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Scientific Research

Antimicrobial activity of *Datura stramonium* ethanolic extract on some pathogenic bacteria causing infection and food poisoning *in vitro*

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ABSTRACT

Datura stramonium is well known as a valuable drug in many diseases including inflammation, wounds, fever and toothache, and its extract has significant antimicrobial activity. Therefore, this study was performed to isolate the ethanolic extract of *D. stramonium* leaves and to investigate its antimicrobial activity. In this study, *D. stramonium* extract was obtained using the maceration method. Antimicrobial methods of disk diffusion agar, well diffusion agar, minimum inhibitory concentration, and minimum bactericidal concentration were used to determine the antibacterial activity of ethanolic extract of *D. stramonium* leaves against *Escherichia coli*, *Salmonella typhimurium*, *Staphylococcus aureus*, and *Bacillus cereus*. The antimicrobial activity of the extract was concentration dependent and the highest diameter of growth inhibition zone was observed at a concentration of 60 mg/ml of the extract. According to disk diffusion agar and well diffusion agar tests, *S. aureus* and *S. typhimurium* had the highest and lowest diameter of growth inhibition zones, respectively. The minimum inhibitory concentrations for *E. coli*, *S. typhimurium*, *S. aureus* and *B. cereus* were 16, 16, 8 and 8 mg/ml, respectively, and the minimum bactericidal concentrations for these bacteria were 256, 256, 64, and 128 mg/ml, respectively. In general, Gram-positive bacteria were more sensitive to the extract than Gram-negative types. Ethanolic extract of *D. stramonium* leaves can be used as a natural antimicrobial agent to prevent the growth of pathogenic bacteria and treat related diseases.

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1- Introduction

Food products may be contaminated at various stages during production, processing, distribution, preparation and/or final consumption. The risk of food contamination largely depends on the health status of food producers, their personal hygiene, knowledge and practices regarding food hygiene [1]. According to the definition of the World Health Organization, diseases transmitted through food or caused by food[1] Diseases of an infectious or toxic nature that are caused by consuming food or water are called [2, 3]. Poisoning, infection and toxic infections are three types of foodborne diseases [4]. Diseases of animal origin can be transmitted between humans and animals through direct contact, indirect environmental contact, and/or through food consumption. About 60% of human diseases originate from animals, and approximately 75% of emerging human infectious diseases are transmitted from vertebrates to humans [5].

Foodborne pathogens are microorganisms (e.g., bacteria, viruses, and fungi) as well as some parasites and are the main cause of food spoilage and foodborne illness. Food-borne microbes are major problems that affect food safety and cause human infections after consumption of animal products contaminated with microorganisms or their toxins [6-8]. In recent years, foodborne pathogens have become an important public health problem worldwide, and their impact on health and economy is well known. According to various reports, a large number of people around the world suffer from foodborne diseases every year, and about 600 million people (10 people in the world) become ill due to the consumption of contaminated food [9].

Foodborne diseases are major health problems in developed and developing countries, but developing countries suffer the largest share of the burden of foodborne diseases. According to the World Health Organization, 30% of the population in developed countries suffer from foodborne diseases every year, and up to 2 million deaths are estimated in developing countries [10].

Among the bacteria that cause food poisoning, some of them are particularly important in terms of frequency and/or disease severity. The bacteria produce toxins that cause food poisoning, resulting in symptoms ranging from digestive disorders to paralysis and death. Gram-negative bacteria have been reported to account for approximately 69% of foodborne illness cases [11]. Although there are 31 pathogens that have been identified as the cause of foodborne illness, bacterial pathogens including*Staphylococcus aureus*, Species*salmonella*, Species*Campylobacter* ،*Listeria monocytogenes* And*Escherichia coli* They are one of the common causes of foodborne diseases and deaths in the world [12-15].

There is a growing interest in using natural food preservatives with antimicrobial properties. These natural antimicrobials are often considered safe compared to synthetic antimicrobials that are commonly accepted by consumers and have been shown to be effective against pathogens. Extracts and essential oils derived from plants are among the natural preservatives and are generally recognized by the FDA as safe and have antimicrobial properties due to their complex mixture of bioactive compounds. For this reason, they are widely used as natural preservatives in the food industry [3, 7, 13, 16].

