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Antimicrobial Activity of *Echinophora platyloba* Aqueous Extract on Gas- Producing Fungi in Doogh

F. Edadi Ebrahimi [a], ***A. Javadi*** [b *]

[a] *M. Sc. Graduate Student of the Department of Food Science and Technology, Shabestar Branch, Islamic Azad University, Shabestar, Iran.*
[b] *Associate Professor of the Department of Food Hygiene, Biotechnology Research Center, Tabriz Branch, Islamic Azad University, Tabriz, Iran.*

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ABSTRACT: Nowadays, the application of natural antimicrobials like plant extracts in food preservation is becoming popular and increasing. The aim of this study is to determine the effect of *Echinophora platyloba* (*E. platyloba*) aqueous extract on gas-producing fungi and sensory properties of doogh. For this reason, the concentration of 0% (Control), 0.125%, 0.25%, 0.5%, 1%, 2%, 4% and 8% of the extract were prepared. Five samples of doogh containing no preservatives or extract was purchased from manufacturer factories. Six types of mold and ten types of yeast were isolated from the samples. The isolated fungi were cultured in the plates containing different concentrations of *E. platyloba* aqueous extract. After determination of the effective concentration of the extract, the sensory properties of samples were evaluated using five-point hedonic scale. The results showed that the *E. platyloba* extract have antifungal properties and its effect against the yeasts was greater than molds. At the extract concentration of 8%, the count of yeasts decreased to zero and the half of mold growth was inhibited. Due to the negative effect on sensory properties of doogh at the extract concentration of 8%, it cannot be used as a preservative, but it might be applied as an aromatic or flavoring agent at lower concentrations in this product.

Keywords: *Doogh, Echinophora Platyloba, Extract, Fungi*.

Introduction

Due to the increasing awareness about the adverse effects of synthetic preservatives on human health (toxic and carcinogenic effects), consumers are concerned about the safety of foods containing these compounds in recent years (Shariat *et al.*, 2014). In the last two decades, because of the increasing microbial resistance to antibiotics, consumers are interested to herbal products with antimicrobial properties (Clark *et al.*, 1996).

Nowadays, public interest is drawn to the use of plant origin compounds as natural food preservatives and there is a scope for producing safe foods that have a natural or green image. Due to the unique properties such as good flavor, inhibitory effect on food spoilage and enhancement of the shelf life, the use of natural compounds like wild growing plants as food preservatives has a great importance (Burt, 2004).

Doogh is one of the traditional beverages in Iran as well as in Eastern Europe, Middle East and Asian countries (Azarikia, 2009). Various types of spices, plant essential oils and extracts are used in doogh production for flavoring and enhancing its acceptability for consumers (Seifi *et al.*, 2014).

*Corresponding Author: Javadi@iaut.ac.ir

Echinophora platyloba (*E. platyloba*) belonging to Apiaceae family, is a yearlong gramineous and aromatic plant that its height varies from 90 to 100 cm. It has cylindrical and glabrous stems, needle-shaped alternate leaves, yellow and small flowers with umbrella-shaped inflorescence, right cone-shaped roots and hazelnut fruit containing small granules.

E. platyloba belongs to the Echinophora genus (Avijgan, 2006) that contains 10 different species. Four species including *E. cinerea, E. platyloba, E.orientalis, E. sibthorpiana* are native to Iran and are distributed in the west and North-West of this country. The first two species are exclusively grow in Iran. The others, beside Iran are grown in Mediterranean region, Armenia, Turkmenistan, Afghanistan, Balkan Peninsula, Crete, Cyprus and Syria (Mozaffarian, 1996).

