Journal of Food Science and Technology (Iran)

Homepage:www.fsct.modares.ir

Scientific Research

Evaluation of the antimicrobial activity of dill aqueous extract and its interaction with chloramphenicol antibiotic: An *in vitro* study

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ARTICLE INFO

Article History:

Received:2025/1/15

Accepted:2025/2/23

Keywords:

Dill; Antimicrobial;

Bioactive extract;

Natural preservative;

Traditional medicine.

DOI: 10.22034/FSCT.22.161.275.

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ABSTRACT

Dill (*Anethum graveolens*) is a traditional medicinal plant widely used for the treatment of various diseases. In this study, the antibacterial potential of dill leaf aqueous extract against *Erwinia amylovora*, *Pseudomonas syringae*, *Xanthomonas campestris*, *Salmonella typhi*, *Staphylococcus epidermidis* and *Listeria monocytogenes* was evaluated. Disk diffusion agar, well diffusion agar, minimum inhibitory and bactericidal concentrations and interaction with the antibiotic chloramphenicol were used for this purpose. Increasing the extract concentration from 20 mg/ml to 110 mg/ml significantly increased the antimicrobial activity. The results of disk diffusion agar and well diffusion agar tests showed that the largest diameter of the inhibition zone was for *Staphylococcus epidermidis* (12.90 mm and 14.37 mm, respectively) and the smallest diameter of the inhibition zone was for *Salmonella typhi* (9.23 mm and 10.38 mm, respectively). The results of the interaction between dill aqueous extract and the antibiotic chloramphenicol showed that in the combined state of the extract with the antibiotic, a synergistic state was observed for all strains. The antibacterial effect shown by this plant provides a scientific basis and, therefore, confirms its traditional use as a home remedy. Isolation and purification of various phytochemicals may produce significant antibacterial agents.

[DOI: 10.22034/FSCT.22.161.275]

1- Introduction

Pathogenic microorganisms pose a significant risk to public health and food safety and can cause a range of diseases ranging from mild gastrointestinal problems to severe and potentially fatal infections. Their remarkable adaptability and growing resistance to conventional treatments complicate efforts to manage these infections. Therefore, gaining insight into how these pathogens cause disease, along with exploring effective measures to mitigate their impact, is essential. The increasing prevalence of antibiotic-resistant strains has led to renewed focus on alternative antimicrobial approaches [1-4].

At the same time, the use of synthetic preservatives has become a standard practice in both the food and pharmaceutical sectors to extend shelf life and ensure product stability. Although these chemical antimicrobial agents are effective in inhibiting microbial growth, they can sometimes lead to adverse health effects and raise environmental concerns. As public awareness of the risks associated with synthetic additives increases, the demand for safer alternatives is increasing. This situation has led to increased research on the efficacy and safety of synthetic preservatives compared to more natural alternatives and has fueled the debate on sustainable food preservation practices [5-14].

Natural preservatives, particularly extracts and essential oils from various plants, have emerged as viable alternatives to synthetic products. These plant-derived compounds are known for their antimicrobial properties, which often originate from phytochemicals that allow plants to protect themselves from pathogens and pests. The increased interest in natural preservatives can be attributed to their broad efficacy, reduced toxicity, and additional health benefits [15-20]. In addition, an increasing number of consumers prefer products labeled “natural,” which has accelerated research on the antimicrobial properties of various plant materials. These findings are not only in line with consumer trends but also support the move towards sustainable and health-oriented food preservation methods [5, 21-29].

Among various plant extracts, dill (*Anethum graveolens*) has received attention for its potent antimicrobial effects [30, 31]. This aromatic herb, commonly used in cooking, holds promise in the fields of food science and technology. Dill aqueous extract contains several bioactive compounds that contribute to its effectiveness as a natural preservative [32-35]. Its dual capacity to enhance flavor while suppressing microbial growth makes it an innovative solution in food safety and preservation. Research shows that dill extract is effective against a wide range of pathogenic microorganisms. The review of natural preservatives such as dill highlights the urgent need for sustainable alternatives to synthetic antimicrobial agents. Therefore, this research paper aimed to investigate the antimicrobial properties of dill aqueous extract.

2- Materials and Methods

2. Extraction

Dill leaves were purchased from Ahvaz. Aqueous extraction was performed by adding 200 ml of water to 20 g of dried dill leaves and then boiling for 10 minutes. After cooling, the extract was filtered and concentrated to a volume of 100 ml [36].

