



## Evaluation of antioxidant activity, total phenol and flavonoid and antibacterial activity of German chamomile

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### ABSTRACT

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German chamomile (*Matricaria chamomilla* L.) is a well-known medicinal plant that is distributed worldwide and is widely used in traditional medicine for the treatment of various diseases. In this study, the essential oil of *M. chamomilla* was extracted by water distillation method and its total phenol content, total flavonoid content, antioxidant activity, and antimicrobial effect were investigated. The total phenol content of the essential oil was 39.70 mg GAE/g and its total flavonoid content was 18.80 mg QE/g. The antioxidant activity of the essential oil was evaluated based on two methods of inhibiting free radicals DPPH and ABTS; the essential oil was able to inhibit free radicals DPPH (58.60 %) and ABTS (61.60 %). The antimicrobial effect of the essential oil against *Bacillus cereus*, *Streptococcus pyogenes*, *Staphylococcus aureus*, *Shigella dysenteriae*, *Enterobacter aerogenes*, and *Salmonella typhimurium* was investigated by disk diffusion agar, well diffusion agar, minimum inhibitory concentration, and minimum bactericidal concentration. The findings of the antimicrobial activity of the essential oil by the disk diffusion agar and well diffusion agar, showed that *S. pyogenes* and *E. aerogenes* were the most sensitive and resistant microbial strains to *M. chamomilla* essential oil, respectively. The results of this study showed that the essential oil of *M. chamomilla* has shown strong antioxidant and antimicrobial activity, which can be a potential candidate for the preparation of antioxidant and antimicrobial drugs.

## 1. Introduction

Despite the use of various preservation methods, food poisoning remains a concern for both consumers and the food industry. Due to the resistance that pathogens create against antibiotics, there is an increasing interest in using natural antibacterial products for food preservation, such as plant and spice extracts and essential oils. In fact, raw natural extracts and essential oils and biologically active compounds of plant species used in traditional medicine may be valuable sources for such new preservatives [1-40].

In addition, in food processing, lipid oxidation not only reduces the nutritional and taste quality of foods, but also produces oxidized products such as free radicals, leading to various undesirable chemical reactions [13]. To prevent or delay this process, common synthetic antioxidants such as Butylated Hydroxyanisole (BHA), Butylated Hydroxytoluene (BHT), Propyl Gallate (PG), and Tert-Butylhydroquinone (TBHQ) have been used for over five decades. However, these synthetic antioxidants are suspected of having negative health effects [41]. Therefore, there is an increasing interest in studies of natural additives as potential antioxidants. Many sources of plant-based antioxidants have been studied in recent years. Among them, the antioxidant properties of many aromatic and medicinal plants have been effective in delaying the process of lipid peroxidation in oils and fatty foods and have attracted the attention of many research groups. Therefore, the demand for these plants in industrial and non-industrial countries is increasing, leading to an increase in their prices [2, 8, 14].

German chamomile (*Matricaria chamomilla* L.) is one of the important medicinal plants native to southern and eastern Europe. It also grows in Germany, Hungary, France, Russia, Yugoslavia, and Brazil. These plants can be found in North Africa, Asia, North and South America, Australia, and New Zealand. Hungary is the main producer of plant biomass and it grows abundantly in poor soils and is a source of income for the poor residents of these areas. The flower of this plant is mainly exported to Germany for essential oil extraction. Chamomile has been used in herbal medicines for thousands of years and is known in ancient Egypt, Greece, and Rome. This plant was considered by the Anglo-Saxons as one of the nine sacred plants given by God to humans. The flowers of German chamomile contain 0.2 to 1.9 % essential oil, which has various applications. Chamomile is mainly used as an anti-inflammatory, antiseptic, and also antispasmodic. German chamomile is a natural source of blue oil (essential oil). The flowers and flower heads are the main parts of essential oil production. The flower oil of chamomile is mainly composed of sesquiterpene derivatives (75 to 90 %). The main components of the essential oil extracted from the flowers are beta-farnesene, farnesol, alpha-bisabolol, chamazulene. The color of the essential oil determines its quality. The blue color of the essential oil is due to sesquiterpene [42].

