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Scientific Research

Investigating the antioxidant potential and antimicrobial effect of Roman (*Anthemis nobilis*) chamomile essential oil: "*in vitro*"

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ABSTRACT

In this study, after preparing Roman chamomile (*Anthemis nobilis*) essential oil, the total phenol and flavonoid content, antioxidant properties, and antimicrobial effects were evaluated using four methods: disk diffusion agar, well diffusion agar, minimum inhibitory concentration, and bactericidal inhibitory concentration. The study included various bacteria such as *Bacillus cereus*, *Streptococcus pyogenes*, *Staphylococcus aureus*, *Shigella dysenteriae*, *Enterobacter aerogenes*, and *Salmonella typhimurium*. The total phenol content was 33.50 mg gallic acid per gram of essential oil, and the total flavonoid content was 14.60 mg quercetin per gram of extract. The antioxidant activity of *A. nobilis* essential oil was 51.70% in the DPPH radical scavenging method and 57.90% in the ABTS radical scavenging method. Among all antimicrobial methods, the essential oil exhibited the highest antimicrobial effect against Gram-positive bacteria *Staphylococcus aureus* and the least effect against *Shigella dysenteriae*. The results suggest that *A. nobilis* essential oil can be used in the production of food and pharmaceutical products as an antioxidant and antimicrobial compound.

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1- Introduction

The emergence of resistance to common antimicrobial compounds is a serious challenge faced by physicians. This necessitates continuous development of newer agents that can inhibit the growth of resistant organisms. Herbal plants have been used for centuries and their therapeutic efficacy has been widely described [1]. Recently, researchers estimate that there are approximately 400,000 plant species worldwide, with about one-third to one-fourth of them utilized by companies for medicinal purposes. For thousands of years, plant products and their modified derivatives have been rich sources for clinically useful drugs. Even today, a significant portion of the global population relies primarily on plants and herbal extracts for healthcare. Fragrant plants have long been recognized and are used as natural flavorings, preservatives for food, in perfumery, and for various medical purposes due to their aromatic and antimicrobial properties [2]. Essential oils derived from plants are recognized as Generally Recognized as Safe (GRAS) compounds and are among the most important natural products extracted from various plants. Due to their high antimicrobial and antioxidant properties, plant essential oils are often used in the food industry as flavor enhancers, antioxidants, and antibacterial agents [3, 4].

Roman chamomile, scientifically known as *Anthemis nobilis*, is a plant from the daisy family (*Asteraceae*) or *Compositae*. It grows to a height of 20 to 30 centimeters and is highly aromatic. The stems of this plant are greenish-white, and its leaves are covered with fine hairs. Roman chamomile contains compounds such as essential oils, tannins, terpenoids, phytosterols, flavonoids, azulene, sulfur, and coumarins [5, 6]. The essential oil from this plant has anti-inflammatory, antihistaminic, and antispasmodic effects. Due to its valerenic acid and cyanogenic glycosides, it also has calming properties. Two hydroperoxide compounds isolated from Roman chamomile exhibit moderate antibacterial activity [7].

Given the existing properties of *A. nobilis*, the aim of this research was to prepare an essential oil from this plant and investigate its total phenol content, total flavonoid content, antimicrobial properties, and antioxidant activity.

2-Materials and methods

2.1. Microbial strains and chemicals used

The microbial strains used included *Enterobacter aerogenes*, *Salmonella typhimurium*, *Shigella dysenteriae*, *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Bacillus cereus*, obtained from the microbial collection of the Food Science and Engineering Department at Khuzestan University of Agricultural Sciences and Natural Resources. The following chemicals were prepared: quercetin solution, Folin-Ciocalteu reagent, ABTS solution, and DPPH solution from Sigma (USA); Muller-Hinton agar, Muller-Hinton broth, and blank discs from Merck (Germany); and 96% ethanol from Ghadir Chemical Industries (Iran). Other chemicals used were of laboratory grade.

2.2. Preparation of chamomile essential oil

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To prepare the essential oil, powdered chamomile plant material was subjected to water distillation in a Clevenger apparatus for 3 hours. The obtained essential oil was stored in dark containers under cool conditions [8].

