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Evaluation of the Sporicidal Activity of Ethanol Extract of Arctium lappa Root against Bacillus cereus

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Abstract

***Background*:** *Bacillus cereus* is one of the most common causes of food spoilage, keratitis, endophthalmitis, and panophthalmitis. These bacteria produce spores which are resistant to chemical and physical agents. Nowadays, the sporicidal properties of plants have been considered as alternatives to chemical sporicidal agents.

***Materials and Methods*:** In this empirical-experimental study the effect of ethanol extract of edible burdock (*Arctium lappa*) root has been studied on *Bacillus cereus* spores. In this investigation, the suspensions of tested microorganisms were cultured in sporulating agar. Sporulation process was assessed by optical microscopy following the staining of spores. Then the produced spores were exposed to various concentrations (100, 150, 200, 250, 300 mg/mL) of ethanol extract of edible burdock (*Arctium lappa*) root and finally the remaining spores were counted. With increasing concentrations of ethanol extract, the number of spores declined.

***Results*:** Pearson correlation showed inverse relation between the spores count and concentration of ethanol extract of edible burdock (*Arctium lappa*) root ($r=-0.765$, $p<0.001$). The most effective extract concentration was 300 mg /mL.

***Conclusion*:** Ethanol extract of edible burdock (*Arctium lappa*) root, has sporicidal activity. Only, the sporicidal nature of ethanol extract has been evaluated by this study; therefore, the assessment of other extracts and essences is necessary.

Introduction

Bacillus cereus is a gram-positive, aerobic and spore-forming microorganism, belongs to Bacillus genus. This bacterium produces toxins during sporulation. It is an important cause of food poisoning and ophthalmic infections such as keratitis, endophthalmitis and panophthalmitis [1, 2]. Food poisoning caused by *B. cereus* is in two types: emetic and diarrheal, the diseases caused by produced toxin [2]. The bacterial spores have been spread widely in nature, water and soil. They may contaminate contact lens and grains including rice resulting keratitis associated with contact lens wear and food poisoning respectively [3, 4]. Many methods, such as high temperature, radiation and chemicals are used to eliminate these resistant forms [3].

Hence, due to limitations in the using of antibiotics and possible complications of other chemical disinfectant, we need sporicidal materials with the least damage and fewer complications. Assay of sporicidal effects of plant extracts can result in finding an agent with plant origin that can be used for decontamination of contact lenses and grains.

In the traditional medicine, the *Arctium lappa*, which belongs to Asteraceae family, Arctium genus, has been used in many regions of the world claiming many therapeutic effects [5-7]. Therapeutic properties of this plant can be summarized as, anti-fungal, antioxidant, anti-HIV [7, 8] anti-inflammatory, anti-viral and anti-poisoning [6, 9].

The main ingredients in this plant consist of five powerful flavonoid-types, terpenoids, inulin, several polyphenols, tannins and poly-acetylene [10, 11]. The purpose of this study was to examine the sporicidal activity of ethanol extract of *A. lappa* root.

Materials and Methods

In an empirical-experimental study sporicidal activity of ethanol extract of *A. lappa* root was investigated.

Preparation of ethanol extract of burdock (*A. lappa*) root: Soxhletting apparatus was used for preparing ethanol extract of herb. First of all, 35 g herbal powder added to 250 mL of, 80% ethanol [11]. Then, they were placed in the device for 48 hours. The extract was filtered and dried using a rotary evaporator. Residues were stored in sterile screw capped bottle at 4ºC away from light until time to use.

Preparation of bacterial suspensions: The *B. cereus* (PTCC: 35668) strain were obtained from Iranian Research Organization for Science and Technology. The microorganism was grown at 37°C for 24 h in Nutrient Agar (Merck) and then seeded in to 1.5 mL nutrient broth (Merck) to produce a turbidity of 0.5 McFarland scale,

which corresponds to a concentration of $1.5x10^8$ colony forming units mL^{-1}.

Preparation of spore suspensions: One milliliter of the standard suspension (1.5×10^8 cfu/mL) was inoculated on Sporulating Agar (Merck) by spread plate method. Then the plates were incubated at 37ºC for seven days to get more than 90% of the spores [12]. Sporulation process was examined by using an optical microscope and stained spores [13].

Free spores were visible at 3rd day, almost 90% of bacteria were in spore phase at the 7th day after staining the background, and vegetative form was seen no more than 8-10.

At seventh day, firstly spores were collected in the sterile Eppendorf by adding 3-5 mL of cooled (4ºC) sterile distilled water to each plate, using speridil, and then they were centrifuged at 4ºC, at 9000 rpm for 15 minutes [12]. This step was repeated twice to remove impurities. Then, spores were suspended in 1 ml of sterile water for 20 minutes in water bath 80ºC, for losing vegetative forms. Finally, spores suspension was kept at 4ºC until treatment with the extracts.