Marino and colleagues (2001) tested the essential oils of marigold, mint, hyssop, chamomile, and mountain mint for inhibitory effects against 9 Gram-negative bacterial strains and six strains of Gram-positive bacteria. The essential oils of marigold, mint, hyssop, and chamomile also had bacteriostatic activity. It seems

that the essential oil of mountain mint is bactericidal at concentrations higher than 400 ppm, probably due to their high content in phenolic compounds. Bacteriostatic activity was more effective against Gram-positive bacteria. On the other hand, bactericidal activity was higher against Gram-negative bacteria [43].

Given the above, this study was conducted with the aim of extracting the essential oil of German chamomile and determining the amount of its phenolic and flavonoid compounds, as well as investigating the antioxidant and antimicrobial activity of the essential oil of this medicinal plant.

## 2. Materials and methods

### 2.1. Essential oil extraction

The German chamomile plant was collected from Fars province. The Clevenger apparatus was used for 4 hours to extract the essential oil from the German chamomile flower. The extraction was carried out in 500 mL of water and the resulting essential oil was stored at a temperature of 4 °C until use [44].

### 2.2. Total phenol and flavonoid contents

The total phenol and flavonoid content of the essential oil was evaluated using the method presented by Alizadeh Behbahani and colleagues [10]. To determine the total phenol content, 10 µL of the essential oil was mixed with 50 µL of Folin-Ciocalteu reagent and stirred for 3 min. Then, 300 µL of sodium bicarbonate was added and the solution was shaken for 2 h. The absorbance of the solution at a wavelength of 765 nm was obtained. Gallic acid solution (0-200 mg/L) was used as a standard. By comparing the absorbance

obtained from the essential oil with the calibration curve of the gallic acid solution, the total phenol content was obtained in terms of milligrams equivalent to gallic acid per gram of essential oil (mg GAE/g). On the other hand, the flavonoid content was calculated using the aluminum chloride method and the response was measured as milligrams equivalent to quercetin mg QE/g essential oil.

### 2.3. Antioxidant effect

The antioxidant activity of the essential oil was determined using the methods of inhibiting free radicals DPPH and ABTS and according to the methods presented by Adewusi and colleagues [45] with the necessary modifications. To evaluate the ability of the essential oil to inhibit the DPPH radical, a mixture of 185 µL of 0.135 mM DPPH solution in methanol and 15 µL of essential oil was prepared. The mixture was kept in the dark at room temperature for 30 min. Then, the absorbance of the mixture at a wavelength of 570 nm was measured. The ability of the essential oil to inhibit the DPPH radical was determined using the following formula:

$$\text{Inhibition (\%)} = [(A \text{ control} - A \text{ sample}) / A \text{ control}] \times 100$$

To determine the ABTS inhibition capacity of the essential oil, a stock solution was prepared by mixing equal volumes of 7 mM ABTS salt and 2.4 millimolar potassium persulfate. After incubating it in the dark for 16 hours at room temperature, the solution was diluted with methanol until its absorbance reached 0.706 at a wavelength of 734 nm. Then, the essential oil was mixed with 2 mL of the ABTS solution and the absorbance at a wavelength of 734 nm was recorded. The ABTS inhibition

capacity of the essential oil was calculated using the following formula:

$$\text{Inhibition (\%)} = [(A \text{ control} - A \text{ sample}) / A \text{ control}] \times 100$$

#### 2.4. Antimicrobial effect

The antimicrobial effect of German chamomile essential oil against *Bacillus cereus*, *Streptococcus pyogenes*, *Staphylococcus aureus*, *Shigella dysenteriae*, *Enterobacter aerogenes*, and *Salmonella typhimurium* was evaluated according to the methods of disk diffusion agar, well diffusion agar, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) [10, 11, 40].

