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Scientific Research

Investigating the chemical and antimicrobial properties of *Anthemis cotula* essential oil against *Salmonella typhimurium*, *Enterobacter aerogenes*, *Staphylococcus aureus*, *Shigella dysenteriae*, *Bacillus cereus* and *Streptococcus pyogenes*

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ABSTRACT

This study aimed to extract essential oil from the medicinal plant *Anthemis cotula* (Mayweed) and evaluate its total phenolic content, total flavonoids, antioxidant properties, and antimicrobial activity. The analysis of total phenolic content in the essential oil using the Folin-Ciocalteu method revealed a value of 29.80 mg GAE/g. Additionally, the total flavonoid content was determined to be 13.47 mg QE/g. The essential oil exhibited significant antioxidant activity based on its ability to scavenge free radicals using the DPPH and ABTS methods. The antioxidant activity against DPPH and ABTS radicals was found to be 53% and 55%, respectively. Furthermore, the antimicrobial activity of the essential oil was assessed against various pathogenic bacteria, including *Salmonella typhimurium*, *Enterobacter aerogenes*, *Staphylococcus aureus*, *Shigella dysenteriae*, *Bacillus cereus*, and *Streptococcus pyogenes*. The disk diffusion agar, well diffusion agar, and minimum inhibitory concentration methods demonstrated that the essential oil exhibited remarkable antibacterial activity. Among the tested strains, *Staphylococcus aureus* and *Shigella dysenteriae* were the most sensitive and resistant, respectively, to the essential oil. These findings highlight that Mayweed essential oil serves as a natural source of bioactive compounds with antioxidant and antibacterial effects against pathogenic bacteria.

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1- Introduction

In recent years, the increase in therapeutic chemical failure and antibiotic resistance demonstrated by microbial pathogens has prompted the screening of medicinal plants for their potential antimicrobial activity. The research and utilization of drugs and dietary supplements derived from plants have rapidly expanded. Pharmacists, botanists, microbiologists, and chemists are actively exploring the natural world to discover plant-based chemical compounds that can be used for treating various diseases [1-10].

Additionally, some synthetic antioxidants, such as hydroxybutyl toluene and hydroxybutyl anisole, are commonly used in processed foods. However, their use is limited due to suspicions of toxic and carcinogenic effects associated with these compounds. Consequently, there has been a growing interest in developing and isolating natural antioxidants from plant sources, especially edible plants with high antioxidant capacity [11-16].

Plant polyphenols constitute a diverse group of secondary metabolites characterized by an aromatic ring containing one or more hydroxyl substitutions. In plants, these compounds serve various functions, including protection against ultraviolet radiation, participation in growth and reproduction, and acting as components of pigments, essences, flavorings, and more. Plant phenolics, such as flavonoids, function as free radical scavengers, metal chelators, and singular oxygen quenchers. Their high redox potential contributes to their antioxidant properties. Moreover, they exhibit a wide range of biological activities, including antimicrobial and anticancer effects, many of which can be attributed to their antioxidant properties. Studies have shown that a diet rich in phenolic content contributes to the prevention of oxidative stress-related degenerative diseases, such as cancer, neurological disorders, and cardiovascular diseases [11, 17-19].

Genus *Anthemis* (Mayweed) belongs to the family *Asteraceae* (*Compositae*) and is now recognized as the second-largest genus within the *Anthemideae* tribe, comprising approximately 210 species. In folk medicine, various species of Mayweed are used to treat gastrointestinal discomfort, hemorrhoids, dysmenorrhea, and stomach pain. Additionally, members of this genus exhibit antibacterial, antispasmodic, anti-inflammatory, liver-protective, anticholinesterase, anti-biofilm, and antioxidant activities. Phytochemical studies of several Mayweed plants have revealed the presence of sesquiterpene lactones, polyacetylenes, flavonoids, and essential oils [20].