2.2. Antimicrobial activity

The antimicrobial activity of dill aqueous extract against the microorganisms *Erwinia amylovora*, *Pseudomonas syringae*, *Xanthomonas campestris*, *Salmonella typhi*, *Staphylococcus epidermidis* and *Listeria monocytogenes* was investigated according to the disk diffusion agar, well diffusion agar, interaction with antibiotics,

[DOI: 10.22034/FSCT.22.161.275]

and minimum inhibitory and bactericidal concentrations methods.

2.2.1. Disk diffusion agar

A loop of each bacterial stock culture was plated on Mueller-Hinton agar, then paper disks (Whatman filter paper, 6 mm in diameter) soaked in different concentrations of extract were placed on the surface of the medium. Extract concentrations (20, 50, 80 and 110 mg/ml) were prepared using sterile distilled water. All culture media were incubated for 24 h at 37°C, then the diameter of the zone of inhibition was carefully measured using a ruler. All experiments were performed in triplicate [37].

2.2.2. Well diffusion agar

To measure the diameter of the zone of inhibition produced by the plant extract, Mueller-Hinton agar medium was prepared and poured into a Petri dish. Then, some of the microbial suspension was spread on the culture medium using an L-shaped spreader. In the next step, several wells with a diameter of 6 mm were created on the surface of the culture medium and 20 µl of the extract with concentrations of 20, 50, 80 and 110 mg/ml were poured into the wells. The cultures were kept in an incubator at 37°C for 24 h and the diameter of the zones of no growth around the wells was measured and expressed in millimeters [29].

2.2.3. Minimum inhibitory (MIC) and bactericidal (MBC) concentrations

The minimum inhibitory concentration of dill leaf extract was determined using broth microdilution. For this purpose, 8 consecutive concentrations (2, 4, 8, 16, 32, 64, 128, 256 and 512 mg/ml) of dill leaf extract were prepared in Mueller Hinton Broth medium. Bacteria were inoculated in the culture medium and incubated for 24 h at 37°C. Subsequently, the concentration of the extract that prevented bacterial growth was considered as the minimum inhibitory concentration [38].

To determine the minimum bactericidal concentration of dill leaf extract, the contents of all tubes that were devoid of microbial growth were cultured in Mueller Hinton Agar medium. Incubation was performed at 37°C for 24 h and plates without microbial colony formation were considered as the minimum bactericidal concentration [39].

2.2.4. Interaction of dill extract with the antibiotic chloramphenicol

In this method, concentrations equivalent to half the minimum inhibitory concentration were used. Standard microbial culture was performed as a lawn on Mueller Hinton agar culture medium containing the extract and the chloramphenicol antibiotic disk was gently placed on the surface of the culture medium using sterile forceps. After incubation of the samples at 37°C for 24 h, the zone of non-growth was measured and recorded in millimeters [1].

2.3. Statistical analysis

The tests were repeated 3 times. The results were analyzed using Minitab software (version 16) and Tukey's test at a confidence level of 95%.

3. Results and Discussion

The results of the antimicrobial test of dill aqueous extract based on the disk diffusion agar method are presented in Figure 1. According to the results, increasing the extract concentration from 20 to 110 mg/mL increased the diameter of the zone of inhibition from 7.54 to 14.20 mm ($p < 0.05$). In addition, the largest diameter of the zone of inhibition was observed for *Staphylococcus epidermidis* (12.90 mm) and the smallest diameter of the zone of inhibition was observed for *Salmonella typhi* (9.23 mm) ($p < 0.05$). According to the interaction effect results,

[DOI: 10.22034/FSCT.22.161.275]

Staphylococcus epidermidis with a 16.50 mm diameter of the zone of inhibition in the presence of a concentration of 110 mg/mL of the extract and *Salmonella typhi* with a 6.50 mm diameter of the zone of inhibition in the presence of 20 mg/mL were the most sensitive and resistant strains to the extract, respectively.