In the disk diffusion agar test, the essential oil was sterilized using a 0.22 micrometer syringe filter. Then, blank disks were soaked in the essential oil for 15 min. In the inoculation stage, the above bacteria were used at an inoculation rate equivalent to the 0.5 McFarland standard. The microbial plates were rotated at a 60-degree angle to ensure that the microorganisms covered the entire surface of the medium. The disks that had been soaked in the essential oil were then placed on the surface of the medium. The culture medium was incubated at a temperature of 37 °C for 24 h and the diameter of the non-growth halo around the disks was measured.

In the well diffusion agar test, Mueller Hinton agar culture medium was prepared and poured into a Petri dish. Using an L-shaped spreader, some microbial suspension was spread on the culture medium. Then, several wells with a diameter of 6 mm were created on the surface of the culture medium. Next, 20 µL of essential oil was poured into the wells.

The culture medium was kept at a temperature of 37 °C for 24 h and finally, the diameter of the non-growth halo around the wells was measured.

To measure the minimum inhibitory concentration, 125 µL of microbial suspension (equivalent to 0.5 McFarland) was added to each well of a 96-well plate and then the essential oil (concentrations of 2, 4, 8, 16, 32, 64, 128, 256, and 512 mg/mL) was added. Incubation was carried out for 24 h at a temperature of 37 °C. Then, 25 µL of triphenyl tetrazolium chloride solution (5 mg/mL) was added to each well. A dark red color appeared in the wells where the microbe had grown. The lowest concentration at which no microbial growth and color change were observed was considered as the minimum inhibitory concentration.

To determine the minimum bactericidal concentration, 100 µL of medium from each well (no red color in the plate) was cultured on Mueller Hinton agar medium and incubated at a temperature of 37 °C for 24 hours. The minimum concentration that prevented colony formation was considered as the minimum bactericidal concentration.

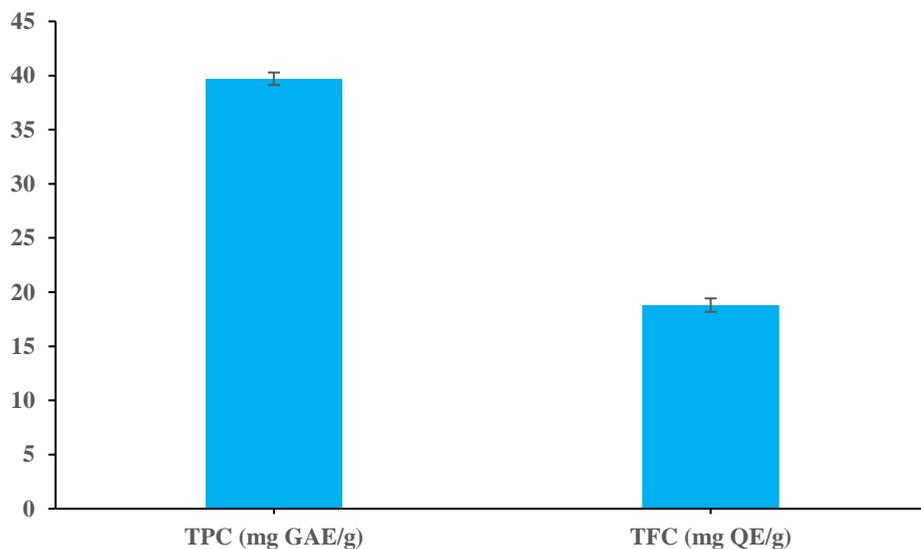
#### 2.5. Statistical analysis

The data were analyzed using Minitab software (version 21). Tukey's test was used at a 95% confidence level to determine the difference between the mean data. It should be noted that the experiments were repeated three times.

