, 24]. The similar antioxidant effects were observed with tested Allium species, which was probably to extract similar components during the steam distillation process. Various antimicrobial effects

were observed with the tested species, it can be due to the difference in the species genetics and/or climatic conditions [25]. Plant organ, extraction method, extraction solvent, sensitivity of bacteria, concentration of the extract, and the growth medium are other variables influencing antimicrobial properties [26]. Satyal et al. evaluated the chemical compositions of A. vineale and A. sativum volatile oils [27]. Their GC-MS analysis proved the presence of a wide spectrum of sulfur-containing compounds (>90 %) such as disulfide, 3-methyl/2,4-dimethyl/3,4dimethylthiophenes, 1,2-dithiolane/dithiolene, [1-propenyl] 2-thiopent-3-yl/propyl 4-thiohept-2-en-5-yl/dimethyl/diallyl sulfides/disulfides, methyl/methyl allyl/allyl propyl/methyl methylthiomethyl/allyl methylthiomethyl/1propenyl/1-propenyl propyl sulfides/disulfides, diallyl/methyl propyl/methyl propenyl/dimethyl/allyl methyl/allyl propyl/allyl 1-propenyl trisulfides, 4-methyl-1,2,3trithiolane, 2/3-vinyl-4*H*-1,2/1,3-dithiines, 1,2,3trithia-4-cyclohexene, allicin. methyl (methylsulfinyl)methyl sulfide, 5-methyl-1,2,3,4-tetrathiane, 1,4-dihydro-2,3diallyl/dimethyl/allyl benzoxathiin 3-oxide, methyl tetrasulfide, 4-methyl-1,2,3,5,6pentathiepane and cyclooctasulfur. It appears that the antimicrobial properties of Brassica and Allium families originate from their volatile sulfur compounds [28, 29]. Blocking effects of diallyl trisulfide, diallyl disulfide, dimethyl trisulfide, diallyl tetrasulfide and allyl isothiocyanate, which are among the compounds present in Allium and Brassica species, have been proven against S. aureus, E. coli, E. aerogenes, L. mesenteroides, P. pentosaceus, L. plantarum, C. albicans, C. utilis, S. cerevisiae, P. membranifaciens, Z. bisporus and Z. rouxii with MICs of from 1 to above 1000 ppm [30]. These components react with thiol (SH) groups of cell

wall proteins to inhibit the growth microorganisms [31]. Antibacterial and antifungal potentials of ethanolic extracts of Allium sativum L. from China and Turkey were investigated on bacterial species including Enterobacter, Bacillus, Listeria, Enterococcus, Salmonella, Klebsiella, Escherichia, Pseudomonas and Staphylococcus as well as C. albicans yeast [32]. Inhibition zones were ranged from 7 to 30 mm; Turkish samples was more effective than Chinese samples in killing microorganisms. Antimicrobial effects of essential oils of an A. sativum and three A. cepa (red, yellow and green) in the concentration range of 50-500 ml/l were assessed on S. Enteritidis, S. aureus, P. cyclopium, F. oxysporum and A. niger strains [33]. A. sativum and green A. cepa were identified as the best and worst antimicrobial agents, respectively. The lowest sensitivities were observed with essential oils against F. oxysporum and S. aureus.

A direct relationship is usually observed between antimicrobial properties and antioxidant potentials of compounds. The antioxidant ability of plants is mainly dependent on their phenolic content. These phenolic compounds prevent oxidative stress via reduction and neutralization of free radicals, coordination to metal ions, and scavenging singlet and triplet oxygen [34]. Phenolic compounds play an important role in the antimicrobial properties of plants. They kill microorganisms by destroying cell walls and proteins, membrane and enzyme dysfunction, and disrupting DNA and RNA replication [35]. It is found that the antibacterial properties of essential oils can be caused by the following factors: 1) The hydrophobic property of the essential oil allows it to penetrate the bacterial cell membrane and causes the release of ions and other internal components of the cells; 2) Phenolic derivatives present in the essential oils such as carvacrol, eugenol, and thymol destroy the cytoplasmic membrane of the cell and interfere with the proton and electric current; 3) Some components especially cinnamaldehyde can attach to cell proteins such as decarboxylase to prevent its vital function; 4) The presence of alphatriptin and molecules containing SH group is effective in inhibiting the growth of many microorganisms [36].

