Antibacterial Effects of Ethanol and Acetone Extract of *Plantago major* L. on Gram Positive and Gram Negative Bacteria

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**INTRODUCTION**

Medical usage of herbs dates back to prehistoric era. Since then, herbal cures has played key roles in medical services all around the world ¹. As traditional medicines, use of herbs is very popular and important for 80% of the population in Africa, Asia, Latin America and Middle East Countries ². Like many other countries in the world, medical usage of herbs has been very popular in Turkey for many years, too. Despite of the improvement in synthetic organic chemistry of industrialized countries, directly or indirectly herbal medicines take place as 25% in all medicines in the prescriptions ³. Especially increasing microbiologic resistance of bacteria ³ leads people to search new solutions. Because of this, studies of herbs and obtained herbal medicine raw materials became more important.

*Plantago* is the most important genus of Plantaginaceae familia and is used in traditional medical area for many purposes. *Plantago coronopus* L., *Plantago major* L., *Plantago media* L. and *Plantago lanceolata* L. are the most commonly used plantago species in Turkey ⁴. *Plantago major* L. is known as “büyük sinirli ot” in Turkish culture ⁵.

*Plantago major* L. includes 5 biologically active class materials; benzoic compounds (vanillic acid), flavonoids...
Antibacterial Effects of...

(baicalein, baicalin, luteolin), iridoid glycoside (aucubin), phenolic compounds (cafeic acid, chlorogenic acid, ferulic acid, p-coumaric acid) and triterpenes (oleanolic acid, ursolic acid) 6.

Ettnopharmacological studies show in recent years that; Plantago major L. is used as cure of many disease all around the world 7. The pharmacological activites of this herb was reported to show depressing effect on scar formation 8, anti-microbial activity 5,8 and the level of blood cholesterol 8 in these studies. P. major’s main cafeic derivative, “Plantomagocide” has effectiveness as antiinflammatory and antioxidant and antibacterial. Flavanoids, which is isolated from P. major, are known with their killing effect on carcinoma cells, beside of their anti allergic and antiviral and anti inflammatory and vasodilator capacities 9.

The purpose of this study is to evaluate the in vitro antibacterial effectiveness of P. major L, which has many bioactive components.

MATERIAL and METHODS

Collecting of Herbal Material

Plantago major L. samples, which was used in this study, were collected in Yukarı Çiyrikli Village of Tuzluca town of Igdir city (Turkey) in July 2009. Leaves and other parts of herbs were dried seperately. The identification of herbs were made by Biolog Mehmet Nuri Yılmaz (Kafkas University, Faculty of Science and Literature, Biology Department, Kars, Turkey).

Extraction Procedure

Two different solvents (acetone and ethyl alcohol) were used on leaves. The powdered herb material (20 g) was extracted with 500 ml solvent in soxhlet apparatus for 3 h. It was filtered from Whatman No.1 filter paper. Filtrate was concentrated in evaporator at 35°C. Extract was diluted with 10 ml dimethylsulfoxide and then it was sterilised with 0.45 μm membrane filters and kept at -20°C till the usage 5.

Test Microorganisms

Gram positive bacteria tested are Bacillus cereus (ATCC 11778), Bacillus subtilis (ATCC 6633), Staphylococcus aureus (ATCC 29213), Staphylococcus epidermidis (ATCC 12228) plants, Gram negative bacteria; Escherichia coli (ATCC 25922), Klebsiella pneumonia (ATCC 4352), Proteus mirabilis (CCM 1944), Pseudomonas aeruginosa (ATCC 27853) and Salmonella enteritidis (KUEN 349) plants. Microorganisms were provided from Department of Microbiology at Istanbul University.

Culture Media

In order to maintain used plants, 7% sheep blood added Nutrient agar (HiMedia M001) was used. As feedlots, Ca++ and Mg++ aktions added Müller-Hinton Broth (OXOID CM 405) (CAMHB) was used in macrodilution tube method in order to determine the quantitative antibacterial effect 10.