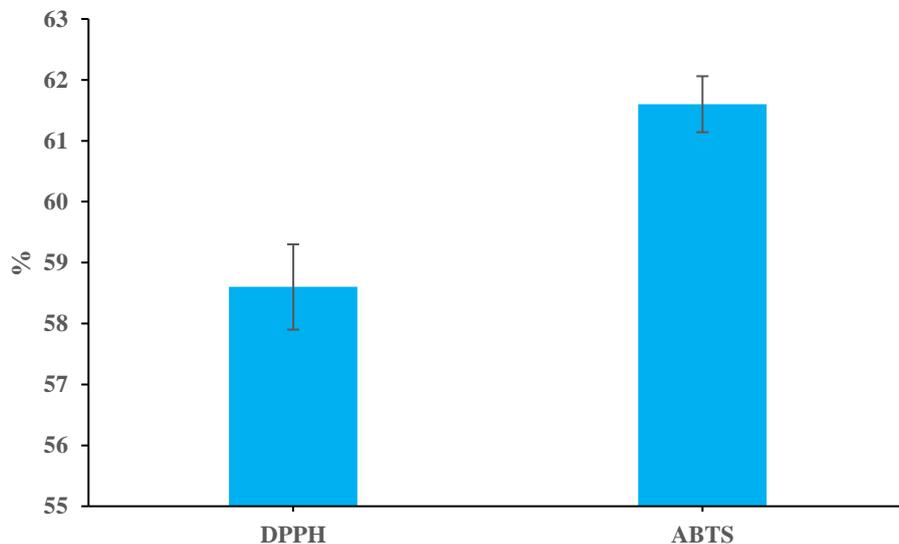
### 3. Results and discussion

The total phenol and flavonoid content of *M. chamomilla* essential oil were respectively equal to 39.70 mg GAE/g and 18.80 mg QE/g (Fig. 1). In addition, the antioxidant activity of *M. chamomilla*

essential oil based on DPPH and ABTS radical inhibition methods was respectively equal to 58.60% and 61.60% (Fig. 2).



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



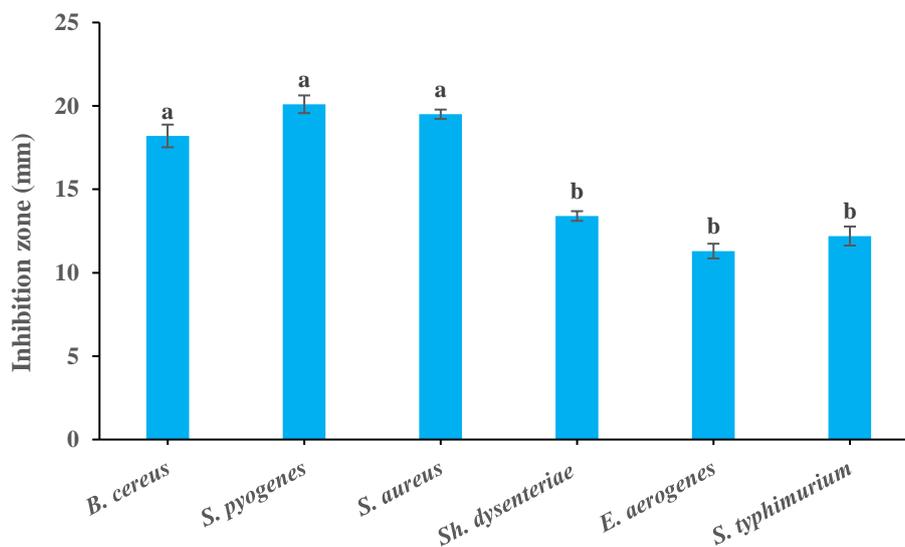
**Figure 2.** Antioxidant effect of *Matricaria chamomilla* essential oil based on DPPH and ABTS radical scavenging methods.