Genus Datura-*Datura* (Family*Solanaceae*) of important medicinal species including*D. stramonium* ، *D. fierce* ،*D. harmless* And*D. metal* Composed. These species have been used for medicinal and recreational purposes since ancient times. Datura species have several traditional and medicinal uses, for example, antiasthmatic, anesthetic, sedative, antihemorrhoidal, expectorant, sedative, and antitumor. tatore (*D. stramonium* Linn). The most common species of the Datura genus is native to Asia, but it is abundantly found in tropical, subtropical, and temperate regions around the world. Tatore is well known as a valuable medicine in many diseases including inflammation, rheumatoid arthritis, wounds, gout, asthma and bronchitis, fever and toothache [17]. Tatura plant extract and essential oil have significant antimicrobial activity [18, 19]; Therefore, the aim of this study is to extract the ethanolic extract of tatorre and investigate its antimicrobial activity against pathogenic bacteria.*Escherichia coli* ،*Salmonella Typhimurium* ، *Staphylococcus aureus* And*Bacillus cereus* Was.

2- Materials and methods

1-2- Collecting the tatore plant and preparing the extract

Tatore plant was collected from its natural habitats in different regions. Identification and confirmation of the genus and species of plant samples was done. The collected leaf was washed with water to remove any dirt and contaminants such as insects, germs and soil that could affect the results and then completely dried at room temperature. The dried part was turned into powder using a mill. The powdered samples were weighed and kept in closed containers until the extraction. Extraction was done using cold soaking method, in which 200 grams of powdered plant sample

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was soaked in 1000 ml of 80% ethanol in a flask to obtain crude hydroalcoholic extract. The mixture was placed in a shaker at 160 rpm for 72 hours at room temperature and then filtered through filter paper. The extract was concentrated using a rotary evaporator to remove ethanol. Finally, the concentrated extract was kept in a closed container in the refrigerator (temperature 4°C) [18].

2-2- Preparation of microbial strains

Antimicrobial activity of ethanolic extract of tatore against bacteria*Escherichia coli* ،*Salmonella Typhimurium* ،*Staphylococcus aureus* And*Bacillus cereus* It was checked.

Bacterial strains were obtained from the Department of Food Industry Science and Engineering, Faculty of Animal Science and Food Industry, Khuzestan University of Agricultural Sciences and Natural Resources. The lyophilized bacterial culture was opened under sterile conditions and recultured in Mueller Hinton broth for 24 hours at 37°C. In order to prepare fresh microbial suspension, stock culture was given in slant culture nutrient agar, then it was washed with sterile Ringer's solution and thick microbial suspension was prepared. This suspension was used to adjust the turbidity of the experimental suspension. The turbidity at 630 nm of each sample was adjusted using a spectrophotometer. The turbidity of the microbial suspension based on 0.5 McFarland or 10 $CFU/ml^8 \times 1.5$ was prepared [20].

3-2- Antimicrobial activity of tatore extract

Antimicrobial tests of agar diffusion disc, agar well, minimum inhibitory concentration and minimum lethal concentration were performed to investigate the antimicrobial effect of ethanol extract of tatorre. At Agar disk diffusion test, concentrations of 15, 30, 45 and 60 mg/ml of the extract were prepared in the appropriate solvent. Then, the blank disks were immersed in the extract solutions for 15 minutes until they were completely soaked. Finally, discs previously immersed in certain concentrations of the extract were fixed on the surface of the culture medium. After that, the Petri dishes were placed at 37°C for 24 hours for the bacteria. The antimicrobial effect was determined by the diameter of the growth halo around the discs [21].

In the agar well method, 5 wells were created on the surface of Mueller Hinton agar culture medium using a Pasteur pipette and 0.1 ml of bacterial suspension was spread on the culture medium. The concentrations of 15, 30, 45 and 60 mg/ml extract were prepared. In the next step, 20 microliters of each concentration was poured into the well and after being kept in a greenhouse for 24 hours at a temperature of 37°C, the diameter of the halo of non-growth was measured in millimeters and reported as the antimicrobial activity of the ethanolic extract of tatorre [13].

The minimum inhibitory concentration of the extract against bacterial strains was evaluated using the microdilution method. The final concentration of the extract in the 96-well plate was 512-4 mg/ml. Then the 96-well plate was kept at 37°C for 24 hours. After incubation, 20 µl of 5% 2,3,5-triphenyltetrazolium chloride was added to each well. The first concentration in which no red color was observed was considered as the minimum inhibitory concentration of the extract [3, 22].