#### 5. Conclusion

The antioxidant capacity and antimicrobial activity of essential oils of four *Allium* species and *F. garcinia*, *D. verna*, and *Th. arvense* (Brassicaceae) from Iran were determined for the first time. Moderate antioxidant and moderate to excellent antimicrobial effects were observed with them. Although AZA showed the best antioxidant effects, no antimicrobial effect was observed with it on tested microbial strains. FFH showed more effective and broad-spectrum antimicrobial effects compared to others. Essential oil of FFH can be introduced as a potent antimicrobial agent, its inhibitory effects may be ameliorated by climate change.

#### **Author contributions**

H. B. supervised the data collection and prepared the final version of the manuscript. M. D. and A. D. contributed to the plant sampling and identification as well as manuscript preparation. N. M. prepared the essential oils. Z. E. collected the laboratory data. All authors have approved the final version of the manuscript.

#### **Conflicts of interest**

The authors declare no conflict of interest associated with this manuscript. The authors alone are responsible for the content of the paper.

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#### References

- **1.** Bolouri P, Salami R, Kouhi S, Kordi M, Asgari Lajayer B, Hadian J and Astatkie T. Applications of essential oils and plant extracts in different industries. *Molecules* 2022; 27(24): 8999. doi: 10.3390/molecules27248999.
- **2.** Tisserand R and Young R. Essential oil safety: a guide for health care professionals. Amsterdam: Elsevier Health Sciences; 2<sup>nd</sup> Ed. 2013.
- **3.** Dreger M and Wielgus K. Application of essential oils as natural cosmetic preservatives. *Herba Pol.* 2013; 59(4): 142-156. doi: 10.2478/hepo-2013-0030.
- **4.** Olujimi OO, Fatoki OS, Odendaal JP and Okonkwo JO. Endocrine disrupting chemicals (phenol and phthalates) in the South African environment: a need for more monitoring. *Water SA* 2010; 36(5): 671-682. doi: 10.4314/wsa.v36i5.62001.
- **5.** Carson PA. Hazardous chemicals handbook. Amsterdam: Elsevier; 2<sup>nd</sup> Ed. 2002.
- **6.** Vergis J, Gokulakrishnan P, Agarwal RK and Kumar A. Essential oils as natural food antimicrobial agents: a review. *Crit. Rev. Food Sci. Nutr.* 2015; 55(10): 1320-1323. doi: 10.1080/10408398.2012.692127.
- **7.** Bhavaniramya S, Vishnupriya S, Al-Aboody MS, Vijayakumar R and Baskaran D. Role of essential oils in food safety: Antimicrobial and antioxidant applications. *Grain Oil Sci. Technol.* 2019; 2(2): 49-55. doi: 10.1016/j.gaost.2019.03.001.
- **8.** Block E. Garlic other alliums. Cambridge: RSC publishing; 2010.
- **9.** Li Q-Q, Zhou S-D, He X-J, Yu Y, Zhang Y-C and Wei X-Q. Phylogeny and biogeography of *Allium* (Amaryllidaceae: Allieae) based on nuclear ribosomal internal transcribed spacer and chloroplast rps16 sequences, focusing on the inclusion of species endemic to China. *Ann. Bot.*