2.3. Measurement of total phenol content

In this method, 20 µL of *A. nobilis* essential oil was mixed with 110 µL of Folin-Ciocalteu reagent. Next, 70 µL of sodium carbonate solution was added to the sample. After 30 min at room temperature, its absorption at 760 nm was recorded. Gallic acid was used to create the standard curve. The total phenol content was reported in mg of gallic acid per gram of essential oil (mg GAE/g) [9].

2.4. Determination of total flavonoid content

1.0 mL of essential oil (equivalent to 1.0 mg) or quercetin (ranging from 0 to 5.0 mg) was mixed with 3.0 mL of 5% sodium nitrite solution. Then, 3.0 mL of 10% w/v aluminum trichloride was added. After 6 min, 2.0 mL of 1 M sodium hydroxide was added. Finally, the absorption of the sample was measured at 510 nm. The total flavonoid content was reported in mg of quercetin per gram of essential oil (mg QE/g) [10].

2.5. Measurement of antioxidant activity

2.5.1. Measurement of DPPH free radical scavenging

In this method, 2.0 mL of the sample was mixed with 1.0 mL of 0.2 mM DPPH solution in ethanol. The samples were kept in a dark place at room temperature for 30 min. The absorption of the sample was measured at 517 nm. The control sample was prepared according to the mentioned method, with the difference being that distilled water was used instead of the sample. Finally, the antioxidant activity of *A. nobilis* essential oil against DPPH radicals was calculated using the following formula [11]:

$$\text{Activity (\%)} = \frac{\textit{Abs sample} - \textit{Abs control}}{\textit{Abs control}} \times 100$$

2.5.2 Measurement of ABTS free radical scavenging

After preparing the ABTS cation, the obtained essential oil was mixed with ABTS and left in the dark for 10 min. Then, the absorption of the sample was recorded at 734 nm, and the inhibition power (%) was measured according to the following formula [12]:

$$\text{Activity (\%)} = \frac{\textit{Abs sample} - \textit{Abs control}}{\textit{Abs control}} \times 100$$

2.6. Measurement of antimicrobial activity

2.6.1. Disk diffusion agar

After culturing the microorganisms on Muller-Hinton agar plates, disks impregnated with the essential oil were placed on the agar medium. After 24 hours of incubation at 37°C, the diameter of growth inhibition zones was measured in mm [13].

2.6.2 Well diffusion agar

After creating wells with a diameter of 6 mm in plates containing Muller-Hinton agar, microbial suspensions were inoculated onto the surface of the agar. Twenty µL of the prepared concentrations were poured into the wells. The plates were incubated at 37°C for 24 hours. At the end of the incubation period, the diameter of non-growth zones around the wells was measured in mm using a ruler [14].

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2.6.3. Minimum inhibitory concentration (MIC)

Essential oil concentrations of 2, 4, 8, 16, 32, 64, 128, 256, and 512 mg/mL were prepared in Muller-Hinton broth. Then, 100 µL of each concentration and 10 µL of each of the used bacteria were added to 96-well plates. The plates were incubated at 37°C for 24 hours. After incubation, the lowest concentration at which no color change was observed was reported as the minimum inhibitory concentration [15].

2.6.4. Minimum bactericidal concentration (MBC)

For wells without color change in the minimum inhibitory concentration test, 100 µL of the sample was added to plates containing Muller-Hinton agar. The plates were incubated at 37°C for 24 hours. At the end of the incubation period, triphenyl tetrazolium chloride (5 mg/mL) was added to each well, and the plates were incubated for 30 min. The lowest concentration at which no color change was observed was reported as the minimum bactericidal concentration [16].

2.7. Statistical analysis

The results were analyzed using one-tailed variance in SPSS version 18. The significance of differences between means was examined using the Duncan test at a 95% confidence level ($p<0.05$). All tests were conducted in triplicate.

3-Results and discussion

The total phenol content of *A. nobilis* essential oil was 33.50 mg of gallic acid per gram of extract, and the total flavonoid content was 14.60 mg of quercetin per gram of extract (Figure 1). The phenol content of cultivated *A. nobilis* in Iraq was reported to be 33.05% [17]. Additionally, the phenol content in the extracts of *Anthemis cretica subsp. argaea* and *Anthemis fumariifolia* was 48.51% and 31.94%, respectively, while the flavonoid content in these two chamomile species was 11.49% and 12.88%, respectively [18].

