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## Assessment of the plantago major extract for antimicrobial activities

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### ABSTRACT

Plants have been used for their medicinal properties for thousands of years. From ancient times, different scientists and physicians had used various types of herbs to treat diseases and many books and treatises had been written to describe different effects of different plants on different maladies. Emerging the drug resistant pathogens in recent decades prompted the scientists to evaluate the effects of the medicinal plants against pathogens. *Plantago major* is one these herbs which its values has been praised since time immemorial. *P. major* has been used to treat different kinds of maladies including infectious diseases. Thus, we conducted this survey to evaluate the antimicrobial activities of this herb against three common pathogens: *Staphylococcus aureus*, *Escherichia coli* and *Listeria monocytogenes*. To achieve this purpose, the activity of the *P. major* both aqueous and alcoholic extract against mentioned pathogens was scrutinized by disc diffusion method and the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the seed extracts were determined as well. Our results showed that the MIC of the aqueous extract was between 0.022 mg/mL and 0.045 mg/mL for *S. aureus* and between 0.09 mg/mL and 0.181 mg/mL for both *E. coli* and *L. monocytogenes*; however, the aqueous extract did not show any bactericidal activity and no inhibitory zone was detected by disc diffusion method. Strains which were used in this inquiry were completely resistant to the alcoholic extract and thus its MIC and MBC remained undetermined and no inhibitory zone was detected.

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### KEYWORDS

*Plantago major*;  
Antimicrobial activities;  
Minimum inhibitory  
concentration;  
Minimum bactericidal  
concentration;  
Disc diffusion.

### INTRODUCTION

Plants have been used for their medicinal properties for thousands of years. According to some inscriptions and other remains of ancient worlds, Egyptians and Chinese were the first who used and even planted herbs<sup>[1]</sup>. Ancient Greeks also were aware of the thera-

peutic features of some plants. Hippocrates, the forefather of Greece medicine, and his follower, Aristotle were very fond of using herbs for treatment of various diseases. "Enquiry into Plants" written by Theophrastus, the successor to Aristotle, consists of ten volumes of which the seventh is about medicinal plants<sup>[2]</sup>. In the first century A.D. Dioscorides, another famous Greek

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physician and botanist wrote an encyclopedia about herbal medicine named “De Materia Medica” in which he mentioned nearly 600 herbs and their medicinal features<sup>[3]</sup>.

In the eighth and ninth century A.D., which may be considered as Islamic golden age, many Iranian physicians such as Avicenna and Rhazes made great efforts in the field of herbal medicine and wrote many worthwhile treatises and books alluding to medicinal plants such as “The Canon of Medicine” and “al-Hawi”. Ibn al-Baitar was another Muslim physician who lived during the thirteenth century A. D and wrote an enormous pharmacopoeia called “Kitab al-jami li-mufradat al-adwiya wa al-aghdhiya” listing nearly 1400 plants, foods and medicinal herbs<sup>[4]</sup>.

Folk medicine has utilized various kinds of plants to cure different diseases. *Plantago*, a genus of the family Plantaginaceae, is one of these herbs which has been used for assorted intentions in different parts of the world since centuries ago. *P. lanceolata*, also known as ribwort plantain and narrow-leaf plantain, has been used to treat ulcers, bronchial catarrh and pharyngeal mucositis and believed to have antimicrobial and nematocidal properties<sup>[5,6]</sup>. *P. major*, another species of this genus, also known as broadleaf plantain has been used as a remedy for different kind of conditions including: skin diseases, Infectious diseases, tumors, high fever, pains, colds, hepatitis and diseases of gastrointestinal tract and respiratory organs<sup>[7]</sup>.