- 2010; 106(5): 709-733. doi: 10.1093/aob/mcq177.
- **10.** Dolatyari A, Moazzeni H and Fritsch RM. Karyological studies of Iranian *Allium* (Amaryllidaceae) species with a focus on sect. *Acanthoprason*. 2. Meiotic chromosomes. *Plant Biosyst*. 2023; 157(4): 821-834. doi: 10.1080/11263504.2023.2200779.
- **11.** Dolatyari A, Saeidi Mehrvarz S, Shahzadeh Fazeli SA, Naghavi MR and Fritsch RM. Karyological studies of Iranian *Allium* L. (Amaryllidaceae) species with focus on sect. *Acanthoprason*. 1. Mitotic chromosomes. *Plant Syst. Evol*. 2018; 304(5): 583-606. doi: 10.1007/s00606-017-1489-5.
- **12.** Ye C-L, Dai D-H and Hu W-L. Antimicrobial and antioxidant activities of the essential oil from onion (*Allium cepa* L.). *Food Control* 2013; 30(1): 48-53. doi: 10.1016/j.foodcont.2012.07.033.
- **13.** Li M, Zhao X and Xu M. Chemical composition, antimicrobial and antioxidant activity of essential oil from *Allium tenuissimum* L. flowers. *Foods* 2022; 11(23): 3876. doi: 10.3390/foods11233876.
- **14.** Yasin G, Jasim SA, Mahmudiono T, Al-Shawi SG, Shichiyakh RA, Shoukat S, Kadhim AJ, Iswanto AH, Saleh MM and Fenjan M. Investigating the effect of garlic (*Allium sativum*) essential oil on foodborne pathogenic microorganisms. *Food Sci. Technol.* 2022; 42: 1-6. doi: 10.1590/fst.03822.
- **15.** Hedge IC and Rechinger KH. Cruciferae. In: Rechinger KH. Flora Iranica. Graz: Akademische Druck- u. Verlagsanstalt; 1968: 372-408.
- **16.** Beyzaei H, Khosravi Z, Aryan R and Ghasemi B. A green one-pot synthesis of 3(5)-substituted 1,2,4-triazol-5(3)-amines as potential antimicrobial agents. *J. Iran. Chem. Soc.* 2019;

- 16(12): 2565-2573. doi: 10.1007/s13738-019-01714-2.
- **17.** Ebrahimnezhad Z, Dehghani M and Beyzaei H. Assessment of phenolic and flavonoid contents, antioxidant properties, and antimicrobial activities of *Stocksia brahuica Benth. Int. J. Basic Sci. Med.* 2022; 7(1): 34-40. doi: 10.34172/ijbsm.2022.07.
- **18.** Beyzaei H, Kamali Deljoo M, Aryan R, Ghasemi B, Zahedi MM and Moghaddam-Manesh MR. Green multicomponent synthesis, antimicrobial and antioxidant evaluation of novel 5-amino-isoxazole-4-carbonitriles. *Chem. Cent. J.* 2018; 12(1): 1-8. doi: 10.1186/s13065-018-0488-0.
- **19.** Sadat-Hosseini M, Farajpour M, Boroomand N and Solaimani-Sardou F. Ethnopharmacological studies of indigenous medicinal plants in the south of Kerman, Iran. *J. Ethnopharmacol.* 2017; 199: 194-204. doi: 10.1016/j.jep.2017.02.006.
- **20.** Ullah I, Iqbal T, Ullah F, Aslam MM, Mehmood S, Khan M, Rehman SU and Hussain M. Phytochemical screening, antimicrobial and antioxidant properties of *Douepia tortuosa* Camb., a crucifer endemic to Pakistan. *Pak. J. Bot.* 2024; 56(3). doi: 10.30848/pjb2024-3(39).
- **21.** Zarei H, Fakheri BA, Naghavi MR and Mahdinezhad N. Phylogenetic relationships of Iranian *Allium* species using the matK (cpDNA gene) region. *J. Plant Biotechnol*. 2020; 47(1): 15-25. doi: 10.5010/JPB.2020.47.1.015.
- **22.** Thakur A, Singh S, Dulta K, Singh N, Ali B, Hafeez A, Vodnar DC and Marc RA. Nutritional evaluation, phytochemical makeup, antibacterial and antioxidant properties of wild plants utilized as food by the Gaddis-a tribal tribe in the Western Himalayas. *Front. Agron.* 2022; 4: 1010309. doi: 10.3389/fagro.2022.1010309.
- **23.** Barbu IA, Ciorîță A, Carpa R, Moț AC, Butiuc-Keul A and Pârvu M. Phytochemical