The chemical composition and in-vitro antibacterial activity of *Echinophora cinerea* Boiss. (*E. cinerea*) essential oil (EO) has been evaluated previously. It was demonstrated that the EO has antibacterial properties and the greatest effect has been reported against *Staphylococcus aureus* (Zarali *et al.*, 2016). Moghtader et *al.* (2013) also reported a significant inhibitory effect of *E. platyloba* EO against *Aspergillus flavus* in comparison to gentamicin. Phenolic compounds in *E. platyloba* EO like other plant EOs is considered as the best sources of natural antioxidants. Antibacterial activity of *E. platyloba* EO was reported against *Staphylococcus aureus*, but its effect was poor against *Staphylococcus* and *Escherichia coli*. *Pseudomonas aeruginosa* was also resistant to this EO. According to antibacterial and antioxidant properties of *E. platyloba* EO, this EO can be applicable in food, pharmaceutical and cosmetic industry (Pass *et al*., 2012). Due to the demonstration of the antimicrobial effects reported of *E. platyloba*, this study is aimed to determine the antimicrobial effect of *E. platyloba* aqueous extract against gas producing fungi in doogh. Since the preparation of aqueous extract is economical and can be easily used in doogh at the industrial scale in Iran, only this type of extract was applied in the present study.

Materials and Methods

- *Plant material*

E. platyloba was provided from Maragheh city located in East-Azerbaijan Province of Iran. The taxonomic of the plant materials was confirmed by the Agricultural Jihad Organization of East Azerbaijan province of Iran.

- *Preparation of the aqueous extract of Echinophora platyloba*

Twenty grams of powdered *E. platyloba* was dissolved in distilled water (at a ratio of 1:10 w/v) for 32 hours. In order to remove plant residues, Whatman filter paper (pore size 0.22 µm) was used. The solvent was removed from the extract using a rotary evaporator (Model RV-10, Germany) (at 50 °C, pressure of 0.05 atm and speed of 30 rpm). In order to obtain fully-dried extract, it was placed into an incubator (LABINCO, Netherlands) at 37° C. The powdered extract was dissolved in 10% dimethyl sulfoxide (DMSO) at the ratio of 1: 2 (w/v). In order to clarify the extract solution and remove the remaining solids, the solution was centrifuged at 3500 rpm for 5 minutes (Allerga, Germany).

- *Providing of doogh samples*

Five samples of doogh containing no preservatives or extract were purchased from factories. The pH and acidity levels of the samples were measured.

- *Isolation of fungi from doogh samples*

For this purpose, the samples were incubated at the ambient temperature (25-26 °C) for 10 days. Fungi were isolated after their growth on the surface of samples. They

were cultured on Sabouraud Dextrose agar (SDA) (Merck, Germany) and Yeast extract Glucose Chloramphenicol agar (YGCA) (Merck, Germany) for the isolation of molds and yeasts, respectively. The plates were incubated at 20±2°C for 5 days.

- *Inhibitory effect of Echinophora platyloba extract against molds in doogh*

In order to evaluate the antifungal activity of the extract, SDA medium was used. 15 ml of the medium containing 8%, 4%, 2%, 1%, 0.5%, 0.25% and 0% (Control) of extract were poured on sterile plates. Three plates were provided for each concentration of extract. Isolated molds from samples in the previous step were cultured on the plates and incubated for 5 days at 20±2°C. The diameter of molds growth was measured daily. The molds were identified using slide culture technique.

- *Inhibitory effect of Echinophora platyloba extract against yeasts in doogh*

For this purpose, YGCA medium was used. 15 ml of the medium containing 8%, 4%, 2%, 1%, 0.5%, 0.25% and 0% (Control) of the extract was transferred to sterile plates. Each concentration of extract was prepared in three replicates. Yeast suspension was prepared using turbidity measurement method after adding yeast colonies in the ringer solution and comparison with half McFarland standard. Using pour-plate method, 1 mL of yeast suspension was inoculated in the center of each plate containing YGCA and *E. platyloba* extract. Plates were incubated at 20±2°C for 5 days and yeasts colonies were enumerated.

- *Evaluation of sensory properties of doogh samples containing the extract*

After determining the effective concentration of the extract against fungi, doogh samples containing extract were prepared. They were stored for 5 days and their sensory properties were evaluated on the 1st and 5th day of storage using 5-point hedonic scale. The color, odor, taste, gas production and general acceptability of the samples were examined by ten skilled judges.

