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Antibacterial Properties of an Iranian Ethnomedicinal Plant

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Abstract

Ulmusulmaceae (*U. ulmaceae*) is a native plant in Asia especially Iran, the plant has been in use as an antioxidant and anti-inflammatory agent in food production in west of Iran (in Kermanshah). In comparison to many other pharmaceutical-industrial plants, there is very little data about the antibacterial activities of *U. Ulmaceae* aqueous extract collected from Kermanshah province against *Escherichia coli O157:H7* (*E. coli*) (ATCC No. 25922). Hence, the aim of the recent study was evaluation of antibacterial properties of the aqueous extract of *U. ulmaceae*on *E. coli*. The antibacterial activities of *U. Ulmaceae* were assessed by macro-dilution method in Mueller-Hinton broth medium, agar disk and agar well diffusion methods. The outcome revealed that by increasing the concentration of the extract, the inhibition zone increased in many of the samples. Also, the aqueous extract of *U. ulmaceae* have excluded the growth of *U. Ulmaceae* and destroyed it. The results indicated that in tested bacterium, there was a considerable difference in terms of sensitivity to *U. Ulmaceae* and the most sensitivity was observed in agar disk diffusion method. Thus, the present research indicates the antibacterial effects of the *U. ulmaceae* on *E. coli*, offering to use as antibacterial supplement towards the development of new therapeutic agent.

Keywords

Ulmusulmaceae, aqueous extract, Antibacterial properties, Macro-dilution method, Agar well diffusion method, Agar disk diffusion method.

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INTRODUCTION

Infection is the incursion of an organism's body tissues by disease-causing agents such as virus, bacterium, and fungus. Infections diseases due to bacterial species also stay as a serious clinicalproblem. Antibiotics provide the primary basis for the treatment of bacterial infections. However, overuse of antibiotics has become the primary factor for the emergence and dissemination of multi-drug resistant strains of different groups of microorganisms[1]. Kanamycin is an antibiotic that can treat a number of bacterial infections. It destroys gram-negative and some gram-positive bacteria by disrupting the growth of the bacterial cell wall. But this antibiotic like other antibiotics have many side effects. Common side effects of Kanamycin include diarrhea, stomach upset and allergy[2].

Plants are invaluable resources useful in daily life as food additives, pharmaceuticals, or directly in medicine[3,4]. Plants have been screened for their potential uses as other remedies for the treatment of different infectious diseases[5-7]. Most plants have been shown to possess antibacterial agents active on bacteria in vitro[8-11]. Some medicinal plants used in traditional Iranian medicine are effective in treating several diseases caused by bacterial and oxidative stress[12-14]. A plant extract is a substance or an active with favourable effects that is removed from the tissue of a plant, to be used for a particular purpose. The antibacterial activities of extracts have been identified for many years, and their rudiment have found applications as naturally occurring antibacterial agents in the field of pharmaceutical botany, medical and clinical microbiology, food maintenance, etc[15]. Plants extracts have antibacterial activity on a wide range of bacteria, and most of extract compounds have phenolic groups in their structure[16]. The original benefit of plant extracts is that they do not increase the antibiotic resistance because they have a considerable role in the defence system of the plant to microbial diseases due to their intrinsic antioxidative and antimicrobial effects[17, 18]. In Iranian medicine, plant extracts are consumed by the population for the prevention, control, and treatment of diseases such as bacterial diseases[19,20]. *Ulmusulmaceae* (*U. ulmaceae*) is the member of plants family called *Ulmaceae*. *U. ulmaceae* has long been used in Asian countries as a medicinal plant. *U. ulmaceae*is one of the edible plants which have generated a lot of interest throughout

human history as a medicinal panacea. Several extracts of the plant are traditionally used in treating different inflammatory and bacterial diseases[21]. Likely, the antibacterial effects of the plant are related to its phenolic, flavonoid, and flavones compounds. These components gets attached to the bacterial outer membrane proteins, deactivate the matrix metalloproteinase and inhibit growth of bacteria or destroyed bacteria[22-24].

The aim of the current study was evaluation antibacterial activities of hydroalcoholic extract of SS against BS in west of Iran (in Kermanshah).