By developing chemotherapy, the traditional medicine was dragged into oblivion. Antimicrobial agents, since discovery have made a great difference in the treatment of infectious diseases. The Introduction of penicillin to the market in 1945, brought up the belief that infectious diseases could be eradicated, but by emerging of the resistant and multi-drug resistant (MDR) strains of pathogens, this belief seems to be unreachable<sup>[8]</sup>. *Escherichia coli* and *Staphylococcus aureus* are those which soon developed antimicrobial resistance. MDR strains of *E. coli* are now isolated from both hospitals and community with a high prevalence<sup>[9]</sup>. *S. aureus* which is considered as a major nosocomial pathogen, soon became resistant to penicillin, the first antibiotic widely used to treat staphylococcal infections, by acquiring the  $\beta$ -lactamase producing plasmid. Shortly after introduction of methicillin, a synthetic penicillin, me-

thicillin-resistant *S. aureus* (MRSA) strains were isolated in 1960<sup>[10]</sup>. Nowadays *S. aureus* strains show resistance and reduced susceptibility to most available antibiotics, as well as cross-resistance to a wide range of antibiotics<sup>[11]</sup>.

*Listeria monocytogenes*, a gram-positive food-borne pathogen responsible for several out breaks in past decades, is another bacteria which has gained resistance against many antibiotics including tetracycline, gentamicin, chloramphenicol, erythromycin, streptomycin, sulfamethoxazole and rifampin due to the selective pressure induced by misuse of antibiotics as well as their overuse<sup>[12-14]</sup>. Furthermore, use of antibiotics, especially tetracycline, as supplements in animal food, which then ingested by human, played a probable role in developing antibiotic-resistant species of *Listeria*<sup>[12]</sup>.

According to what we mentioned, the emergence of MDR pathogens has led to treatment failure and caused a great cost to health systems. Thus, making use of natural sources seems to be a solution to this problem. Many studies have been done during the past decades to evaluate the antimicrobial effects of different kinds of herbs, including *Plantago major*, a well-known medicinal herb in different cultures. Some researchers have declared that *P. major* exhibits antimicrobial effects on some pathogens<sup>[15]</sup>.

In this research, we tried to determine the antibacterial activities of *Plantago major* against *Escherichia coli*, *Staphylococcus aureus* and *Listeria monocytogenes*.

## MATERIALS AND METHODS

### Preparation of the extracts

*P. major* seeds were purchased from a credential herb and spice grocery in Mashhad. Samples then were confirmed by the “Research center for plant sciences” of Ferdowsi University. Samples then were sent to “Johar-Taem” corporation for extraction. To determine the weight of the desiccated substance of the extract, specific amount was evaporated in 60 °C, then transported to desiccator and weighted by digital scale.

### Preparation of the pathogens

The *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922) and *Listeria*

*monocytogenes* (PTCC 1298) strains were obtained from the “Department of Food science & Technology” of Ferdowsi University. The stocked pathogens, which were stored in a  $-70^{\circ}\text{C}$  refrigerator, were thawed at room temperature and after cultivation on nutrient agar were incubated at  $37^{\circ}\text{C}$  for 18 hours.

In order to prepare the McFarland standard 0.5 mL of 0.048 M  $\text{BaCl}_2$  (Merck, Germany) was added to 99.5 mL of 0.18 M  $\text{H}_2\text{SO}_4$ . The absorbance of the standard was measured in a spectrophotometer to ensure that it was in the acceptable range (0.08-0.13 at a wavelength of 625 nm)<sup>[16]</sup>.

Inoculums were prepared by the growth method: colonies, previously cultivated on Mueller-Hinton agar, were transferred to Mueller-Hinton broth by a sterile loop, then incubated in  $37^{\circ}\text{C}$  until the visible turbidity was the same as that of a 0.5 McFarland standard. Then the absorbance was measured by a spectrophotometer<sup>[16]</sup>.