- *Data analysis*

Data analysis was performed using SPSS software (version 22) and ANOVA test. The graphs were provided using Excel software (version 2013).

Results and Discussion

The mean value of pH and acidity of purchased Doogh samples were 3.8 and 42°D, respectively. Penicillium and Acremonium were detected as the genus of molds in the samples.

Sixteen species of fungi including 6 species of molds and 10 species of yeasts were isolated. The results of antifungal activity test showed that the inhibitory effect of the extract on molds was improved by increasing the extract concentration and storage time. At every time interval of storage, the lowest diameter of the inhibition zone was related to the extract concentration of 8% (Figure 1).

The differences between the concentrations of extract showed that there were significant differences between the control (0%), 0.5%, 1%, 2%, 4%, and 8% of the extract ($p<0.05$). The extract concentrations of 8% in doogh had the greatest antimicrobial activity as compared to other concentrations ($p<0.05$) (Figure 2).

The results concerned with the color, odor, taste and general acceptability of the samples indicated that the control sample 0% on the 1st day of storage period and the sample containing extract concentration of 4% on the fifth day had the best and least quality characteristics, respectively (Figure 3).

Regarding to the gas production, samples containing 4% on the 5th day of storage had

the best quality in this aspect. The lowest quality was related to the control sample (0%) on the 5th day of storage (Figure 4).

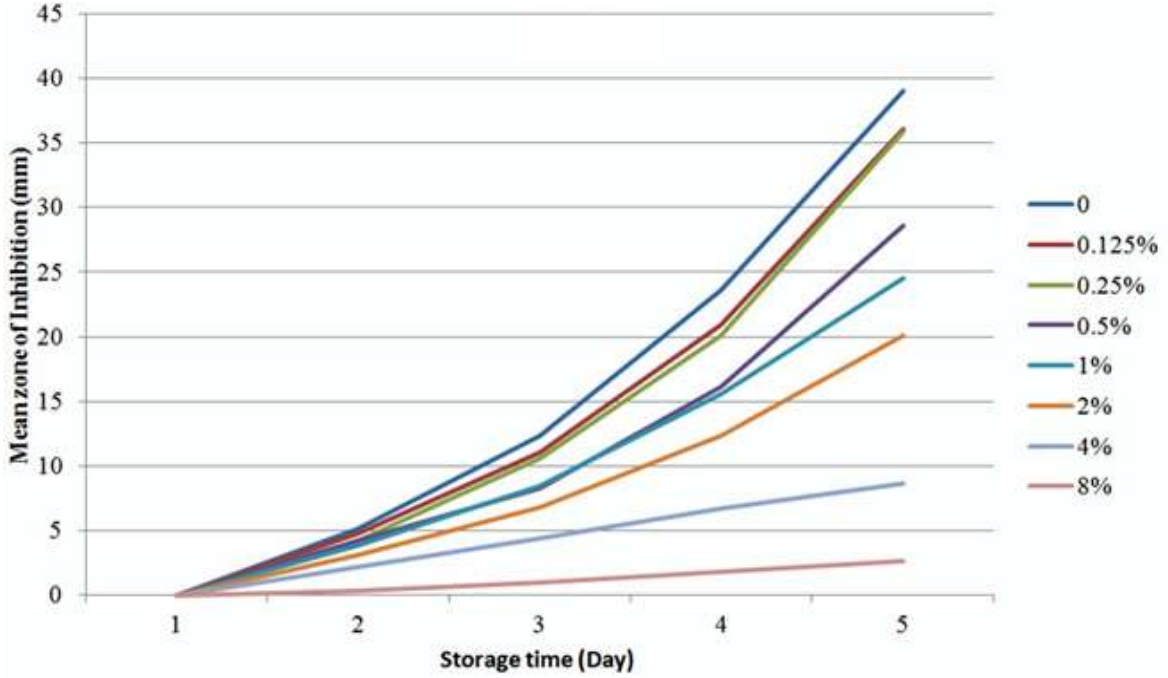

Fig. 1. Growth Inhibition of isolated molds from doogh samples by the aqueous extract of *Echinophora platyloba* during 5 days of storage.