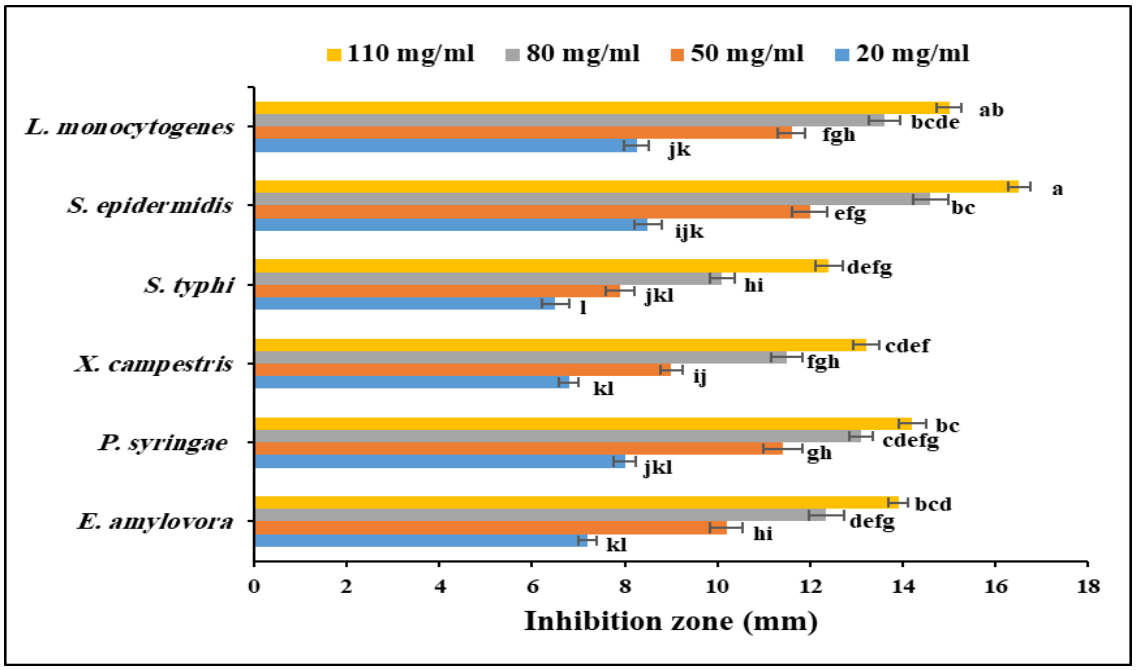

Figure 1. The antibacterial activity of *Anethum graveolens* extract based on disc diffusion agar method. Treatments labeled with different letters show significant differences at $p < 0.05$.

Figure 2 shows the findings of the antimicrobial effect of the extract against pathogenic microorganisms based on the well diffusion agar method. The concentration of the extract had a significant effect on the diameter of the zone of inhibition, and the diameter of the zone of inhibition increased from 8.27 mm in the presence of 20 mg/ml of the extract to 15.68 mm in the presence of 110 mg/ml of the extract. *Staphylococcus epidermidis* and *Salmonella typhi* were the most sensitive (zone diameter = 14.37 mm) and the most resistant (zone diameter = 10.38 mm) strains to the aqueous dill extract, respectively. In general, the lowest (7.00 mm) and highest (18.10 mm) diameters of the zone of non-growth were observed in the presence of 20 mg/ml for *Salmonella typhi* and 110 mg/ml for *Staphylococcus epidermidis*, respectively.