MATERIALS AND METHODS

U. ulmaceae plants were collected in Kermanshah, Iran and cleared of contaminants. Plants were washed, air dried for 7-8 days, and ground into powder before being placed into a Soxhlet apparatus for extraction with distilled water with increasing polarity to extract phyto-constituents separately at 20ºC for 3-4 h. Whitman filter paper No.1 was used to filter the extract. Pressure was reduced to evaporate and dry the filtrates (after drying, powder of aqueous extract are obtained.

Lyophilized *Escherichia coli O157:H7* (*E. coli*) (ATCC No. 25922) provided by The Iranian Research Organization for Science and Technology was activated on Tryptic Soy broth at 37°C for 18 h. Then 60 µl of the broth was transferred to Nutrient agar and incubated at 37°C for another 24 h; cell concentration was then adjusted to obtain final concentration of 10^8cfu/ml in Muller Hinton broth. Mueller-Hinton Agar was prepared according to the manufacturer's instruction (Oxoid, UK), autoclaved and dispensed at 20 ml per plate in 12 x 12cm Petri dishes. Set plates were incubated overnight to ensure sterility before apply.

Agar disk and agar well diffusion were used as screen tests to assess the antibacterial activities of *U. ulmaceae* based on standard protocol. *U. ulmaceae,* 1g/ml, was diluted 6 fold (v/v) and 60 µl of each dilution was poured on each disk and well. After 24h incubation diameters of growth inhibition zones were measured. Distilled water was used as negative control and Kanamycin as positive control. Minimum inhibitory concentration (MIC) is the lowest concentration which prevents bacterial growth despite removing or inhibiting growth of bacteria. Minimum bactericidal concentration (MBC) is the lowest

concentration of an agent which causes death to test bacteria calculated by 60 µl in 6 dilutions on agar plates. After incubation the lowest concentration which stops growth was noted as MBC. For MIC the macrobroth dilution method was used[25].

RESULTS

In agar disk diffusion, the widest zone was seen in 0.50 g/ml concentration (The value of growth inhibition zone was 12 mm in this dilution). No inhibition zone was observed due distilled water. Growth inhibition zones due to different dilutions are listed in figure 1.