Mayweed Chamomile (*Anthemis cotula*) is an annual herbaceous plant with a strong and noticeable odor. It typically grows to a height of 30 to 60 centimeters. In traditional herbal medicine, it is well-known for treating conditions such as psoriasis, fever, gastrointestinal issues, bloody diarrhea, and gout-related arthritis [20]. However, limited studies exist regarding the antioxidant and antimicrobial activity of Mayweed Chamomile essential oil in scientific literature. In a study by Lo'ay and colleagues (2023), essential oils were extracted from the leaves and flowers of Mayweed Chamomile grown in Jordan using water distillation. Sesquiterpene hydrocarbons were the predominant

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constituents in the oils extracted from both leaves and flowers. γ-Muurolene and artemadendrene were major compounds obtained from the flower essential oil, while γ-muurolene and trans-caryophyllene ether were identified as primary constituents in the leaf extract. Apigenin and chlorogenic acid were the main compounds identified in the alcoholic extract of the flowers, while all the extracts were evaluated for total antioxidant activity. Compared to positive controls (α-tocopherol and ascorbic acid), they exhibited significant antioxidant activity [20].

Given the above information, the objective of this study was to extract *A. cotula* essential oil, determine its total phenolic and flavonoid content, investigate its antioxidant activity, and assess its antimicrobial effects.

2- Materials and methods

2.1. Chemicals

The materials used in this research were of laboratory grade and were purchased from Merck, Germany.

2.2. Microbial strains

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 Agricultural Sciences and Natural Resources University of Khuzestan.

2.3. Essential oil extraction

Fresh Mayweed chamomile aerial parts were dried in the shade for one month at room temperature. Then the dried samples were turned into powder. The amount of 200 g of the powder was hydro-distilled for 4 h using a Cloninger apparatus. Then the obtained essential oil was dried over anhydrous sodium sulfate and immediately stored at 4°C [20].

2.4. Total phenol content

A certain volume of Folin Ciocalto's reagent (0.12 mL) was mixed with 0.02 mL of essential oil and 1.97 mL of distilled water. After keeping at room temperature for 3 min, sodium carbonate solution (0.37 mL; 200 g/L) was added. The solution was kept in a dark room for 2 h and then its absorbance was read at 750 nm. The total phenolic content of the essential oil was reported as mg gallic acid equivalent per gram of essential oil (mg GAE/g) [21].

2.5. Total flavonoid content

For the determination of flavonoids, the colorimetric method of aluminum chloride presented by Albarak and Aksoy [11] was used with a slight modification. For this purpose, 0.5 mL of essential oil was mixed with 1.5 mL of methanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1 M potassium acetate and 2.8 mL of distilled water. The mixture was kept at room temperature for 30 min. The absorbance of the reaction mixture was measured at 415 nm and the results were expressed as milligrams of quercetin equivalent per gram of essential oil (mg QE/g).

2.6. Antioxidant activity

2.6.1. DPPH free radical scavenging

In this method, 37.5 µL of essential oil or methanol (as control) was mixed with 2 mL of DPPH solution. After keeping the mixture for 30 min at room temperature in a dark place, its absorbance was measured at a wavelength of 517 nm. The inhibitory

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activity was calculated using the following formula [2]:

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

2.6.2. ABTS free radical scavenging

ABTS radical is also widely used to evaluate antioxidant capacity, which basically involves the conversion of colorless ABTS to blue by adding ammonium persulfate. Briefly, ABTS solution was prepared by adding 7.4 mM ABTS and 2.6 mM ammonium persulfate. Then essential oil was added to it and the mixture was kept for 16 h at 37°C in the dark. The absorbance was measured at 734 nm and the inhibition percentage was calculated according to the formula presented in the DPPH radical inhibition test section [22].

2.7. Antimicrobial activity

2.7.1. Disk diffusion agar

First, microorganisms were cultured on Mueller Hinton agar culture medium. Then, sterile discs soaked in essential oil were fixed on the medium. The plates were kept in an incubator at 37°C for 24 h. At the end, the diameter of the inhibition zone was measured in millimeters [5].

2.7.2. Well diffusion agar

This test was performed using a suspension with McFarland standard turbidity of 0.5. The essential oil was sterilized by 0.22-micron syringe filter. Wells with a diameter of 6 mm were created on the Mueller Hinton agar medium and then a specific volume of microbial suspension was poured into them. The Petri dish was incubated at 35°C for 24 h and the diameter of the inhibition zone around the wells was determined [23].