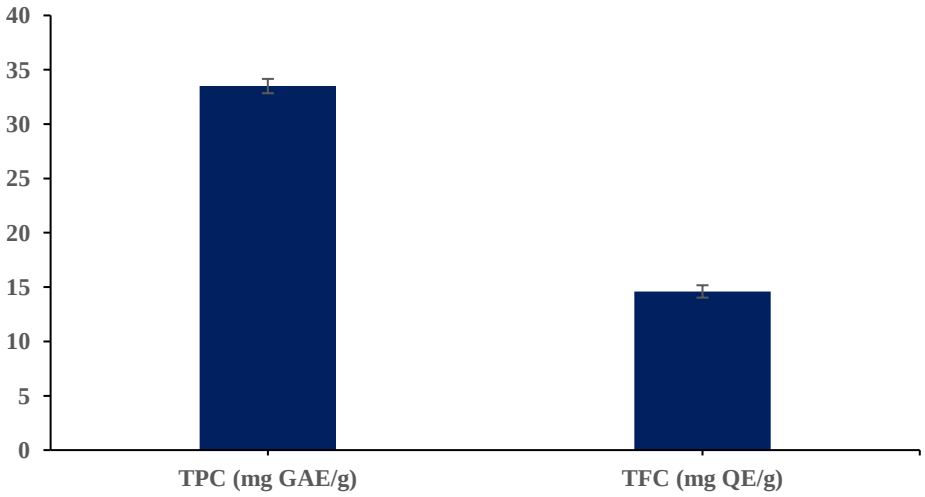

Figure 1. Total phenol content (TPC) and total flavonoid content (TFC) of *A. nobilis* essential oil. GAE = Gallic acid equivalent; QE = Quercetin equivalent.

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The antioxidant activity of *A. nobilis* essential oil was evaluated using both the DPPH and ABTS free radical scavenging methods (Figure 2). The inhibition power against DPPH radicals was 70.51%, and against ABTS radicals, it was 90.57%. In a specific study, the antioxidant activity of *A. nobilis* extract was found to be higher than that of the synthetic antioxidant butylated hydroxytoluene [19]. Other studies have also reported the antioxidant activity of chamomile, including research by Yue et al. (2021), Huiwen et al. (2022), and Albayrak and Aksoy (2013) [18, 20, 21]. The variation in antioxidant activity and phenolic compounds among different chamomile species is attributed to factors such as cultivation methods, harvest time, extraction techniques, climatic conditions, drying methods, and differences in measurement methods for phenolic compounds and antioxidant activity [22, 23].

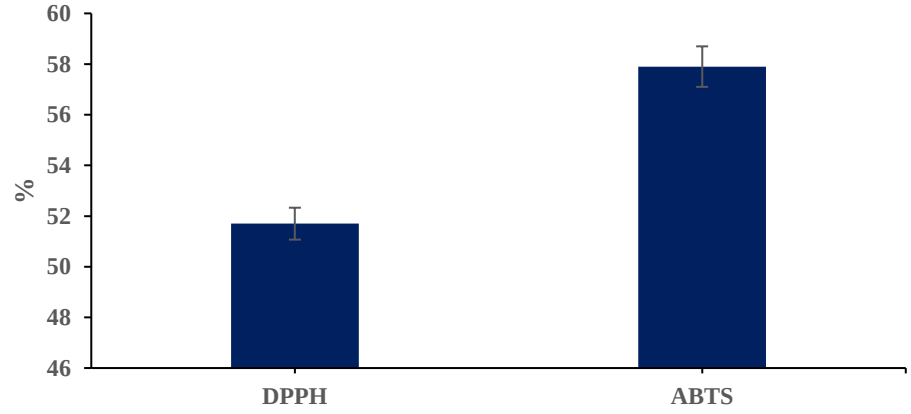

Figure 2. Antioxidant effect of *A. nobilis* essential oil based on DPPH and ABTS radical scavenging methods.

The results of the antimicrobial activity of *A. nobilis* essential oil using the disk diffusion method are shown in Figure 3. It can be observed that *Staphylococcus aureus* exhibited the largest growth inhibition zone (80.18 mm), while *Shigella dysenteriae* had the smallest growth inhibition zone (20.10 mm), making them the most sensitive and resistant bacteria to the essential oil, respectively ($p<0.05$).