#### Determination of the minimum inhibitory concentration (MIC)

To ascertain the MICs, we utilized broth micro-dilution method. Ninety five microliters of Muller-Hinton broth along with 5  $\mu\text{L}$  of bacterial suspensions (0.5 McFarland) were added to each plate of a 96-well sterile microtitre tray. A hundred microliters of each dilution was transferred to the plates. The first well was filled with 11.6 mg/mL concentration of the aqueous extract (2.05 mg/mL for alcoholic extract), then the concentration was half folded from the next well to the last. Two plates of each row were designated to be as positive (without the extract) and negative (without the organism) controls (Figure 1). One row for each extract

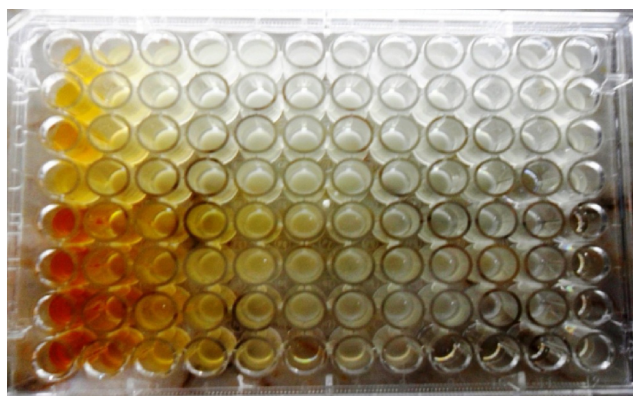


Figure 1: MIC test

(aqueous and alcoholic) designated as control row; each plate of the control rows only was filled with 95  $\mu\text{L}$  of Muller-Hinton broth along with 100  $\mu\text{L}$  of each dilution. The tray then was incubated at  $37^{\circ}\text{C}$  for 24 hours. After that, absorbance of each plate was read on an ELISA reader.

To determine the MICs of selected antibiotics (i.e. gentamicin, chloramphenicol and erythromycin), standard antibiotic powders were obtained, and stock solutions and dilution ranges were prepared as suggested by J. M Andrews<sup>[16]</sup>. In order to interpret and illustrate the results, data were transformed to graphs by Slide-Write.

#### Determination of the minimum bactericidal concentration (MBC)

Previously prepared solutions for determining MICs were used to ascertain minimum bactericidal concentration for both aqueous and alcoholic extracts, as well as mentioned antibiotics. In order to do that, 10  $\mu\text{L}$  of each plate was cultivated on nutrient agar and incubated at  $37^{\circ}\text{C}$  for 24 hours. After incubation, the plate with the lowest concentration which had no growth on it was considered as MBC.

#### Disc diffusion method

Disc diffusion was performed based on Kirby-Bauer method. Ten  $\mu\text{L}$  of diluted extracts were inoculated on blank discs. After cultivation of 15  $\mu\text{L}$  of bacterial suspension (0.5 McFarland) on Mueller-Hinton agar, discs were placed on plates. Then after 24 hours incubation at  $37^{\circ}\text{C}$ , the diameter of inhibitory zones were measured<sup>[17]</sup>.

## RESULTS

The absorbance of each plate is mentioned in TABLE 1. For *Staphylococcus aureus*, the minimum inhibitory concentration of the aqueous extract was 0.022 mg/mL. The MIC range for this pathogen was considered to be between 0.022 mg/mL and 0.045 mg/mL (Figure 2). The MIC of the aqueous extract estimated to be between 0.09 mg/mL and 0.181 mg/mL against both *E. coli* and *L. monocytogenes* (Figures 3 & 4).

The alcoholic extract did not show any inhibitory

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TABLE 1 : Absorbance of the samples at 625 nm

Plate No.		1	2	3	4	5	6	7	8	9	10	11	12
Plate Contents													
Extract	Organism												
Aqueous	<i>S. aureus</i>	1.106	0.542	0.471	0.407	0.349	0.339	0.331	0.311	0.299	0.317	0.285	0.252
	<i>E. coli</i>	1.050	0.465	0.420	0.366	0.344	0.330	0.330	0.333	0.333	0.335	0.321	0.330
	<i>L. monocytogenes</i>	1.239	0.509	0.418	0.363	0.354	0.331	0.332	0.333	0.332	0.314	0.319	0.339
	Control Row	1.055	0.478	0.386	0.375	0.360	0.336	0.328	0.310	0.291	0.288	0.026	0.311
Alcoholic	<i>S. aureus</i>	0.994	0.681	0.528	0.438	0.375	0.344	0.330	0.330	0.330	0.326	0.328	0.045
	<i>E. coli</i>	0.964	0.584	0.364	0.368	0.364	0.328	0.318	0.301	0.308	0.300	0.297	0.027
	<i>L. monocytogenes</i>	1.092	0.708	0.529	0.410	0.370	0.345	0.328	0.320	0.323	0.326	0.328	0.030
	Control Row	0.643	0.510	0.350	0.315	0.284	0.269	0.240	0.156	0.173	0.150	0.029	0.063

effect on the pathogens in the mentioned range, and all of the isolates were completely resistant to the extract