- characterization and antimicrobial activity of several *Allium* extracts. *Molecules* 2023; 28(10): 3980. doi: 10.3390/molecules28103980.
- **24.** Karunanidhi A, Ghaznavi-Rad E, Jeevajothi Nathan J, Abba Y, Belkum AV and Neela V. *Allium stipitatum* extract exhibits in vivo antibacterial activity against methicillin-resistant Staphylococcus aureus and accelerates burn wound healing in a full-thickness murine burn model. *eCAM*. 2017; 2017: 1914732. doi: 10.1155/2017/1914732.
- **25.** Ncube NS, Afolayan AJ and Okoh AI. Assessment techniques of antimicrobial properties of natural compounds of plant origin: current methods and future trends. *Afr. J. Biotechnol.* 2008; 7(12): 1797-1806. doi: 10.5897/AJB07.613.
- **26.** Hadacek F and Greger H. Testing of antifungal natural products: methodologies, comparability of results and assay choice. *Phytochem. Anal.* 2000; 11(3): 137-147. doi: 10.1002/(SICI)1099-1565 (200005/06)11:3 <137::AID-PCA514>3.0.CO;2-I.
- **27.** Satyal P, Craft JD, Dosoky NS and Setzer WN. The chemical compositions of the volatile oils of garlic (*Allium sativum*) and wild garlic (*Allium vineale*). *Foods* 2017; 6(8): 63. doi: 10.3390/foods6080063.
- **28.** Kyung KH. Antimicrobial activity of volatile sulfur compounds in foods. In: Qian MC, Fan X and Mahattanatawee K. Volatile Sulfur Compounds in Food. Washington: American Chemical Society; 2011: 323-338. doi: 10.1021/bk-2011-1068.ch016.
- **29.** Kim S, Kubec R and Musah RA. Antibacterial and antifungal activity of sulfurcontaining compounds from *Petiveria alliacea* L. *J. Ethnopharmacol.* 2006; 104(1-2): 188-192. doi: 10.1016/j.jep.2005.08.072.
- **30.** Kim JW, Kim YS and Kyung KH. Inhibitory activity of essential oils of garlic and onion

against bacteria and yeasts. *J. Food Prot.* 2004; 67(3): 499-504. doi: 10.4315/0362-028X-67.3.499.

- **31.** Kyung KH. Antimicrobial properties of *Allium* species. *Curr. Opin. Biotechnol.* 2012; 23(2): 142-147. doi: 10.1016/ j.copbio. 2011.08.004.
- **32.** Yetgin A, Canlı K and Altuner EM. Comparison of antimicrobial activity of *Allium sativum* cloves from China and Taşköprü, Turkey. *Adv. Pharmacol. Pharmaceu. Sci.* 2018; 2018: 9302840. doi: 10.1155/2018/9302840.
- **33.** Benkeblia N. Antimicrobial activity of essential oil extracts of various onions (*Allium cepa*) and garlic (*Allium sativum*). *LWT-Food Sci. Technol.* 2004; 37(2): 263-268. doi: 10.1016/j.lwt.2003.09.001.
- **34.** Rudrapal M, Khairnar SJ, Khan J, Dukhyil AB, Ansari MA, Alomary MN, Alshabrmi FM, Palai S, Deb PK and Devi R. Dietary polyphenols and their role in oxidative stress-induced human

diseases: insights into protective effects, antioxidant potentials and mechanism(s) of action. *Front. Pharmacol.* 2022; 13: 806470. doi: 10.3389/fphar.2022.806470.

35. Foroughi A, Pournaghi P, Zhaleh M, Zangeneh A, Zangeneh MM and Moradi R. Antibacterial activity and phytochemical screening of essential oil of Foeniculum vulgare. Int. J. Pharm. Clin. Res. 2016; 8(11): 1505-1509. 36. Swamy MK, Akhtar MS and Sinniah UR. Antimicrobial properties of plant essential oils against human pathogens and their mode of action: an updated review. Evid. Complement. Alternat. Med.2016; 2016: 3012462. doi: 10.1155/2016/3012462.