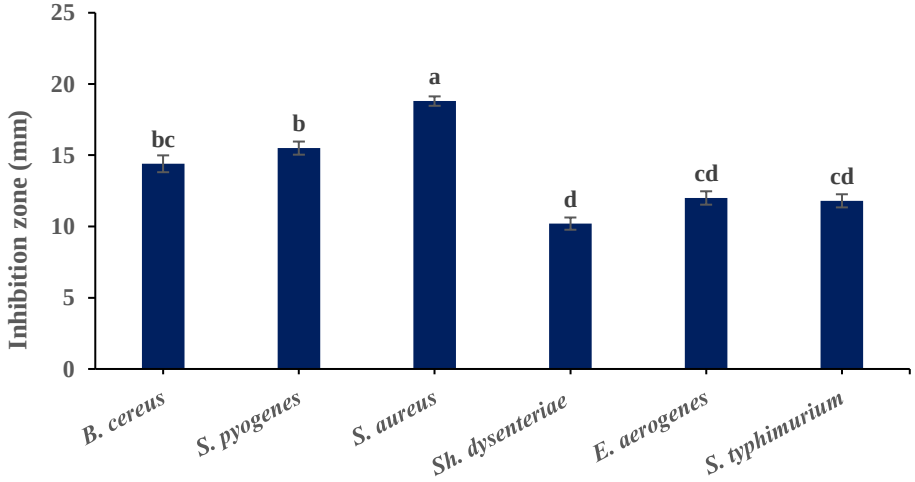

Figure 3. Antibacterial effect of *A. nobilis* essential oil based on disc diffusion agar method

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Furthermore, considering the results of the well diffusion agar test shown in Figure 4, it can be seen that in this method, *Staphylococcus aureus* also demonstrated the highest sensitivity with a growth inhibition zone diameter of 20.10 mm, while *Shigella dysenteriae* exhibited the highest resistance with a growth inhibition zone diameter of 90.10 mm ($p<0.05$).

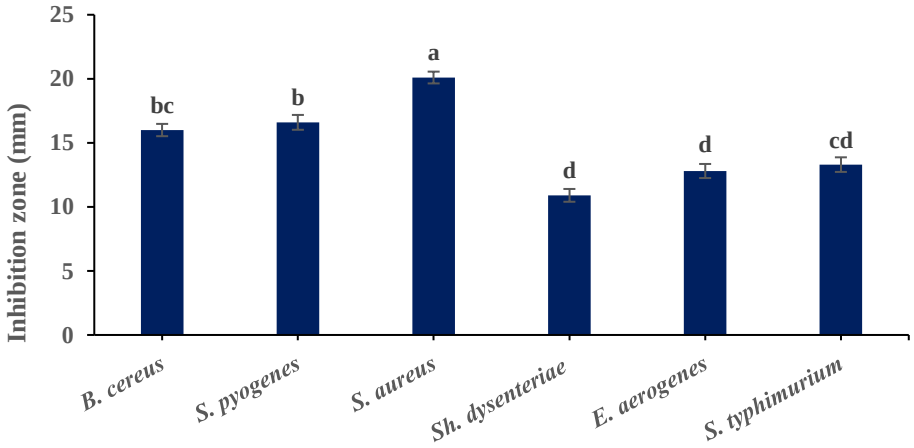

Figure 4. Antibacterial effect of *A. nobilis* essential oil based on well diffusion agar method.

A comparison between the disk diffusion agar and well diffusion agar tests reveals that the growth inhibition zones in the disk diffusion agar method are larger than those in the well diffusion agar method. This difference may be attributed to direct contact between the essential oil and microorganisms in the well diffusion agar method, resulting in a stronger effect on them. In contrast, in the disk diffusion method, the essential oil exhibits a weaker effect on bacteria after passing through the disk surface [24].The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) results for *A. nobilis* essential oil are shown in Table 1. It is observed that *Staphylococcus aureus* had the lowest MIC (4 mg/mL) and MBC (128 mg/mL), while *Shigella dysenteriae* exhibited the highest MIC (128 mg/mL) and MBC (greater than 512 mg/mL).

Table 1. Antibacterial effect of *A. nobilis* essential oil based on minimum inhibitory concentration and minimum bactericidal concentration methods.