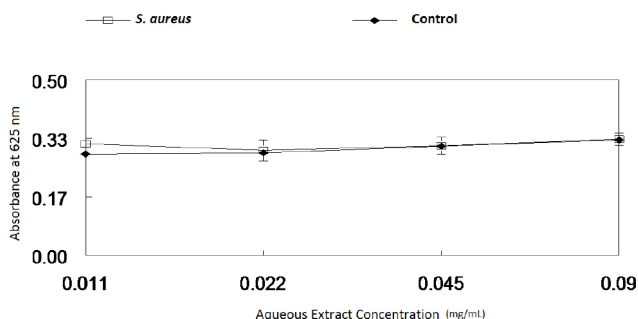


Figure 2 : MIC of the aqueous extract for *S. aureus*

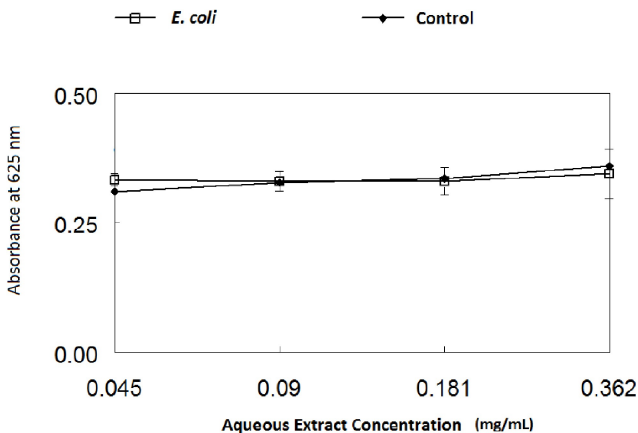


Figure 3 : MIC of the aqueous extract for *E. coli*

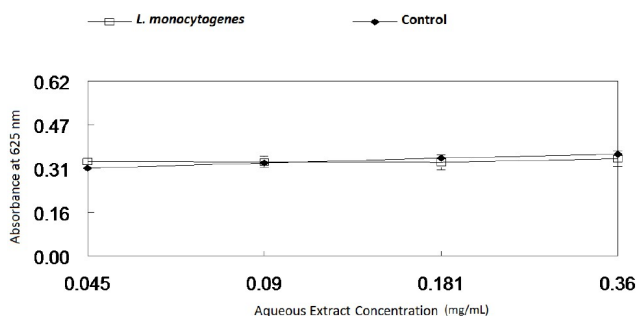


Figure 4 : MIC of the aqueous extract for *L. monocytogenes*

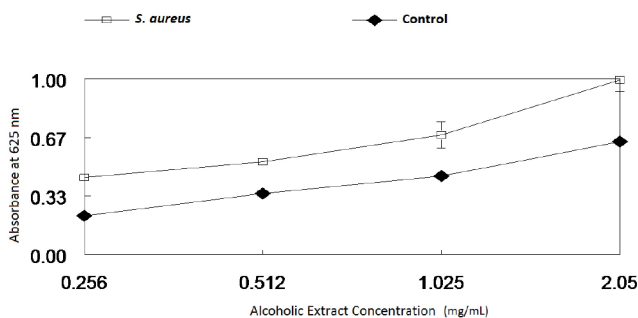


Figure 5 : MIC of the alcoholic extract for *S. aureus*

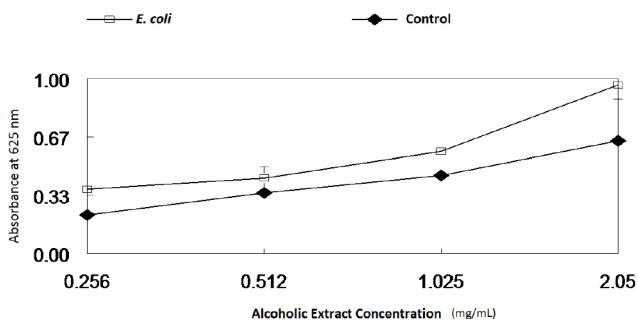


Figure 6 : MIC of the alcoholic extract for *E. coli*

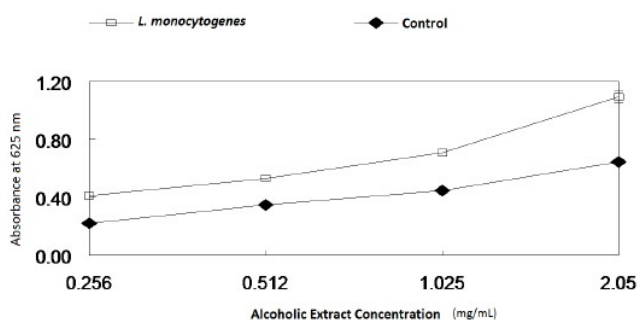


Figure 7 : MIC of the alcoholic extract for *L. monocytogenes*

(Figures 5 to 7).

In this study we were not able to ascertain any bactericidal activity against any of tested pathogens for neither the aqueous extract nor the alcoholic extract; thus MBCs of the extracts remained undetermined. In addi-

tion, no inhibitory zone was detected with the disc diffusion method, and all pathogens were resistant to tested concentrations.

The diameter of inhibitor zones of antibiotic discs measured as follows: 20 mm for gentamicin on *S. aureus*, 25 mm for Chloramphenicol on *E. coli* and 21 mm for erythromycin on *L. monocytogenes*.

## DISCUSSION

Every plant has a defense mechanism against pests and herbivores. Some uses mechanical defense, such as thigmonasty, and some utilize chemical compounds as defense mechanism. Plants also may produce antimicrobial metabolites which can derange the growth of microorganisms<sup>[18]</sup>. Tannin is one of these compounds which is believed to act as an antibacterial agent by depriving the bacteria of essential proteins<sup>[19]</sup>. Flavonoids are another chemical substances which are produced by some plants and are active against a wide range of microorganisms. These compounds play their antimicrobial role by binding to the cell membrane and destroying it<sup>[20]</sup>.