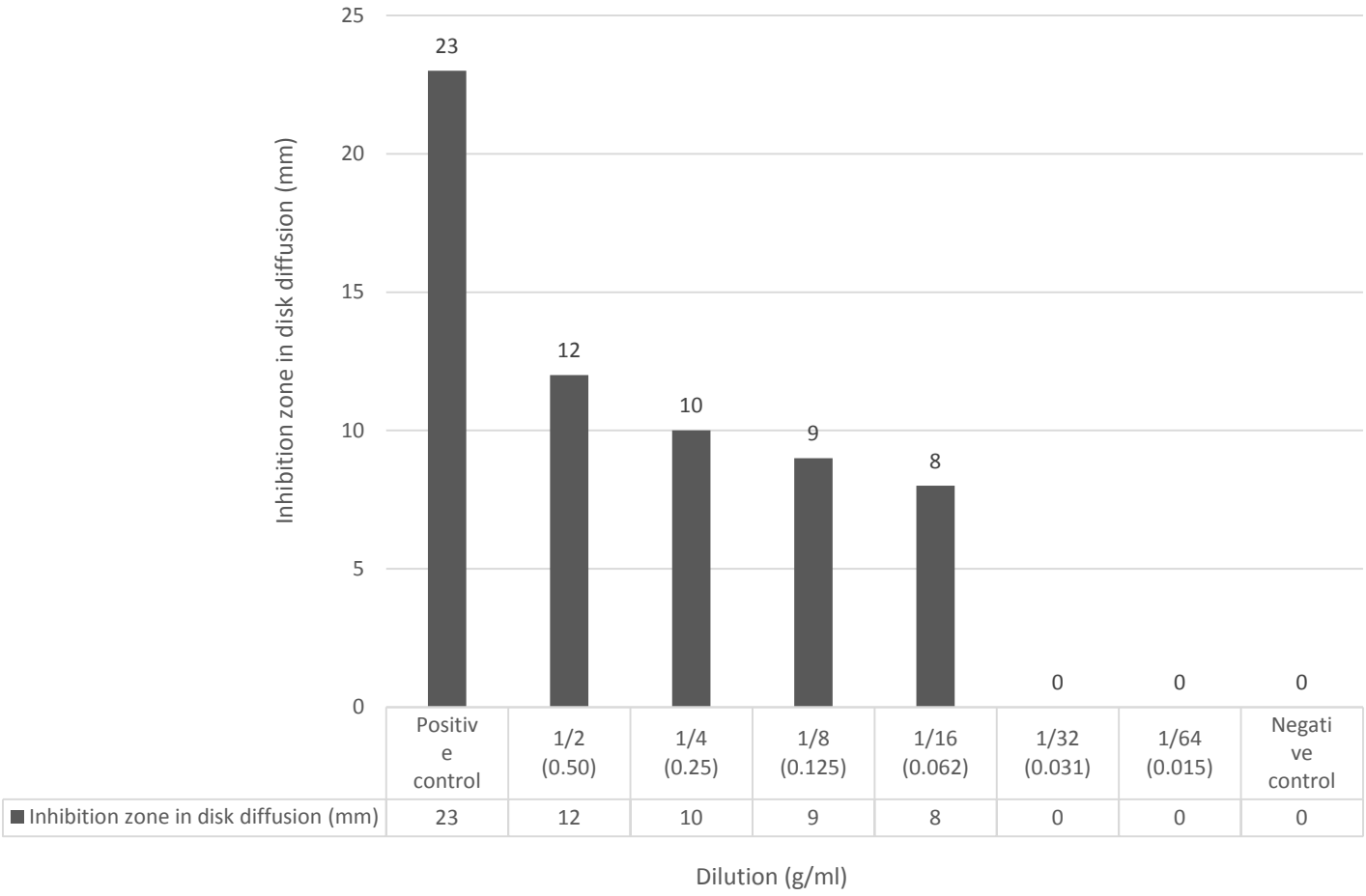

Fig 1 The diameters of growth inhibition zones in agar disk diffusion test in different dilutions of *U. ulmaceae*

In agar well diffusion, the widest zone was seen in 0.50 g/ml concentration (The diameter of growth inhibition zone was 10 mm in this dilution). There was no inhibition zone in *E. coli* due to 0.015, 0.007, and 0.003 g/ml concentrations. No inhibition zone was observed due to distilled water. The data are discoverable in figure 2.