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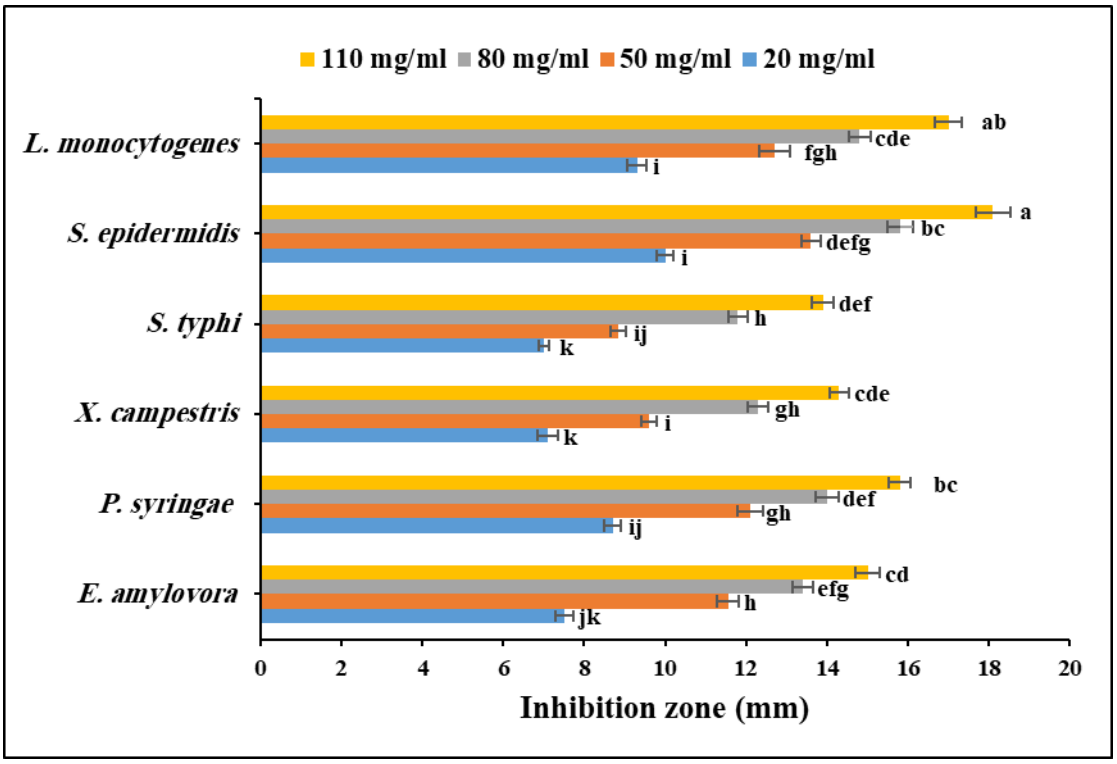

Figure 2. The antibacterial activity of *Anethum graveolens* extract based on well diffusion agar method. Treatments labeled with different letters show significant differences at $p < 0.05$.

According to the results of Figure 3, the lowest minimum inhibitory concentration (4 mg/mL) was observed for *Staphylococcus epidermidis* and *Listeria monocytogenes* and the highest minimum inhibitory concentration (16 mg/mL) was observed for *Salmonella typhi* and *Xanthomonas campestris*. Regarding the minimum bactericidal concentration, *Salmonella typhi* with 512 mg/mL and *Staphylococcus epidermidis* and *Listeria monocytogenes* with 64 mg/mL were identified as the most resistant and sensitive strains to dill aqueous extract, respectively (Figure 4).

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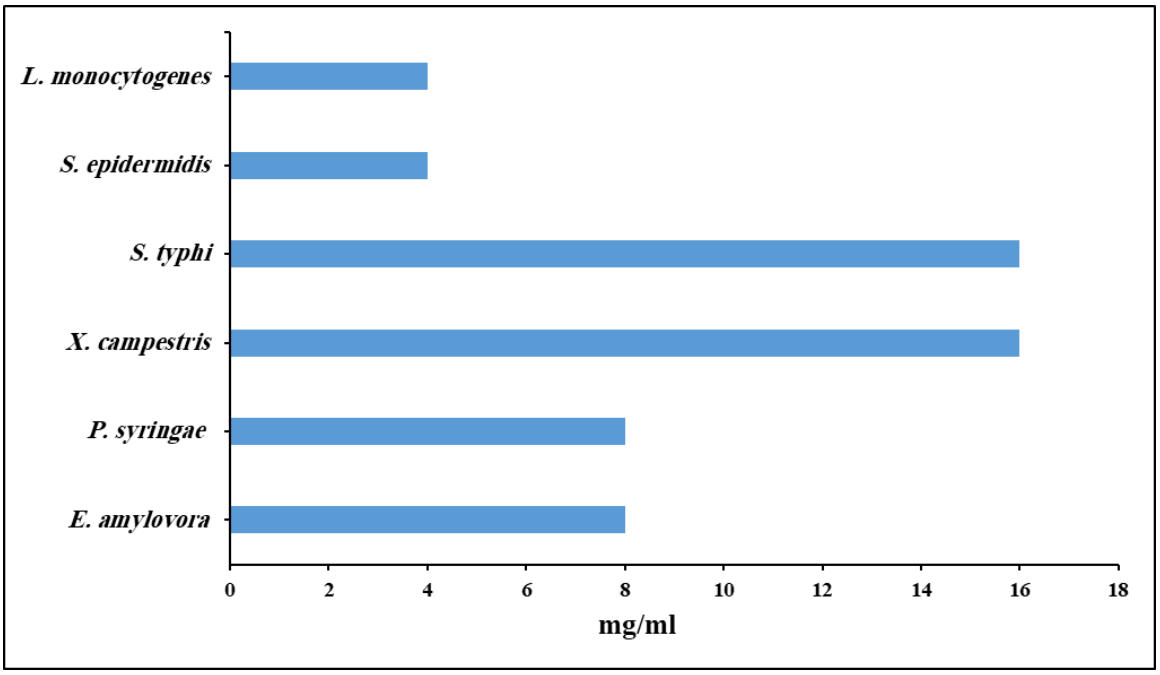