2.7.3. Minimum inhibitory concentration (MIC)

After preparing a stock of 512 mg/mL of essential oil, successive dilutions of 256, 128, 64, 32, 16, 8, 4 and 2 mg/mL were prepared. 100 µL of each dilution and 10 µL of each bacteria were added to the wells of 96-well plates. After 24 h of incubation, Triphenyltetrazolium chloride reagent (5 mg/mL) was added to each well and the plate was again incubated for 30 min. At the end, the lowest concentration in which no color change was observed was reported as the minimum inhibitory concentration [3].

2.7.4. Minimum bactericidal concentration (MBC)

100 µL of the wells without color change in the minimum inhibitory concentration test were cultured in the plates containing Mueller Hinton's medium and the plates were kept at 37°C for 24 h. In the end, the concentrations without growth were reported as the minimum bactericidal concentration [4].

2.8. Statistical analysis

All tests were performed in three repetitions. The results of this research were analysed using SPSS version 18 software, through one-way ANOVA. Duncan's test was used at the 95% confidence level ($p<0.05$) to check the significance of the difference between the means.

3-Results and discussion

Figure 1 shows the total phenol and flavonoid content of *A. cotula* essential oil. The results show that *A. cotula* essential oil contains 29.80 mg GAE/g total phenol and 13.47 mg QE/g total flavonoid. Lo'ay et al. (2023) showed that the aqueous extract of

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A. cotula leaves contained the highest amount of chlorogenic acid (77.25%) compared to other methanolic and butanol extracts. The methanol extract of the leaf contained cinnamic acid, vitexin, luteolin and apigenin as main components. While butanol extract was characterized by the presence of apigenin, quercetin and luteolin [20]. In line with the results of this research, Albayrak and Aksoy (2013) evaluated the antioxidant and antimicrobial activity of the methanol extracts of two native chamomile species in Turkey and reported that the total phenolic content of *A. cretica subsp. argaea* (48.51 mg GAE/g) was higher than *A. fumariifolia* (31.94 mg GAE/g). However, the total flavonoid content of *A. fumariifolia* (12.88 mg QE/g) was higher than that of *A. cretica subsp. argaea* (11.49 mg QE/g). This change may be due to environmental conditions that can change the constituents of the plant [24]. Due to the high content of phenolic acids and flavonoids in *A. cotula* essential oil, this plant can be considered as a promising source for natural antioxidant agents.

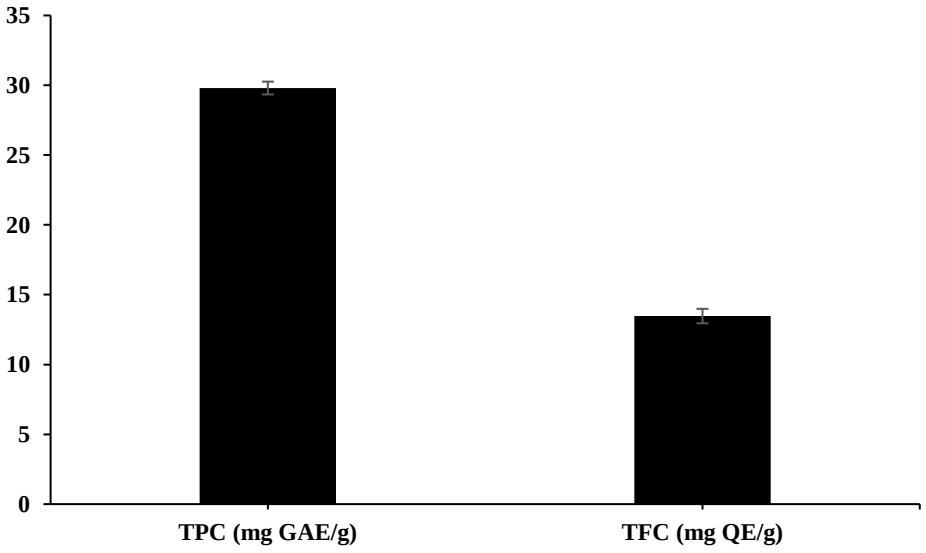

Figure 1. Total phenol content (TPC) and total flavonoid content (TFC) of *Anthemis cotula* essential oil. GAE = Gallic acid equivalent; QE = Quercetin equivalent.