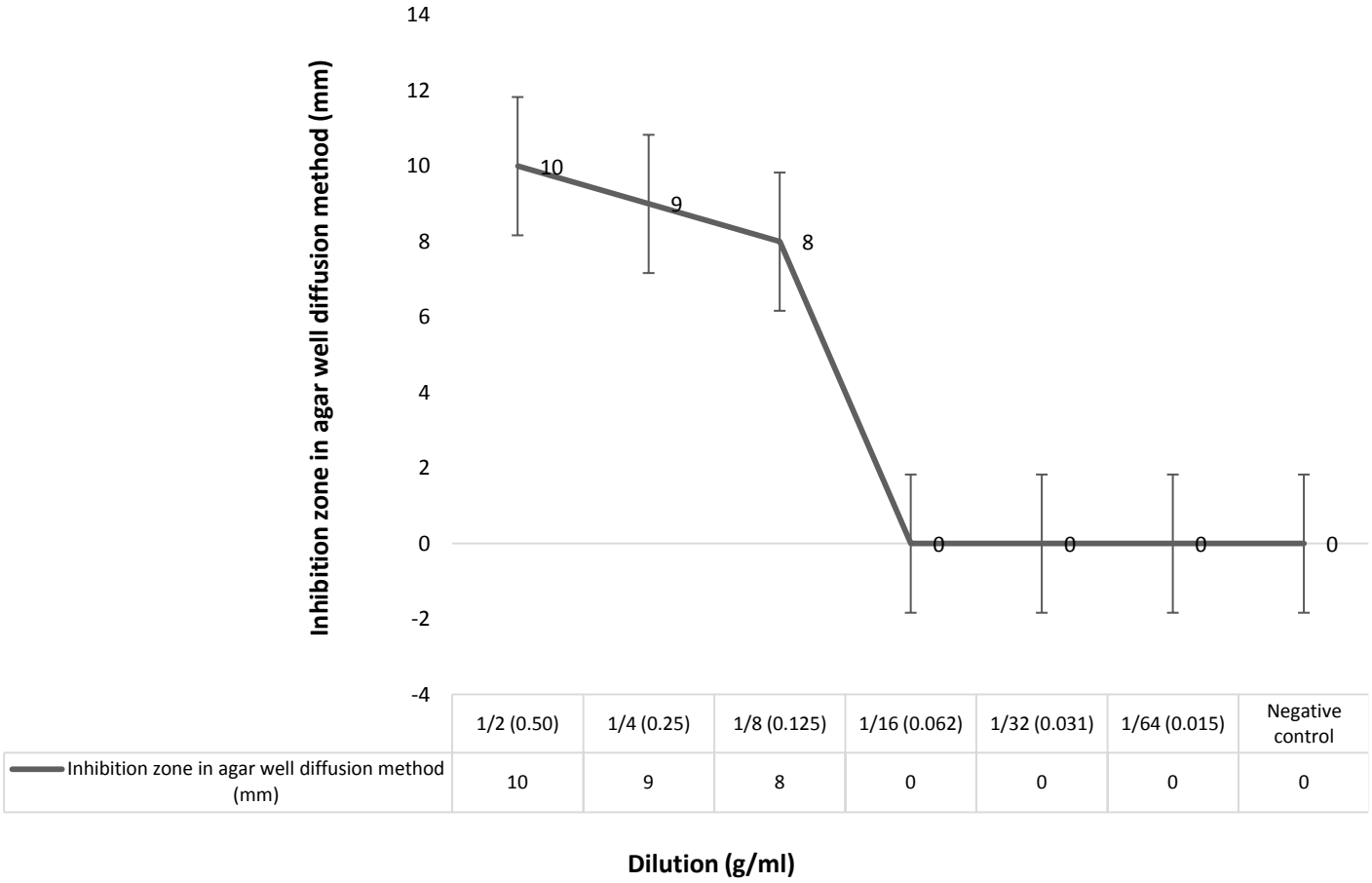

Fig 2 The diameters of growth inhibition zones in agar well diffusion test in different dilutions of *U. ulmaceae*

In the examined bacterium, MIC and MBC values were 0.125 and 0.50 g/ml concentration, respectively.

DISCUSSION

The antibacterial results indicated that the *U. ulmaceae* prevented the bacterium and the activities were dependent upon concentration. In agar disk diffusion test, the

widest inhibition zone was seen in 0.50 g/ml concentration (The value of growth inhibition zone was 12 mm in this dilution, and the value of growth prevention zone of Kanamycin against *E. coli* was 23 mm) and no inhibitory effects of distilled water against the *E. coli*. In agar well diffusion test, the widest zone was seen in 0.50 g/ml concentration (10 mm) and no inhibitory effect of *U. ulmaceae* in 0.062, 0.031 and 0.015g/ml concentrations. In this study, the results demonstrated that *U. ulmaceae* with 0.125 g/ml concentration has inhibited from the growth *E. coli*, also in 0.50 g/ml concentration has removed. There are correspondences between these results and the prior study. The previous study indicated that *U. Ulmaceae* have antibacterial activity against *Staphylococcus aureus* ATCC No. 25923. In their study, *U. ulmaceae* aqueous extract with 0.125 g/ml concentration has inhibited from the growth *Staphylococcus aureus*, also in 0.5 g/ml concentration has removed. Also demonstrated that in agar disk diffusion test, the widest inhibition zone was seen in 0.5 g/ml concentration and the value of growth inhibition zone was 12 mm in this dilution and no inhibitory effects of distilled water against the SA. In past study, in agar well diffusion test, the widest zone was seen in 0.5 g/ml concentration (10 mm) and no inhibitory effects of extract of UU in 0.062, 0.031, 0.015 g/ml concentrations[21].

CONCLUSION

From the study it can be concluded that by increasing the concentration of the extract, the inhibition zone in many of samples increased. Aqueous extract of *U. ulmaceae* have inhibited the growth of *E. coli* ATCC No. 25922 in 0.125 concentration and eradicated it in 0.50 g/ml concentration. Also, in this work we find considerable discrepancy in terms of *E. coli* sensitivity to *U. ulmaceae* aqueous extract with greater effects found in disk diffusion. Thus, the present research indicates the antibacterial activities of the *U. ulmaceae* on *E. coli*, offering to use as antibacterial supplement towards the development of new therapeutic agent.

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