Figure 3. The antibacterial activity of *Anethum graveolens* extract based on minimum inhibitory concentration (MIC) method.

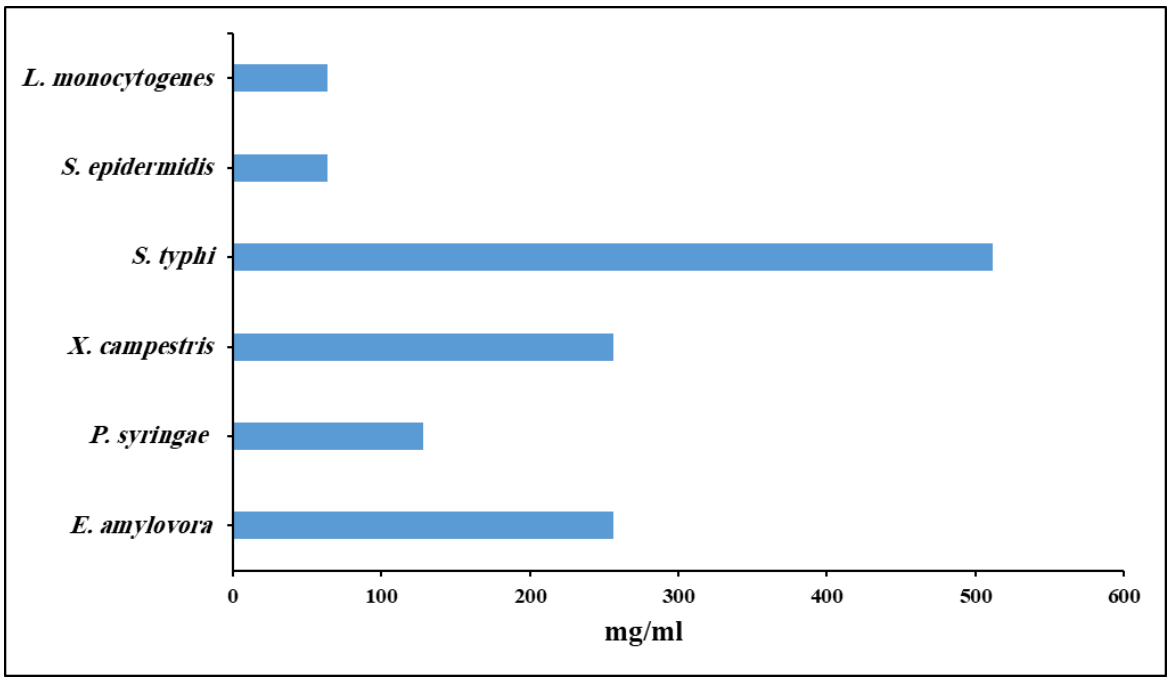

Figure 4. The antibacterial activity of *Anethum graveolens* extract based on minimum bactericidal concentration (MBC) method.

The results of the interaction between dill aqueous extract and the antibiotic chloramphenicol are shown in Figure 5. The results showed that in the combined state of the extract with the antibiotic, a synergistic state was observed for all strains; so that the average diameter of the microbial growth inhibition zone significantly increased from 14.35 mm for the antibiotic chloramphenicol to 17.50 mm for the antibiotic + extract combination. In this regard, the smallest diameter of the growth inhibition zone was 11 mm for *Salmonella typhi* bacteria in the presence of

[DOI: 10.22034/FSCT.22.161.275]

chloramphenicol and the largest diameter of the growth inhibition zone was 20.70 mm for *Staphylococcus epidermidis* bacteria in the presence of the chloramphenicol + dill aqueous extract combination.