Figure 2 presents the results of the antioxidant effect of *A. cotula* essential oil. The antioxidant activity of essential oil against DPPH free radical was equal to 53.80%. In addition, the essential oil was able to inhibit ABTS free radicals and its activity was observed as 55.60%. The antioxidant activity of *A. cotula* extract and essential oil has been reported by Lo'ay et al. (2023) [20]. The antioxidant efficiency of different extracts obtained from *A. cotula* leaves and flowers is mainly attributed to their high content of phenolic and flavonoid compounds, such as especially rich in chlorogenic acid, cinnamic acid, vitexin, apigenin, quercetin and luteolin [25, 26]. In relation to *A. cotula* essential oil, the observed antioxidant activity can be related to its high sesquiterpene hydrocarbon content, which are known as strong antioxidants [27]. The antioxidant activity of the extract and essence of other

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chamomile species has also been reported in different studies [11, 28, 29].

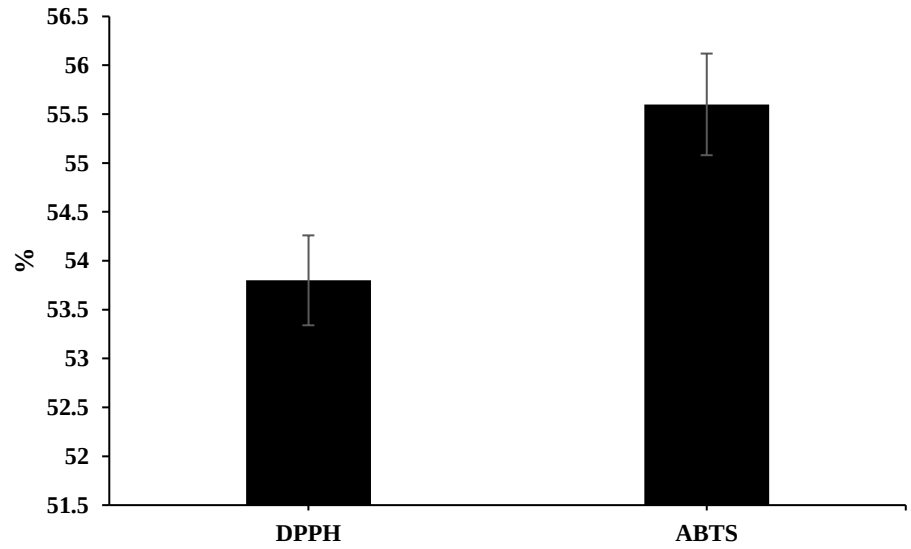

Figure 2. Antioxidant effect of *Anthemis cotula* essential oil based on DPPH and ABTS radical scavenging methods.

Figure 3 shows the findings of the antimicrobial effect of *A. cotula* essential oil based on the disk diffusion agar method. *Staphylococcus aureus* and *Shigella dysenteriae* bacteria were the most sensitive and resistant strains against essential oil with the highest and lowest diameter of inhibition zones, respectively ($p < 0.05$). So that the average diameter of inhibition zone for these bacteria was 19 and 12.10 mm, respectively. The average diameter of inhibition zone for *Bacillus cereus*, *Streptococcus pyogenes*, *Enterobacter aerogenes* and *Salmonella typhimurium* was 16.30, 17.80, 14.50 and 13.30 mm, respectively.