Fig. 3 shows the results of the antimicrobial effect of *M. chamomilla* essential oil based on the disk diffusion agar method. The average diameter of the non-growth halo

varied from 11.30 mm to 20.10 mm. *S. pyogenes* and *E. aerogenes* bacteria were respectively the most sensitive and resistant strains to *M. chamomilla* essential oil ( $p < 0.05$ ). In general, Gram-positive bacteria

(*B. cereus*, *S. pyogenes*, and *S. aureus*) were more sensitive to the essential oil compared

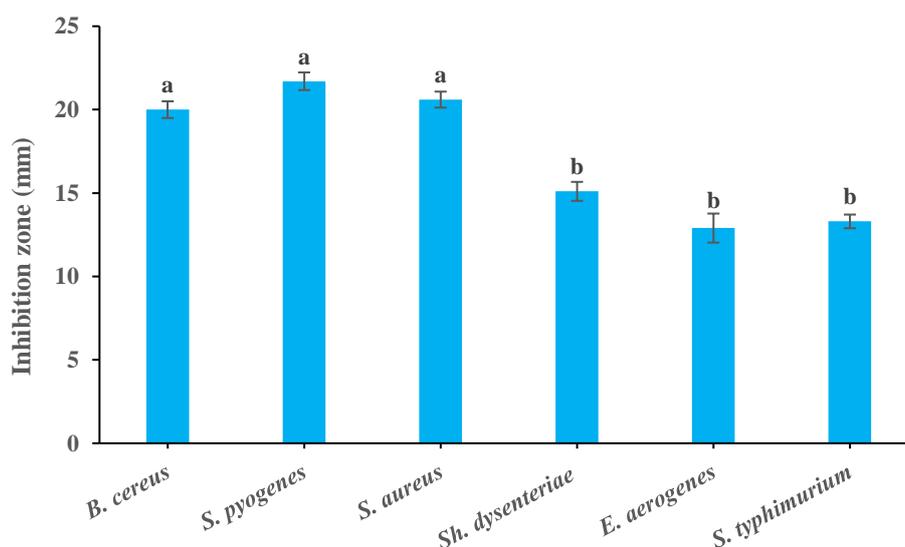
to Gram-negative types (*Sh. dysenteriae*, *E. aerogenes*, and *S. typhimurium*).



**Figure 3.** Antibacterial effect of *Matricaria chamomilla* essential oil based on disc diffusion agar method.

According to the results of the well diffusion agar test (Fig. 4), the average diameter of the non-growth halo for *B. cereus*, *S. pyogenes*, *S. aureus*, *Sh. dysenteriae*, *E. aerogenes*, and *S.*

*typhimurium* bacteria was respectively equal to 20, 21.70, 20.60, 15.10, 12.90, and 13.30 mm. In this regard, *S. pyogenes* and *E. aerogenes* bacteria were respectively the most sensitive and resistant strains to the essential oil, which is in line with the findings of the disk diffusion agar test.



**Figure 4.** Antibacterial effect of *Matricaria chamomilla* essential oil based on well diffusion agar method.

The results of the antimicrobial tests, minimum inhibitory concentration, and

minimum bactericidal concentration of the essential oil are presented in Table 1.

According to the results, Gram-positive bacteria had a lower minimum inhibitory concentration and minimum bactericidal concentration compared to Gram-negative bacteria, indicating their higher sensitivity to *M. chamomilla* essential oil.

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

Bacterial type	Minimum inhibitory concentration (mg/mL)	Minimum bactericidal concentration (mg/mL)
<i>B. cereus</i>	4	64
<i>S. pyogenes</i>	4	64
<i>S. aureus</i>	4	128
<i>Sh. dysenteriae</i>	32	> 512
<i>E. aerogenes</i>	64	> 512
<i>S. typhimurium</i>	64	> 512