Bacterial type	Minimum inhibitory concentration (mg/mL)	Minimum bactericidal concentration (mg/mL)
B. cereus	16	256
S. pyogenes	8	256
S. aureus	4	128
Sh. dysenteriae	128	> 512
E. aerogenes	64	> 512
S. typhimurium	64	> 512

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The greater antimicrobial effect of *A. nobilis* essential oil on Gram-positive bacteria (such as *Staphylococcus aureus*) compared to Gram-negative bacteria (such as *Shigella dysenteriae*) may be due to differences in morphological structures between these microorganisms. Gram-negative bacteria have an outer phospholipid membrane that carries lipopolysaccharide components, making their cell wall impermeable to antimicrobial chemicals. On the other hand, Gram-positive bacteria have only one outer layer of peptidoglycan, which is not an effective barrier to penetration. Therefore, the cell wall of Gram-negative organisms, being more complex than that of Gram-positive organisms, acts as a more effective barrier. Despite these differences in permeability, some extracts still exert varying degrees of inhibition against Gram-negative organisms [25]. Various studies have reported the antimicrobial properties of extracts and essential oils from different chamomile species. For instance, *A. nobilis* essential oil has demonstrated significant antimicrobial activity against bacteria such as *Staphylococcus epidermidis*, *Staphylococcus aureus*, and *Escherichia coli*, when compared to the antibiotic gentamicin [26]. Another study explored the antimicrobial effects of essential oils obtained from German chamomile (*Matricaria chamomilla*) and wild chamomile (*Matricaria recutita*) against bacteria including *Bacillus subtilis*, *Listeria monocytogenes*, *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella typhimurium* [27]. These findings highlight the potential of chamomile essential oil as a bioactive compound for nutraceutical and medical applications, given its antioxidant, antimicrobial, and antiproliferative activities. The greater antimicrobial effect on Gram-positive bacteria compared to Gram-negative bacteria may be attributed to differences in their morphological structures. Gram-negative bacteria have an outer phospholipid membrane that makes their cell wall impermeable to antimicrobial chemicals, whereas Gram-positive bacteria have only one outer layer of peptidoglycan, which is less effective as a barrier. Therefore, chamomile essential oil holds promise as a valuable component in pharmaceutical and food industries [28].

4- Conclusion

The results of this study indicate that *A. nobilis* essential oil contains significant amounts of phenolic and flavonoid compounds, resulting in high antioxidant activity. Additionally, its broad-spectrum antimicrobial effects cover a wide range of pathogenic microorganisms and spoilage agents. Although its antimicrobial effects are more pronounced against Gram-positive bacteria, it remains effective against Gram-negative bacteria. These findings position chamomile essential oil as a promising bioactive compound for various applications in the pharmaceutical and food sectors.

5-Acknowledgements

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بررسی پتانسیل آنتی اکسیدانی و اثر ضدمیکروبی اسانس بابونه رومی: مطالعه در شرایط آزمایشگاهی

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چکیده

در این مطالعه، پس از تهیه اسانس بابونه رومی (*Anthemis nobilis*) میزان فنول و فلاونوئید کل، خاصیت آنتی اکسیدانی و اثر ضد میکروبی آن به ٤ روش دیسک دیفیوژن، چاهک آگار، تعیین حداقل غلظت مهار کنندگی و کشندگی بر باکتری‌های مختلف شامل *باسیلوس سرئوس*، *استرپتوکوکوس پیوژنز*، *استافیلوکوکوس اورئوس*، *شیگلا دیسانتری*، *انتروباکتر ائروژنز* و *سالمونلا تیفی موریوم* مورد بررسی قرار گرفت. مقدار فنول کل برابر با ۳۳/۵ میلی‌گرم گالیک اسید در گرم اسانس و میزان فلاونوئید کل برابر با ۱٤/٦۰ میلی‌گرم کوئرستین در گرم عصاره به دست آمد. میزان خاصیت آنتی اکسیدانی اسانس بابونه رومی در روش مهار رادیکال آزاد DPPH برابر با ۵۱/۷۰ درصد و در مهار رادیکال آزاد ABTS برابر با ۵۷/۹۰ به دست آمد. در تمامی روش‌های ضد میکروبی اسانس تهیه شده بیشترین اثر ضد میکروبی را بر باکتری گرم مثبت *استافیلوکوکوس اورئوس* و کمترین اثر را بر باکتری *شیگلا دیسانتری* نشان داد. نتایج به دست آمده نشان می‌دهد که از اسانس گیاه بابونه رومی می‌تواند در تولید محصولات غذایی و دارویی به عنوان ترکیبی آنتی اکسیدان و ضد میکروب مورد استفاده قرار گیرد.

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