Evaluation of sporocidal effects: The dried extract was dissolved in 0.5% dimethylsulfoxide (DMSO, Merck) and serially diluted to yield 100, 150, 200, 250 and 300 mg/mL.

Eppendorfs tubes containing spores were centrifuged at 4ºC, 9000 rmp for 15 min. Supernatant was removed, and then, 1 mL of each dilution of the extract was added to each Eppendorf tube. They were vortexed and finally incubated at 37ºC for 24 h [13]. Spores were centrifuged in 14500 rpm for 5 minutes, and to prevent the extract effects, they were washed three times with sterile saline [13].

Final spores were dissolved in 1 mL of normal saline consequently; dilution series were set for colony counting. Then 0.1 mL of dilutions, were inoculated to nutrient agar by spread plate method to obtain single colony [13, 14]. In this study, formaldehyde was used as a positive and 5% DMSO as a negative control. The data were analyzed using SPSS-14 software. The selected threshold level for statistical significance was *p*-Value less than 0.05.

Results

The results showed that different concentrations of ethanol extracts of edible burdock root have sporicidal effect. The concentration of 300 mg/mL had shown the greatest level of activity and at this concentration the log of spore counts reduced by 1.46 units, compared to the control. With increasing the extract concentration, this effect increased. Also, with increasing the concentration of the extract, the logarithm of remaining spores reduced (Table 1).

In addition, the Pearson correlation coefficient showed that the extract concentration and the logarithm of the remaining spores are inversely related *(r= -0.756, p<0.001)*.

Table 1. The effect of ethanol extract of edible burdock (Arctium lappa) on Bacillus cereus spores according to logarithmic reduction

Mean logarithm of reduction in spores count compared to control	Extract density (mg/mL)
1±0.23	100
1.17±0.18	150
1.18 ±0.26	200
1.22 ±0.26	250
1.46 ±0.06	300

Results are reported as Mean±SD, (DMSO 5%) Control: negative control, Results of the experiment is the average of 3 times measurement

Discussion

The study focused on sporicidal activity of ethanol extract of edible burdock (*A. lappa*) root. Present study was found that ethanol extracts of *A. lappa* root has anti-microbial effects against *B. cereus* spores.

Recently, with the acceptance of traditional medicine as a method for resolving health problems, and also for combating with microorganisms resistant to common antibiotics, the researchers have been encouraged to examine the antimicrobial properties of herbs.

Moreover, because of their antimicrobial effects, adding these extracts to industrial foods, pharmacological and cosmetic products, is increasing, so it is important to study of different properties of these plants [15].

Edible burdock has been used, for hundreds of years in Europe, North America and Asia for therapeutic purpose [6].

The results of some studies have confirmed the antimicrobial effects of these plants. But there is some controversy about same points, for example Pereira et al. study showed that lyophilized extracts of leaves on *Candida albicans, Lactobacillus acidophilus, Pseudomonas aeruginosa* and the vegetative form of *Bacillus subtilis* were effective [7]. Also, a study was conducted by Takasugi it showed polyacetylene extract of plants; root has antibacterial and antifungal effects [6].

Results of other studies shows aerial root of this plant has an excellent antibacterial effect on vegetative forms of Bacillus spp., *B. cereus*, while it has not been effective on *C. albicans*, *P. aeruginosa* and *Staphylococcus aure*s and *Escherichia coli* [16, 17]. The difference in results can be attributed to the difference in solvents used in extraction, plant growth conditions, and tested organisms.

In a study have been conducted by Cho et al. on 79 plant species, only ethanol extracts of 14 species, have shown antimicrobial activity against *B. cereus* spores [18].

In a study that has carried out by Lawrence et al. on *Bacillus subtilis* spores, 13 plant essences have been studied. In this study all of the essences have shown moderate sporicidal effects, but three plants, including cardamom, juniper, and tea tree leaves had the greatest influence. The mean log reduction of spores was between 0.7±0.18 and 3.12±0.21 [13].

In the present study, the mean logarithm of reduction in spores count in 300 mg/mL concentration of ethanol extract of burdock root was 1.46±0.06.

Considering that tested bacteria and plants in this study are different, the difference in some of the results is justified. In a research that has undertaken by Cho et al. on ethanol extract of *Torilis japonica* antimicrobial properties have confirmed against spores of *B. subtilis* [19]. By comparing the obtained results from some researches on the sporicidal properties of extracts and plant essences, it seems that the effect of extracts is more than plant essence. Therefore, the assessment of sporicidal activity of other extracts and essences of this plant is necessary.

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Authors' Contributions

All authors had equal role in design, work, statistical analysis and manuscript writing.

Conflict of Interest

The authors declare no conflict of interest.

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