The minimum lethal concentration of the extract was determined using the porplate method. Briefly, 100 µl of the solution in wells free of red color [minimum growth inhibitory concentration test] were cultured on Mueller Hinton agar medium. The first concentration that prevented bacterial growth was reported as the minimum lethal concentration of the extract [22, 23].

4-2- Statistical analysis

All experiments were performed in triplicate. SPSS software (version 26) was used to analyze the obtained data. Data were first evaluated using one-way analysis of variance and Duncan's multiple range test to detect significant differences ($p < 0.05$) was used.

3. Results and Discussion

The results of the antibacterial effect of the ethanolic extract of tatorre against pathogenic bacteria based on the agar disk diffusion method are reported in Table 1. Increasing the concentration of the extract caused a significant increase in the diameter of the growth halo. About bacteria*Escherichia coli*, a significant difference was observed between the diameter of the growth halo in different concentrations of the extract. Between the concentrations of 15 and 30 mg/ml of the extract, there was a significant difference between the diameter of the non-growth halo in the case of bacteria*Salmonella Typhimurium* was not observed; But there was a significant difference between the concentrations of 45 and 60 mg/ml together and compared to the concentrations of 15 and 30 mg/ml. In association with*Staphylococcus aureus*, a significant difference was observed between the diameter of the non-growth halo of all concentrations. The results of the diameter of the aura of absence in the case of bacteria*Bacillus cereus* Similar to bacteria*Salmonella typhimurium* Was.

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In the presence of all concentrations of the studied extract, bacteria*Staphylococcus aureus* And*Salmonella typhimurium* respectively, the highest and the lowest diameter of the halo of lack of growth were assigned to themselves. In addition, the diameter of the non-growth halo in the case of Gram-positive bacteria (*Staphylococcus aureus* And*Bacillus cereus*(larger than Gram-negative bacteria)*Salmonella typhimurium* And*Escherichia coli*) which indicates the higher sensitivity of these bacterial strains against the ethanolic extract of tatorre.

Table 1- Antimicrobial activity of *Stramonium datura* ethanolic extract based on agar disk diffusion method (mm)

Concentration / Microorganism	15 mg/ml	30 mg/ml	45 mg/ml	60 mg/ml
Escherichia coli	7.00 ±0.14^{d}	9.10 ±0.21^{c}	11.30 ±0.27^{b}	14.20 ±0.31^{a}
Salmonella typhimurium	6.60 ±0.45^{c}	7.40 ±0.47^{c}	9.80 ±0.26^{b}	12.00 ±0.17^{a}
Staphylococcus aureus	8.20 ±0.33^{d}	10.40 ±0.30^{c}	13.30 ±0.24^{b}	16.40 ±0.28^{a}
Bacillus cereus	7.50 ±0.24^{c}	8.00 ±0.36^{c}	11.50 ±0.13^{b}	14.30 ±0.16^{a}

Values are expressed as mean ±standard deviations, $n = 3$; different letters (a, b, c and d) in each row show significant difference at $p \leq 0.05$.

Table 2 shows the results of the antimicrobial effect of the ethanolic extract of tatore based on the agar well method. In relation to bacteria*Escherichia coli* ،*Salmonella Typhimurium* And*Staphylococcus aureus*, a significant difference was observed between the diameter of the no growth halo in all studied concentrations and increasing the concentration of the extract caused a significant increase in the diameter of the no growth halo. The concentrations of 30 and 45 mg/ml of the extract caused a significant change in the diameter of the halo of non-growth in bacteria.*Bacillus cereus* It did not, but the diameter of the growth halo in the presence of the concentration of 60 mg/ml of the extract was significantly larger than the diameter of the growth halo in the presence of the concentrations of 45, 30 and 10 mg/ml of the extract. It should be noted that the highest and lowest diameters of the non-growth halo are respectively related to bacterial strains*Staphylococcus aureus* And*Salmonella typhimurium* and gram-positive bacteria showed higher sensitivity than gram-negative types against the ethanolic extract of tatorre. In addition, at a constant concentration of the extract, the diameter of the halo of non-growth in the agar well method was larger than that of the agar disk diffusion method.