Determination of Antibacterial Activity

Two different extracts of P. major L. herbs were evaluated with Macrodilution liquid (tube) (MIC) method 10. CAMHB 57-0.003 mg/ml in P. major L’s acetone extract, 85-0.007 mg/ml in ethyl alcohol extract were prepared. 500 µl of each dilution were put in sterilised tubes. From 18 h incubated cultures of tested bacteria in blooded agar, a similar suspension with 0.5 McFarland haze in saline solution was prepared. This suspension was put in each tubes as 500 µl. at the end of each tested batch, positive (herb extract not added) and negative (herb extract added) controls were used. All tubes were incubated at 37°C. Also, Gentamicin sulfate was used as reference antibiotic standard and was evaluated parallelly with herb extract 10.

RESULTS

MIC values of extracts against test bacteria are shown in Table 1.

Bacteria, used in the study, other than E. coli and B. cereus have been detected to be resistant to ethanol extract of P. major L. The ethanol extraction was found to be effective for E. coli and B. cereus strains relatively but only in high concentrations.

Acetone extract of P. major L. was detected to demonstrate effectiveness in different concentrations

<table>
<thead>
<tr>
<th>Plant Name</th>
<th>Minimum Inhibitory Concentration (MIC) (mg/ml)</th>
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<tbody>
<tr>
<td></td>
<td>Bc*</td>
</tr>
<tr>
<td>Plantago Major</td>
<td></td>
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<tr>
<td>Ethanol extract</td>
<td>42.500</td>
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against all strains of the bacteria used. Acetone extract demonstrated the highest effectiveness against *B. cereus* (3.562 mg/ml) while it demonstrated the lowest effectiveness against *B. subtilis*, *P. mirabilis*, *P. aeruginosa* and *S. epidermis* (28.500 mg/ml).

**DISCUSSION**

Antibacterial effectiveness of a paticular plant species are reported to vary according to the geographical area of the plant, the plant part and the extraction method.  

Kahyaoğlu et al. investigated the antimicrobial effectiveness of some plants, including *P. major* L, growing in Elazığ district, against *B. cereus, B. subtilis, Enterobacter aerogenes, E. coli*, *K. pneumoniae, P. aeruginosa, S. aureus, Candida spp. and Saccharomyces cerevisiae* with the disc diffusion method; and they stated that *P. major* L has demonstrated the highest effectiveness against all strains of the bacteria used. Acetone extract, ethanol extract (70%) of the plant, the plant part and the extraction method were reported to vary according to the geographical area of the plant samples.  

In another study, the antimicrobial effectiveness of the ethanol extract (70%) of *P. major* L leaves against *E. coli, P. aeruginosa, B. subtilis, S. aureus, C. albicans, S. cerevisiae* and *Aspergillus niger* was investigated with agar well-diffusion method; and it was stated that *P. major* L has antimicrobial effectiveness against the bacteria and mycotic agents used in the study; and that the most sensitive agent against the extraction was *S. cerevisiae*.

Fabiola Barbieri Holetz et al. investigated the antimicrobial effectiveness of *P. major* L, growing in Igdir district, against *B. cereus, B. subtilis, Enterobacter aerogenes, E. coli, K. pneumoniae* and *B. subtilis* in varying proportions. The authors stated that the most sensitive species of bacteria was *S. aureus;* however, none of the plant extracts was as effective as standard antibiotics against the bacteria used in the study.

Sharifa et al. tested the methanol, ethanol and aqueous extract of *Plantago major* extracts from plantain (*Plantago lanceolata* L.) leaves. The authors found that the extract showed a weak antimicrobial activity on the bacteria species studied.

In the current study, it was found that the acetone extract prepared from samples of *P. major* L plants, growing in Igdir district had antibacterial effectiveness against the bacteria used in; and that ethanol extract had low efficiency values against *B. cereus* and *E. coli*; and it was thought that the difference detected compared with other studies may depend on the geographical area of the plant and the plant part used.

In conclusion, although *P. major* L, having antibacterial effectiveness, does not have the potential to be used for this purpose by itself. However, considering its widespread use in the treatment of open wounds with tissue loss, among the public, it is concluded that it contributes to prevent the formation of infection in wounds with its remarkable antimicrobial effectiveness.

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**REFERENCES**