The antioxidant and antimicrobial activity of *M. chamomilla* essential oil and extracts have been demonstrated in various studies. The antioxidant and antimicrobial activity of *M. chamomilla* essential oil in terms of their potential use as natural antioxidants and antimicrobial agents were investigated by Stanojevic and colleagues (2016). The results obtained confirmed the presence of 52 components, with the highest content of  $\beta$ -farnesene (29.8%),  $\alpha$ -farnesene (9.3%),  $\alpha$ -bisabolol and its oxide (15.7%), chamazulene (6.4%), germacrene D (6.2%), and spiroether (5.6%). The

antioxidant activity was investigated using the DPPH method and this amount was equal to 2.07 mg/mL. The antimicrobial activity of chamomile essential oil was tested using the agar diffusion method, where the essential oil showed a significant antibacterial effect with a non-growth halo diameter from 13.33 mm (in *Listeria monocytogenes*) to 40 mm (on *S. aureus*), and chamomile essential oil had no antimicrobial activity on *Pseudomonas aeruginosa* [46]. In addition, it has been reported that *M. chamomilla* essential oil, compared to the standards used BHT and  $\alpha$ -tocopherol, shows less antioxidant activity [47]. In another study on German chamomile, the essential oil and methanolic extract showed significant antioxidant activity using DPPH sensing and reducing power. This activity varied depending on environmental factors and chemical composition. In fact, the highest activity was achieved by essential oils rich in oxygenated compounds and extract with high phenolic content [48].

The antibacterial effect of *M. chamomilla* essential oil and extract was investigated in several studies. In general, the agar diffusion method, using disks or wells, has the most use for screening the antibacterial activity of essential oils and extracts. Using this technique, Stanojevic and colleagues (2016) reported the antibacterial activity of *M. chamomilla* essential oil. The most sensitive strain was *S. aureus* and the most resistant strain was *P. aeruginosa* [46]. Similarly, Owlia and colleagues [49] did not report any activity using the disk diffusion method against *P. aeruginosa*. However, the essential oil was able to reduce biofilm formation and alginate production and demonstrated its effectiveness in controlling biofilm-

producing bacteria. On the other hand, the results showed that *Bacillus subtilis* was the most sensitive bacteria to *M. chamomilla* essential oil grown in Morocco [50]. In the present study, Gram-positive bacteria showed the lowest minimum inhibitory concentrations. These results can be explained by the difference in cell wall structure as Gram-negative bacteria have a complex and rigid membrane rich in lipopolysaccharide that limits the access of antimicrobial molecules [13, 40].

#### 4. Conclusions

In this research, the antioxidant properties and antimicrobial activity of *M. chamomilla* essential oil were investigated. The antioxidant activity of the essential oil was significant and therefore *M. chamomilla* essential oil can help prevent and treat diseases. The antimicrobial activity of the essential oil was evaluated using the disk diffusion agar method, well diffusion agar, minimum inhibitory concentration, and minimum bactericidal concentration. *S. pyogenes* and *E. aerogenes* were respectively the most sensitive and resistant strains to the essential oil. Therefore, the essential oil under investigation can be recommended as a source of necessary medicinal materials for the preparation of new antimicrobial drugs. However, it is suggested that the effective compounds of the essential oil be identified using the method of gas chromatography coupled to mass spectrometry to provide a better understanding of the antimicrobial and antioxidant activity of the essential oil.

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## ارزیابی فعالیت آنتی اکسیدانی، فنول و فلاونوئید کل و فعالیت ضدباکتریایی بابونه آلمانی

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۱- دانشیار، گروه علوم و مهندسی صنایع غذایی، دانشکده علوم دامی و صنایع غذایی، دانشگاه علوم کشاورزی و منابع طبیعی خوزستان، ملاتانی، ایران.

اطلاعات مقاله	چکیده
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<p>کلمات کلیدی:</p> <p>بابونه آلمانی، اسانس، ضد میکروب، آنتی اکسیدان، ترکیبات فنولی</p> <p>DOI:10.22034/FSCT.21.154.139.</p> <p>* مسئول مکاتبات: Noshad@asnruk.ac.ir</p>	