Table 2- Antimicrobial activity of *Stramonium datura* ethanolic extract based on agar well diffusion method (mm)

Concentration / Microorganism	15 mg/ml	30 mg/ml	45 mg/ml	60 mg/ml
Escherichia coli	7.30 ±0.20^{d}	9.90 ±0.18^{c}	12.00 ±0.34^{b}	14.50 ±0.27^{a}
Salmonella typhimurium	7.30 ±0.16^{d}	9.50 ±0.32^{c}	11.70 ±0.43^{b}	13.90 ±0.36^{a}
Staphylococcus aureus	8.80 ±0.29^{d}	11.00 ±0.40^{c}	14.20 ±0.18^{b}	17.10 ±0.38^{a}
Bacillus cereus	8.20 ±0.27^{c}	10.90 ±0.54^{b}	11.60 ±0.48^{b}	15.00 ±0.43^{a}

Values are expressed as mean ±standard deviations, $n = 3$; different letters (a, b, c and d) in each row show significant difference at $p \leq 0.05$.

The results of tests of the minimum concentration of inhibition and lethality of the ethanolic extract of tatore against bacterial strains are presented in Table 3. Minimum inhibitory concentration for bacteria*Escherichia coli* ،*Salmonella typhimurium* ،*Staphylococcus aureus*

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And*Bacillus cereus* 16, 16, 8 and 8 mg/ml were obtained, respectively, which reflects the higher sensitivity of gram-positive bacteria compared to gram-negative strains. The minimum lethal concentration values for these bacteria were 256, 256, 64 and 128 mg/ml, respectively. According to the results, bacteria*Staphylococcus aureus* The most sensitive bacterial strain against the ethanolic extract was Tatore.

Table 3- Antimicrobial activity of *Stramonium datura* ethanolic extract based on minimum inhibitory/bactericidal concentration method (mg/mL)

Microorganism	MIC (mg/mL)	MBC (mg/mL)
Escherichia coli	16	256
Salmonella typhimurium	16	256
Staphylococcus aureus	8	64
Bacillus cereus	8	128

Medicinal plants contain various metabolites that show antimicrobial activity in vitro and in vivo [21, 22, 24]. The results of this study showed that the ethanolic extract of tatore has a greater antimicrobial effect against Gram-positive bacteria, which is in line with the findings of other researchers [7, 23]. This condition is mainly due to the presence of a single mucopeptide layer in their cell membrane, which makes them more sensitive to antimicrobial agents. In contrast, the cell membrane of Gram-negative bacteria contains a more complex lipopolysaccharide and phospholipid layer with a lower diffusion rate than hydrophobic antimicrobial compounds [14, 15]. In addition, antibacterial activity or inhibition zone was observed more in the agar well method compared to the agar disk diffusion method. In fact, the bacterial species in the agar well method are in direct contact with the extract, but the diffusion rate of the antimicrobial agent from the disk surfaces to the environment determines its inhibitory effect in the agar disk diffusion test [20]. Antibacterial activity of methanolic extracts of aerial parts of two species*D. harmless* And*D. stramonium* (Tatoreh) was investigated by Iftikhar et al. (2005) based on the agar disk diffusion method. extract*D. harmless* Antibacterial activity more against*Bacillus subtilis* ، *Enterococcus faecalis* And*Staphylococcus aureus* shows. The activity was concentration-dependent and the highest level was observed at 2.5 mg/ml. Tatore had slight antibacterial activity against gram-positive bacteria at a concentration of 2.5 mg/ml, and lower concentrations were not effective. Both plant extracts have little activity (or no effect) against*Escherichia coli* And*Pseudomonas aeruginosa* they had. When the results were compared with the antibacterial activity of ampicillin, plant extracts showed equal or better antibacterial activity [25]. The antimicrobial effect of the methanolic extract of tatore seed has been shown in another study. The maximum average diameter of the halo of non-growth (17.67 mm) in the highest concentration of tatore seed extract against*Shigella flexneri* and followed by 15.33 mm vs*Staphylococcus aureus* ،*Escherichia coli* And*Salmonella Typhimurium* was, while the lowest inhibition area (14.33 mm) vs*Pseudomonas aeruginosa* was registered [18]. In the research of Vaza et al. (2015), the methanolic extract of tatora seeds showed the highest inhibition zone (20 mm) against bacteria.*Escherichia coli* Showed, followed*Staphylococcus aureus* (17.50 mm) and*Pseudomonas aeruginosa* (16 mm) was placed [26]. Another study reported that the methanolic extract of tatore fruit growth*Escherichia coli* ، *Staphylococcus aureus* ،*Pseudomonas aeruginosa* and fungal strains*Aspergillus flavus* ،*Aspergillus niger* ، *Fusarium clemorum* And*Rhizopus astolonifera* inhibits [27]. On the other hand, a study conducted by Julius et al. [2018] showed weak antibacterial properties of methanolic extract of Tatura seed against bacteria.*Escherichia coli* ،*Pseudomonas aeruginosa* And*Staphylococcus aureus* showed inhibition zones of 7, 6 and 5 mm at a concentration of 500 mg/ml [28]. The antimicrobial activity of Tatura extract may be due to the main compounds isolated from Tatura such as tropane alkaloids, atropine and scopolamine [29]. The difference in the results of this study with the findings of other researchers can be because the quality and quantity of the extract mainly depends on the variety, growth stage, climate, growth location and time of plant collection and extraction conditions [30-34]. In general, the ethanolic extract of tatore has