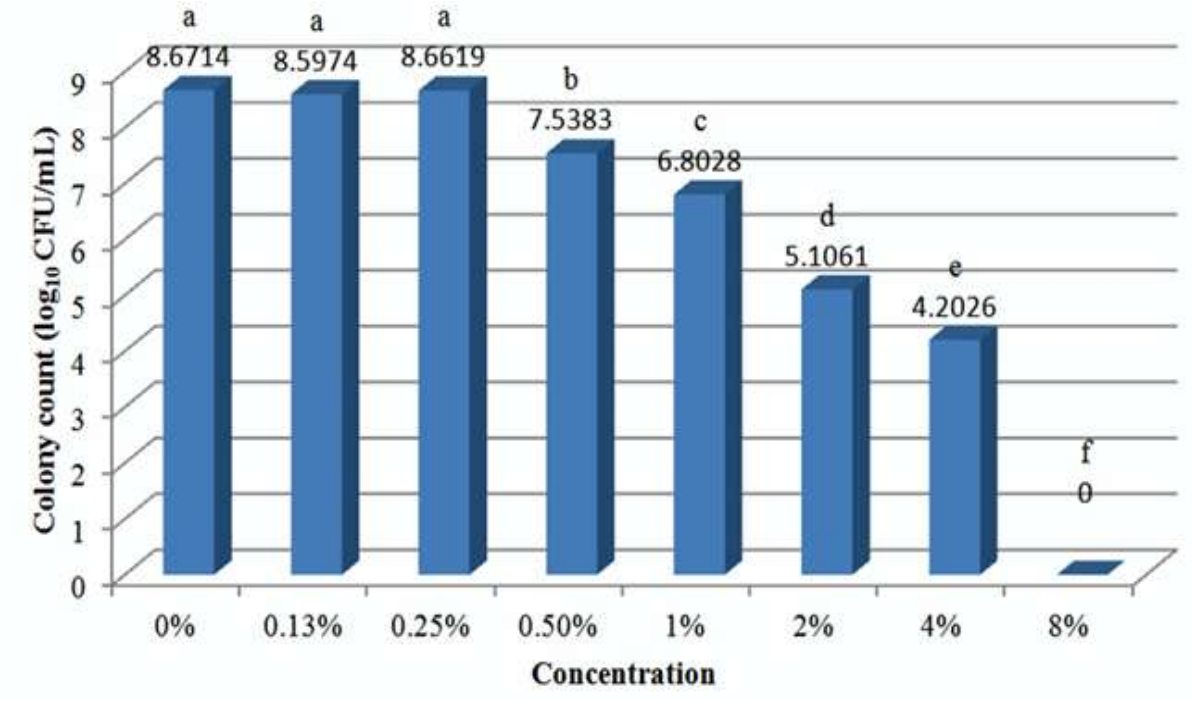

Fig. 2. The colony counting of isolated yeasts (log_{10} CFU/mL) from doogh samples affected by different concentrations of the aqueous extract of *Echinophora platyloba* during 5 days of storage. The difference between the columns with different letter was significant ($p<0.05$).