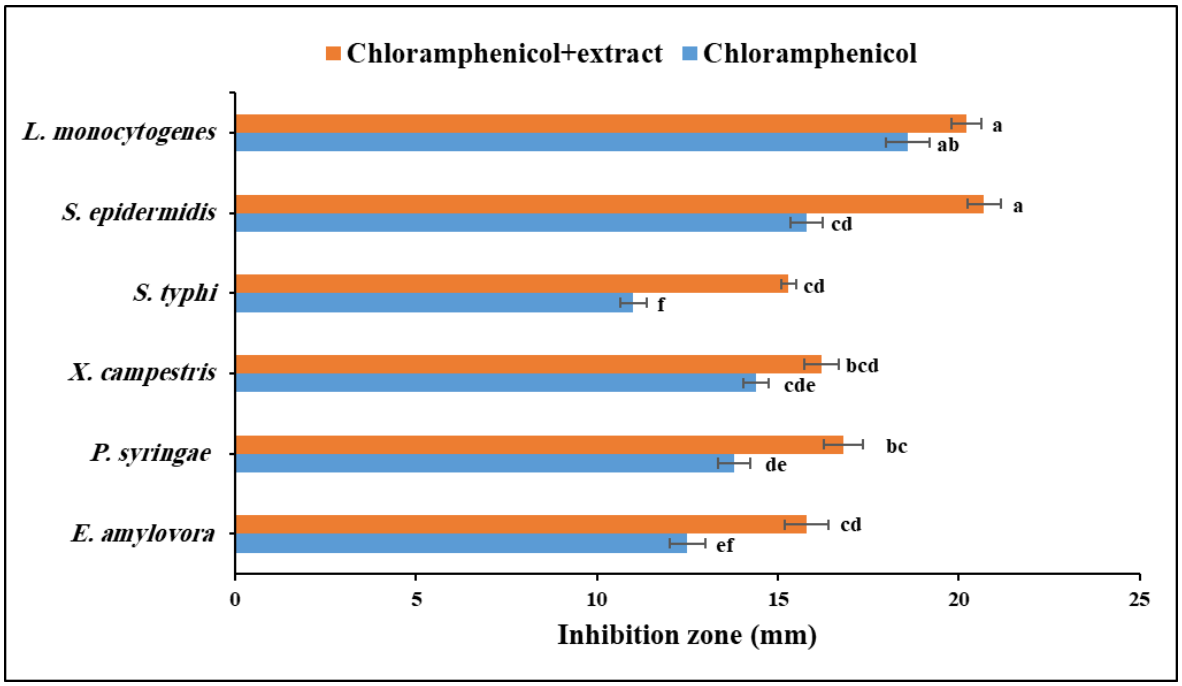

Figure 5. The antibacterial activity of *Anethum graveolens* extract based on interaction method (chloramphenicol+extract). Treatments labeled with different letters show significant differences at $p < 0.05$.

Similar results have been reported by other researchers. Kaur and Arora [40] evaluated the antibacterial activity of aqueous and organic (acetone) extracts of dill seeds using agar diffusion method, minimum inhibitory concentration and viable cell count and compared their antibacterial effect with some standard antibiotics. The aqueous and acetone extracts showed significant antibacterial activity against all bacteria except *Klebsiella pneumoniae* and one strain of *Pseudomonas aeruginosa*. The minimum inhibitory concentrations of aqueous and acetone extracts of the seeds were between 20-80 mg/ml and 5-15 mg/ml, respectively. Viable cell count studies revealed the bactericidal nature of the seed extract. Statistical analysis proved better/equal efficacy of some of these seed extracts compared to standard antibiotics [40].

The antimicrobial potential of aqueous and ethanolic extracts of seeds, leaves, roots, callus and regenerated leaves of dill seedlings was evaluated *in vitro* against important bacterial strains, *Escherichia coli*, *Bacillus subtilis*, *Bacillus cereus*, *Micrococcus luteus* by Jana and Shekhavat [41]. Ethanolic extracts were more potent than aqueous extracts of all plant parts studied. Ethanolic extract of seeds showed strong activity against all bacterial strains. Compared to *in vivo* conditions, plant extracts showed reduced activity *in vitro*. Phytochemical screening of plant parts showed that leaves, stems, roots, laboratory callus and regenerated leaves are rich in tannins, terpenoids, cardiac glycosides and flavonoids. These researchers reported phytochemicals and secondary metabolites responsible for the antibacterial activities of the extract [41].