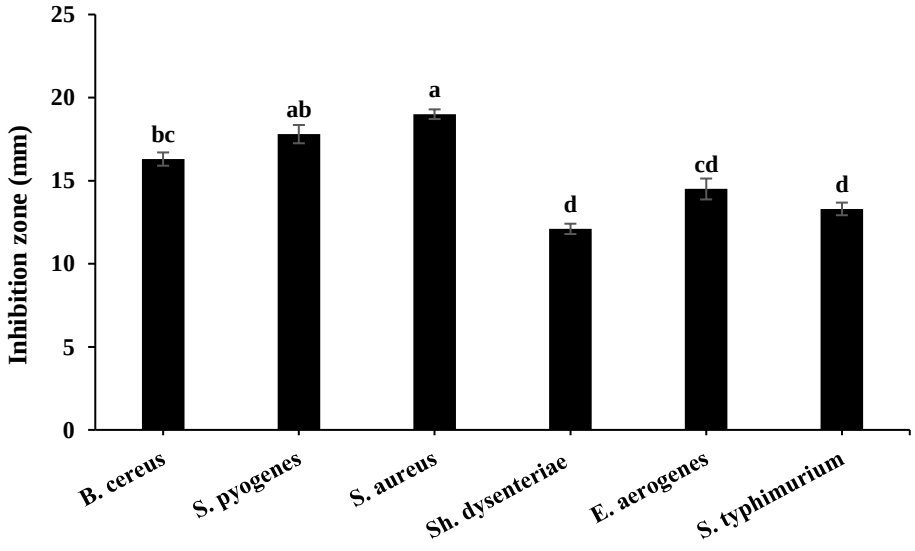

Figure 3. Antibacterial effect of *Anthemis cotula* essential oil based on disc diffusion agar method.

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The results of the well diffusion agar test (Figure 4) were in line with the disk diffusion agar test, and *Staphylococcus aureus* bacteria with an inhibition zone of 20.40 mm were the most sensitive and *Shigella dysenteriae* bacteria with an inhibition zone of 13.80 mm were the most resistant strains to the essential oil ($p < 0.05$).

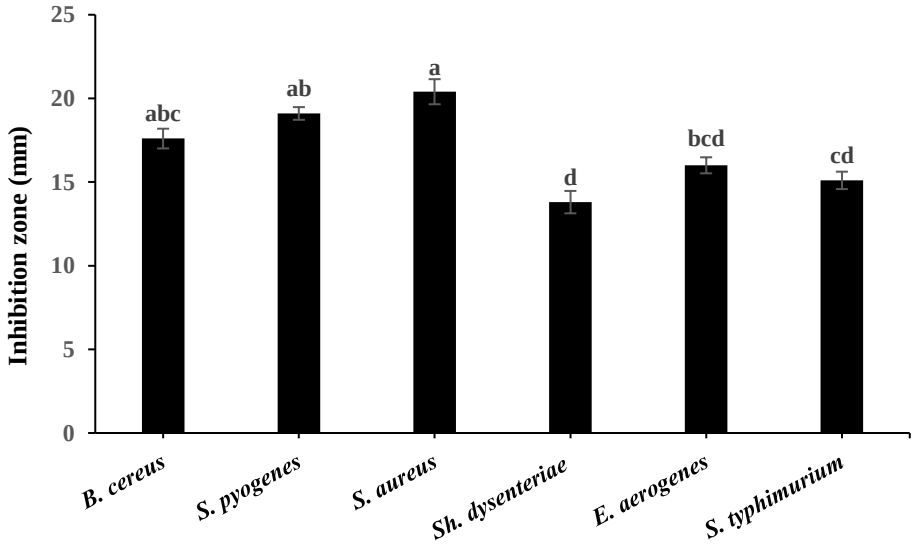

Figure 4. Antibacterial effect *Anthemis cotula* essential oil based on well diffusion agar method.

Table 1 shows the results of the minimum inhibitory and bactericidal concentration of *A. cotula* essential oil. The minimum inhibitory and bactericidal concentration for Gram-positive bacteria (*Staphylococcus aureus*, *Bacillus cereus*, and *Streptococcus pyogenes*) was lower than Gram-negative bacteria (*Enterobacter aerogenes*, *Shigella dysenteriae*, and *Salmonella typhimurium*), which shows the greater sensitivity of these bacteria to essential oil.