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مقاله تحقيقاتي

فعالیت های ضدمیکروبی و آنتی اکسیدانی اسانس هایی از هفت گونه نرگسیان و شب بویان زهرا ابراهیم نژاد'، نفیسه مهدی نژاد'، حمید بیضائی ۱۰۰۰، مهدی دهقانی ۱، علیرضا دولتیاری ۴

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#### اطلاعات مقاله

گل واژگان: Allium عوامل ضدباكتريايي عوامل ضدقارچي آنتی اکسیدان ها اسانسها Fortuynia garcinia

مقدمه: اسانسها در طول تاریخ جهت مصارف مختلفی مورد استفاده قرار گرفتهاند. این مواد معطر در طب جایگزین، رایحه درمانی و محصولات مراقبت شخصی به طور فزایندهای محبوب شدهاند. هدف: در این مطالعه، اسانس هایی از اندامهای هوایی چهار گونه Allium و سه گونه Brassicaceae شامل Brassicaceae Draba verna و Thlaspi arvense از نظر خواص آنتی اکسیدانی، ضدباکتریایی و ضدقارچی مورد ارزیابی قرار گرفتند. **روش بررسی**: خواص مهارکنندگی رادیکالی با استفاده از آزمون DPPH مورد آزمایش قرار گرفت. فعالیت ضدمیکروبی اسانسها علیه نه عامل بیماریزای استاندارد: سه باکتری گرم-مثبت شامل استافیلوکوکوس اورئوس، باسيلوس سرئوس، استر پتوكوكوس پيوژنز، سه باكترى گرم-منفى شامل اشريشيا كلى، سودوموناس أئروژينوزا، سالمونلا انتريكا زير گونه انتريكا و دو قارچ آسپرژيلوس فوميگاتوس، فوساريوم اكسى پروم و همچنين مخمر کاندیدا آلبیکنس مورد بررسی قرار گرفت. ir قرار گرفت. نتایج: مقادیر  $IC_{50}$  سنجش آنتی اکسیدانی در گستره ۱۲۴/۶۶ تا ۱۵۵/۰۴ میکروگرم بر میلی لیتر بودند. Allium zagricum با ۱۲۴/۶۶ IC50 میکروگرم بر میلی لیتر بهترین اثرات آنتی اکسیدانی را در برابر ویتامین E استاندارد (با ۱۰/۴۰ IC<sub>50</sub> میکروگرم بر میلی لیتر) نشان داد. به همین ترتیب، مقادیر ۴۰۰-۲۰۰ MIC میکروگرم بر میلی لیتر به عنوان بهترین اثرات ضدمیکروبی از میوههای Fortuynia garcinii–زاهدان کسب گردید، درحالیکه از مقادیر MIC ثبت شده برای کنترلهای مثبت (۱۶–۰/۰۶ برای آمیکاسین و ۲۵۶–۳۲ برای کلوتریمازول) بالاتر بودند. بهترتیب بهترین اثر آنتیاکسیدانی و ضدمیکروبی را نشان دادند. نتیجه گیری: اسانسهای مستخرج از Allium zagricum و Fortuynia garcinii را می توان در درمان بیماری های عفونی و مرتبط با استرس اکسایشی تجویز کرد.

مخففها: FFH، ميوههاى Fortuynia garcinii-زاهدان؛ FFB، ميوههاى Fortuynia garcinii-زابل؛ FLB، برگهاى Frotuynia garcinii، خابل؛ Allium alamutense Razyfard, Zarre AAR Allium iranshahrii AIR :Thlaspi arvense L. ،TAL :Draba verna L. ،DVL VIE 'Allium zagricum 'AZA 'Allium chrysantherum 'ACH، ويتامين ۴ AMK، أميكاسين؛ CLT، كلوتريمازول؛ PPH؛ ۲،۲-دى فنيل-١-پيكريل هيدرازيل؛ MIC، حداقل غلظت بازدارندگي؛ MBC، حداقل غلظت كشندگي باكتريايي؛ MFC، حداقل غلظت كشندگي قارچي؛ PTCC، مركز منطقهای میكروارگانیسمهای ایران؛ IBRC، مركز منابع زیستی ایران؛ UOZH، هرباریوم دانشگاه زابل؛ SD، انحراف استاندارد؛ -GC MS، كروماتوگرافي گازي-طيف سنجي جرمي؛ EE، راندمان استخراج اسانس؛ DMSO، ديمتيل سولفوكسيد؛ ABTS، ۲،۲'-آزينو-بيس(اتيل بنزوتيازولين-۶-سولفونيک اسيد)

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