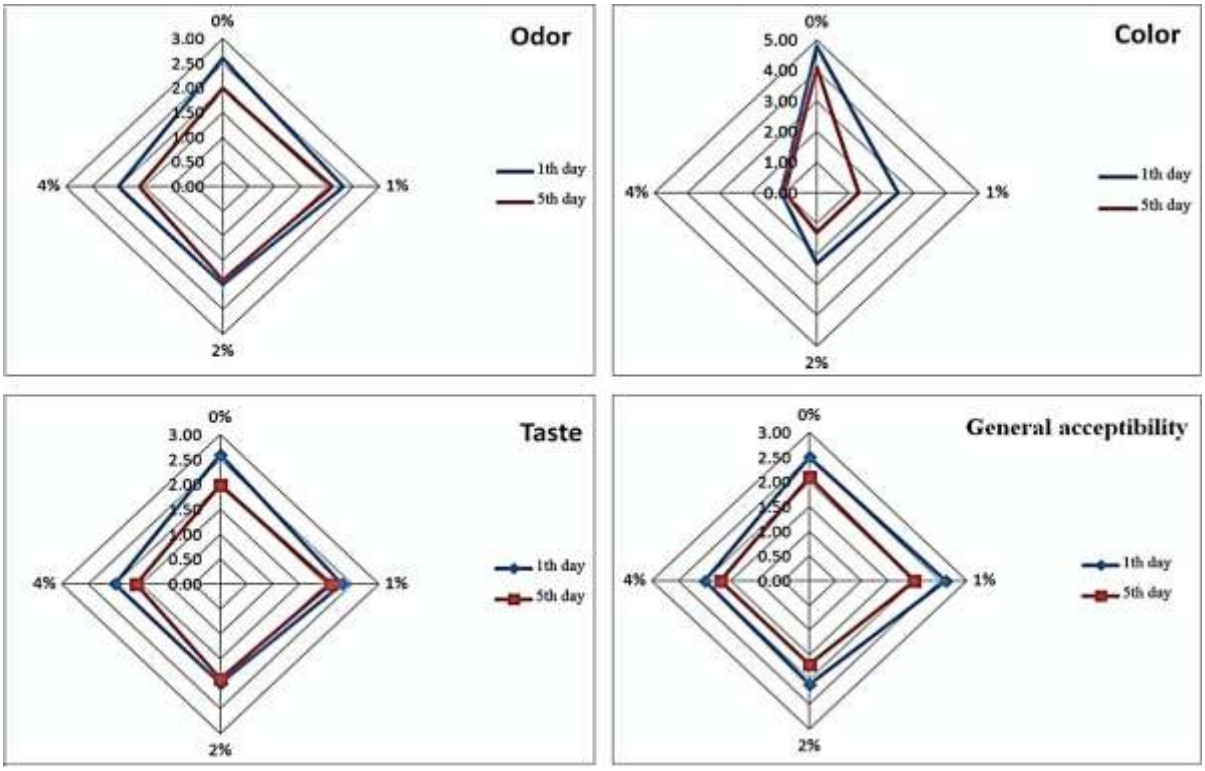

Fig. 3. Sensory properties of doogh samples containing different concentrations of *E. platyloba* extract according to color, odor, taste and general acceptability.

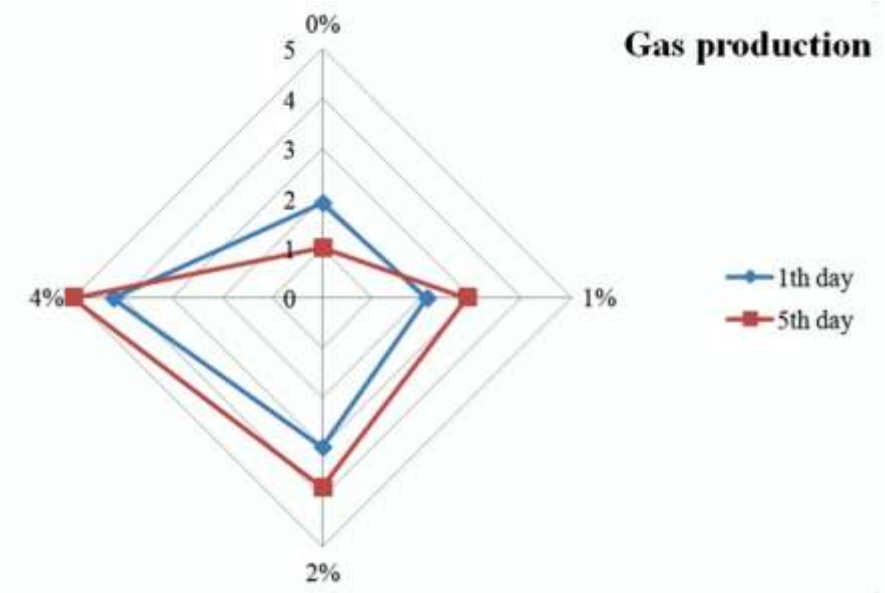

Fig. 4. Sensory properties of doogh samples containing different concentrations of *E. platyloba* extract according to gas production.