Table 1. Antibacterial effect of *Anthemis cotula* 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	32	512
S. pyogenes	16	256
S. aureus	16	256
Sh. dysenteriae	256	> 512
E. aerogenes	128	> 512
S. typhimurium	128	> 512

In general, Gram-negative bacteria are less sensitive to essential oils than Gram-positive bacteria, and this is directly related to the structure of the bacterial cell wall. In

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Gram-negative bacteria, the cell wall is a complex envelope consisting of cytoplasmic membrane, periplasm and outer membrane [30]. The outer phospholipid membrane in Gram-negative bacteria carries structural lipopolysaccharide components that make the cell wall impermeable to antimicrobial chemicals. In relation to Gram-positive bacteria, there is only one outer peptidoglycan layer in the cell wall, which is not an effective permeability barrier [31]. It has been reported that flavonoids (such as patoletin and kaempferol) isolated from *A. cotula* flowers show antimicrobial activity [32]. In fact, the methanol extract of *A. cotula* leaves had an antimicrobial effect against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Micrococcus luteus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Proteus vulgaris* [32]. The results of Bardaweel et al. (2014) showed that *Anthemis palestina* essential oil has antibacterial activity against a wide range of microorganisms. The results showed that Gram-positive bacteria were more sensitive to essential oil treatment than Gram-negative bacteria. In particular, *Staphylococcus epidermidis* was the most susceptible Gram-positive bacterium, while *Escherichia coli* was found to be the most resistant Gram-negative bacterium [33]. The obtained results show the high potential of *A. cotula* essential oil as a bioactive oil for food and medical applications, with antioxidant and antimicrobial activities.

4- Conclusion

The results of this study showed that *A. cotula* essential oil shows significant antioxidant activity and can be used as an alternative medicine to prevent or treat oxidative stress. In addition, the investigated essential oil showed significant antibacterial activity against pathogenic microorganisms. Therefore, *A. cotula* can be considered as a promising source of natural antioxidant and antimicrobial agents. However, further research on molecular mechanisms as well as isolation and identification of chemical compounds responsible for biological activity is necessary for clinical use.

5-Acknowledgements

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بررسی ویژگی های شیمیایی و ضدمیکروبی اسانس بابونه بهاری بر باکتری‌های *سالمونلا تیفی‌موریوم*، *انتروباکتر ائروژنز*، *استافیلوکوکوس اورئوس*، *شیگلا دیسانتری*، *باسیلوس سرئوس* و *استرپتوکوکوس پیوژنز*

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چکیده

این مطالعه با هدف استخراج اسانس از گیاه دارویی بابونه بهاری (*Anthemis cotula*) و بررسی میزان فنول کل، فلاونوئید کل، اثر آنتی‌اکسیدانی و فعالیت ضدمیکروبی آن صورت پذیرفت. بررسی محتوای فنول کل اسانس با کمک روش فولین سیوکالتو نشان داد که اسانس دارای ۲۹/۸۰ mg GAE/g فنول کل می‌باشد. محتوای فلاونوئید کل اسانس برابر با ۱۳/۴۷ mg QE/g بود. بررسی اثر آنتی‌اکسیدانی اسانس با کمک روش‌های مهار رادیکال آزاد DPPH و ABTS نشان داد که اسانس دارای فعالیت آنتی‌اکسیدانی قابل‌توجهی می‌باشد. اثر آنتی‌اکسیدانی در برابر رادیکال‌های آزاد DPPH و ABTS به ترتیب برابر با ۵۳/۸۰ و ۵۵/۶۰ درصد به دست آمد. ارزیابی فعالیت ضدمیکروبی اسانس در برابر باکتری‌های *سالمونلا تیفی‌موریوم*، *انتروباکتر ائروژنز*، *استافیلوکوکوس اورئوس*، *شیگلا دیسانتری*، *باسیلوس سرئوس* و *استرپتوکوکوس پیوژنز* بر اساس روش‌های دیسک دیفیوژن آگار، چاهک آگار و حداقل غلظت مهارکنندگی و کشندگی نشان داد که فعالیت ضد باکتریایی اسانس قابل‌توجه است. باکتری‌های *استافیلوکوکوس اورئوس* و *شیگلا دیسانتری* به ترتیب با بالاترین و کمترین قطر هاله عدم رشد، حساس‌ترین و مقاوم‌ترین سویه‌ها در برابر اسانس بودند. نتایج نشان داد که اسانس بابونه بهاری منبع طبیعی ترکیبات فعال با اثرات آنتی‌اکسیدانی و ضد باکتریایی در برابر باکتری‌های پاتوژن می‌باشد.

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