In addition, the antimicrobial activity of essential oil and dill extracts against

[DOI: 10.22034/FSCT.22.161.275]

Enterococcus cloacae, *Klebsiella pneumoniae*, *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Candida albicans* and *Aspergillus niger* was investigated by Hadi et al. [34]. The results showed that the essential oil was more effective than the aqueous, ethanolic and decoction extracts on most of the microorganisms tested. In addition, the ethanolic extract had antifungal activity that was distinct from the other extracts. The antimicrobial effect of the studied essential oils was primarily attributed to the synergistic interactions between its three main compounds (anethole, estragole and fenchone) [34].

4. Conclusion

The results showed that the aqueous extract of dill had inhibitory activity against the tested bacteria. *Staphylococcus epidermidis* and *Salmonella typhi* were the most sensitive and resistant strains against the aqueous extract of dill, respectively. The antimicrobial activity obtained in this study supports the traditional use of this plant against infectious diseases. Furthermore, the results show hope for the development of many new chemotherapeutic agents or new patterns from such plants that may be used in the future to produce improved therapeutic agents. Therefore, this study confirms the bioactive potential of dill and in addition to its use as a food seasoning and in the pharmaceutical industry, the aqueous extract of this plant can also be used for antimicrobial purposes.

5.Acknowledgements

The authors would like to express their sincere gratitude to the Vice-chancellor for Research and Technology of Agricultural Sciences and Natural Resources University of Khuzestan for supporting this study (1403.38).

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[DOI: 10.22034/FSCT.22.161.275]

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ارزیابی فعالیت ضدمیکروبی عصاره آبی شوید و برهمکنش آن با آنتی بیوتیک کلرامفنیکل: یک مطالعه آزمایشگاهی

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اطلاعات مقاله

تاریخ های مقاله :

تاریخ دریافت: ۱٤۰۳/۱۰/۲٦

تاریخ پذیرش: ۱٤۰۳/۱۲/۵

کلمات کلیدی:

شوید؛

ضدمیکروبی؛

عصاره زیست فعال؛

نگهدارنده طبیعی؛

طب سنتی.

DOI: 10.22034/FSCT.22.161.275.

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چکیده

شوید (*Anethum graveolens*) از گیاهان دارویی سنتی بوده که به طور گسترده‌ای برای درمان بیماری‌های مختلف استفاده می‌شود. در این مطالعه، پتانسیل ضد باکتریایی عصاره آبی برگ شوید در برابر باکتری‌های *اروینیا امیلوورا*، *سودوموناس سیرینگه*، *زانتوموناس کمپستریس*، *سالمونلا تیفی*، *استافیلوکوکوس اپیدرمیدیس* و *لیستریا مونوسیتوژنز* مورد ارزیابی قرار گرفت. از روش‌های دیسک دیفیوژن آگار، چاهک آگار، حداقل غلظت مهارکنندگی و کشندگی و برهمکنش با آنتی‌بیوتیک کلرامفنیکول برای این منظور استفاده گردید. افزایش غلظت عصاره از ۲۰ میلی‌گرم در میلی‌لیتر به ۱۱۰ میلی‌گرم در میلی‌لیتر سبب افزایش معنادار فعالیت ضدمیکروبی گردید. نتایج آزمون‌های دیسک دیفیوژن آگار و چاهک آگار نشان داد که بیشترین قطر هاله عدم رشد برای باکتری *استافیلوکوکوس اپیدرمیدیس* (قطر هاله به ترتیب ۱۲/۹۰ میلی‌متر و ۱٤/۳۷ میلی‌متر) و کمترین قطر هاله عدم رشد مربوط به *سالمونلا تیفی* (قطر هاله به ترتیب ۹/۲۳ میلی‌متر و ۱۰/۳۸ میلی‌متر) بود. نتایج برهمکنش میان عصاره آبی شوید و آنتی‌بیوتیک کلرامفنیکول نشان داد که در حالت ترکیبی عصاره با آنتی‌بیوتیک، حالت سینرژیستی برای تمام سویه‌ها مشاهده شد. اثر ضد باکتریایی نشان داده شده توسط این گیاه یک پایه علمی را فراهم می‌کند و بنابراین، استفاده سنتی آن را به عنوان داروهای خانگی تائید می‌کند. جداسازی و خالص‌سازی فیتوکمیکال‌های مختلف ممکن است عوامل ضد باکتریایی قابل توجهی تولید کند.

[DOI: 10.22034/FSCT.22.161.275]