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excellent antimicrobial activity, which makes it used as a natural source of preservatives in various food products to inhibit microbial growth and thus improve their shelf life and quality.

4 - Conclusion

The results of this study showed that the ethanolic extract of tatore leaf has antibacterial activity depending on the concentration and type of bacteria, and as a result, it has a great potential to be developed as an antibacterial agent for the treatment of bacterial infections. The antimicrobial activity shown by Tatura plant extract against the pathogenic test organisms used in this study can also be used as evidence to provide scientific support for the continuation of the traditional use of this medicinal plant by local communities in the country in the treatment of various diseases caused by pathogenic bacteria in be considered However, more studies are needed to discover the mechanism of antimicrobial activity of the ethanolic extract of tatore and to increase its use in many food products.

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6- Resources

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چکیده

تاتوره به عنوان یک داروی ارزشمند در بسیاری از بیماری‌ها از جمله التهاب، زخم‌ها، تب و دندان درد به خوبی شناخته شده است و عصاره آن دارای فعالیت ضدمیکروبی قابل‌توجهی می‌باشد؛ بنابراین، این مطالعه با هدف استخراج عصاره اتانولی برگ تاتوره و بررسی فعالیت ضدمیکروبی آن صورت گرفت. در این مطالعه، عصاره تاتوره با استفاده از روش ماسراسیون استخراج گردید. روش‌های ضدمیکروبی دیسک دیفیوژن آگار، چاهک آگار، حداقل غلظت مهارکنندگی و حداقل غلظت کشندگی جهت تعیین فعالیت ضد باکتریایی عصاره اتانولی برگ تاتوره در برابر باکتری‌های *اشرشیا کلی*، *سالمونلا تیفی موریوم*، *استافیلوکوکوس اورئوس* و *باسیلوس سرئوس* استفاده گردید. فعالیت ضدمیکروبی عصاره وابسته به غلظت بود و بالاترین قطر هاله عدم رشد در غلظت ۶۰ میلی‌گرم در میلی‌لیتر عصاره مشاهده گردید. مطابق آزمون‌های دیسک دیفیوژن آگار و چاهک آگار، باکتری‌های *استافیلوکوکوس اورئوس* و *سالمونلا تیفی‌موریوم* به ترتیب بالاترین و کمترین قطر هاله عدم رشد را به خود اختصاص دادند. حداقل غلظت مهارکنندگی برای باکتری‌های *اشرشیا کلی*، *سالمونلا تیفی موریوم*، *استافیلوکوکوس اورئوس* و *باسیلوس سرئوس* به ترتیب برابر با ۱۶، ۱۶، ۸ و ۸ میلی‌گرم در میلی‌لیتر بدست آمد و مقادیر حداقل غلظت کشندگی برای این باکتری‌ها به ترتیب ۲۵۶، ۲۵۶، ۶۴ و ۱۲۸ میلی‌گرم در میلی‌لیتر بود. بطور کلی، باکتری‌های گرم مثبت نسبت به انواع گرم منفی در برابر عصاره حساس‌تر بودند. عصاره اتانولی برگ تاتوره را می‌توان بعنوان عامل ضد میکروب طبیعی جهت جلوگیری از رشد باکتری‌های پاتوژن و درمان بیماری‌های مرتبط استفاده نمود.