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RESEARCH ARTICLE

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

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Summary

In this study, minimum inhibitor concentrations of acetone and ethyl alcohol extracts of *Plantago major* L. leaves on predetermined bacteria species was determinbed by Macrodilution liquid (tube) method. Both extracts were tested for nine bacteria species *(Bacillus cereus, Bacillus subtilis, Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Proteus mirabilis, Salmonella enteritidis)*. Ethyl alcohol extract showed no antibacterial activity against all species (except for *E. coli* and *B. cereus*), but acetone extract was effective on selected bacteria species in different concentrations.

Keywords: *Antibacterial effect, Plantago major L, Extract, Gram positive bacteria, Gram negative bacteria*

Gram Pozitif ve Gram Negatif Bakteriler üzerine *Plantago major* L.'nin Etanol ve Aseton Ekstraktlarının Antibakteriyel Etkisi

Özet

Çalışmamızda, *Plantago major* L. yapraklarından elde edilen aseton ve etil alkol ekstraktlarının Makrodilusyon sıvı (tüp) yöntemi ile Minimum inhibitör konsantrasyonları (MİK) tespit edilmiştir. Her iki ekstrakt dokuz farklı bakteri türü *(Bacillus cereus, Bacillus subtilis, Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Proteus mirabilis, Salmonella enteritidis)* üzerinde test edilmiştir. Etil alkol ekstraktının test bakterilerinden *E. coli* ve *B. cereus* dışındaki diğer bakterilere karşı antibakteriyel etkinlik göstermediği saptanırken, aseton ekstraktının ise kullanılan bakteri suşlarına karşı farklı konsantrasyonlarda etkinlik gösterdiği görülmüştür.

Anahtar sözcükler: *Antibakterial etki, Plantago major L, Ekstrakt, Gram pozitif bakteri, Gram negatif bakteri*

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 takes place as 25% in all medicines in the prescriptions [2]. 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

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

Etnopharmacological 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 İstanbul 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 suspansion 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 1. *MIC values of Plantago major L (mg/ml)*
Tablo 1. *Plantago major L'nin MİK Değerleri (mg/ml)*

Plant Name	Minimum Inhibitory Concentration (MIC) (mg/ml)								
	Bc*	Bs*	Sa*	Se*	Ec*	Kp*	Pa*	Pm*	Sen*
Plantago Major Aseton extract	3.562	28.500	14.250	28.500	14.250	14.250	28.500	28.500	7.125
Plantago Major Ethanol extract	42.500	R	R	R	42.500	R	R	R	R

** **Bc:** Bacillus cereus, **Bs:** Bacillus subtilis, **Sa:** Staphylococcus aureus, **Se:** Staphylococcus epidermidis, **Ec:** Escherichia coli, **Kp:** Klebsiella pneumonia, **Pa:** Pseudomonas aeruginosa, **Pm:** Proteus mirabilis, **Sen:** Salmonella enteritidis, **R:** Resistant*

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. epidermidis* (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 [11].

Kahyaoğlu et al.[5] 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 cerevisae* with the disc diffusion method; and determined that *Plantago major* ethanol extraction was effective against *S. aureus, B. cereus, E. 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 extractions was as effective as standard antibiotics against the bacteria used in the study.

In another study [9], 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 they 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*.

Fabíola Barbieri Holetz et al.[12] investigated the effectiveness of *P. major* L. ethanol-water (90-10%) extraction against *S. aureus, Spilanthes acmella, Lippia alba,* and *Achillea millefolium;* and found that the extract showed a weak antimicrobial activity on the bacteria species studied.

Sharifa et al. tested the methanol, ethanol and aqueous extract of sokshlet on *S. aureus, B. subtilis, E. coli, C. albicans* and *C. tropicalis* in terms of antimicrobial activity with disc diffusion method. According to MIC values, the highest antimicrobial effectiveness was observed against ethanol extraction; and it was stated that there was no antimicrobial effectiveness against the extraction with water. *P. major* ethanol and methanol extracts are stated to demonstrate antimicrobial effectiveness against the microorganisms tested, in 100-200 mg/ml concentrations [13].

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 fort 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 antibacterial effectiveness.

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