In this study, the antimicrobial activity of *E. platyloba* aqueous extract was evaluated against gas-producing fungi in doogh and it was found that the extract had a significant antifungal activity. These results are in accordance with the findings of Moghtader (2013), Zarali *et al.* (2016), Mahboubi *et al.* (2009) and Avijgan *et al.* (2006). The

antifungal activity of this extracts was proven against the fungi such as *Trichophyton rubrum, Microsporum gypseum*, Trichophyton mentagrophytes, *Epidermophyton Floccosum*, *Microsporum canis* and *Candida albicans* (Mahboubi *et al.*, 2009; Avijgan *et al.*, 2006). It has been reported that the antifungal activity of *E. platyloba* extract are associated with saponin, flavonoids and alkaloids present in it (Pass *et al.*, 2012). Tannins are also responsible for the antimicrobial activity of this extract. This effect may be related to their inhibitory effect against microbial adhesion, enzymes and transport proteins of cellular membrane (Zargari, 1998). The antifungal activity of *E. platyloba* may also be associated with high percentage of alpha-*phellandrene as a* cyclic monoterpen (Moghtader, 2013). Other components such as carvacrol, p-cymene and linalool are also involved in this effect (Buchanan, 1981).

In the present study, it was found that the antifungal activity of extract was enhanced by the increase in extract concentration and the highest activity was observed at the concentration of 8%. Similar result was also reported by Abbasi and Mahdavi (2016) and Hesarinejad *et al.* (2011).

The sensory properties are the limiting factors in the application of plant extracts and essential oils in food. In this study, the quality of doogh samples in terms of color, odor and taste decreased by the increase of extract concentration.

Plant extracts and essential oils having various chemical components might destroy the microorganisms via different mechanisms. The most important mechanism for this effect is related to the hydrophobicity of their components. These substances can penetrate to the cell and mitochondrial membrane that lead to dysfunction of bacterial cells followed by increased permeability and the leakage of ions and other cell contents (Pauli, 2001).

The antimicrobial effect of plant extracts and essential oils is dependent on lipid composition and net surface charge of microbial membrane. Therefore, the consistency of the composition of antibacterial compounds with cell membrane is necessary for their passing through the microbial cell membrane (Trombetta *et al.*, 2005).

The comparison between the results of various studies about the antibacterial effect of plant extracts is very difficult. One of the reasons for this phenomenon might be related to the application of different extraction methods that can significantly affect the efficiency of the active ingredients in plant extracts. The used solvent for extraction, plant type and strain; the stage of plant growth and harvest; climatic and geographical conditions as well as the used microbial strains for evaluation might affect the level of antibacterial activity of the extracts (Jerković, 2001). The results of various studies demonstrated that most of plant extracts and essential oils can affect the growth of different types of bacteria and fungi (Talei *et al.*, 2007). These findings are in accordance with the results of the present study.

Conclusion

In this research, it was observed that the antifungal activity of *E. platyloba* extract against yeasts was more than molds. Generally, the results of this study showed that the extract concentration of 8% was the most effective concentration against fungi than other concentrations, but this concentration had undesirable effects on the sensory properties of doogh. Therefore, the aqueous extract of *E. platyloba* should be used in low concentrations as an aromatic and flavoring additive and it cannot be employed as a preservative at high concentrations due to the adverse effects on the sensory properties of doogh.

Regarding the antimicrobial properties of *E. platyloba* extract and evaluation of its antifungal activity in other foods such as cheese and yogurt as well as its antibacterial effects against lactic acid bacteria, coliforms and total bacterial count future studies have

been suggested. Due to the traditional use of *E. platyloba* extract in marinades, further studies about its antibacterial effect on fermentative and spoilage microorganisms on these foods would be useful.

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Conflict of interest

The authors do not have any conflict of interest to declare.

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