The mentioned compounds (i.e. Tannin and Flavonoids) are part of active ingredients of the *Plantago major* seeds and green parts; others include: glycosides (such as Aucubin), plantaginins, mucilage, saponin and many other substances<sup>[21]</sup>. As we discussed before some of these components have antibacterial activities, so it is expected that *Plantago major* acts as an antimicrobial agent. This theory was supported by our inquiry which proved the antibacterial activity of the aqueous extract of *P. maior*, notwithstanding that the pathogens used in our study were completely resistant to the alcoholic extract.

Contrary to our results, Jamshidi et al. did not find any antibacterial activity against *S. aureus* for the aqueous extract of *P. major*<sup>[22]</sup>. But Kiaei et al. stated that *P. major* possesses a noteworthy activity against *S. aureus*<sup>[15]</sup>.

Although we were not able to determine MIC for alcoholic extract of *P. major*, another study showed a MIC of 20 mg/mL for the ethanolic extract. Metiner also avowed that the methanolic extract of *P. major* has an anti-staphylococcal activity with a MIC of 14.25 mg/mL<sup>[23]</sup>. Another survey conducted by Sharifa et al.

showed that the alcoholic extract also have activities against *E. coli*<sup>[24]</sup>.

These findings indicate that pathogens are resistant to low concentrations of alcoholic extract of *P. major*, but at greater concentrations the extract shows more desirable effects. These divergence also could be due to different methods of extraction and different parts of plant which were used for extraction, as well as the environment where the plant grew; because every plant has a unique growth pattern and its features and even its ingredients are related to seasonal changes, weather, soil and many other factors<sup>[25]</sup>.

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