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Gram-negative antimicrobial spectrum of *Ulmus pumila*

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Abstract
Antibiotic resistance by gram-negative bacteria is becoming a major global health crisis. The objective of this study is to examine the antibacterial activity of *Ulmus pumila* against a variety of infectious gram-negative bacteria. The extraction of *Ulmus pumila* leaves and flowers were carried out using 95% ethanol. The homogenate was filtered and sterile paper discs were soaked in the filtrate. Discs infused with extracts or only 95% ethanol, were placed on bacterial plates and incubated. Antibacterial activity was tracked by measuring the zones of inhibition. In this research, *Ulmus pumila* displayed antibacterial activity against *P. vulgaris, E. coli, K. pneumoniae, and E. cloacae*. With the results of this experiment and the previous activity of *Ulmus pumila* against gram-positive bacteria, there is much potential for *Ulmus pumila* to be developed into a broad-spectrum antibacterial agent.

Keywords: Gram-negative, bacteria, antibiotic resistance, antimicrobial, microbiology, plants, herbal medicine, antibacterial

Introduction
Antibiotic resistant bacterial strains are becoming a major crisis for healthcare systems around the world [1]. Antibiotic resistance has created billions of dollars in healthcare costs and high levels of patient mortality annually [2, 3]. The development of new antibiotics has been scarce, and has further contributed to this crisis [2]. The development of new antibiotics, particularly against gram-negative bacteria, is necessary to prevent a global health crisis [4, 5]. Plants have been and remain crucial as a key source of active ingredients for the development of new antibiotics [6]. This research aims to determine if *Ulmus pumila* demonstrates antibacterial activity against gram-negative bacteria.

Ulmus pumila, also known across much of the world as Siberian Elm, is an invasive species that is native to, many parts of Asia and some parts of North America [7]. *Ulmus pumila* may reach 70 feet in height, and can adapt to survive in a wide range of harsh conditions [7, 8]. *Ulmus pumila* has previously been examined as a potential chemotherapeutic therapy and has demonstrated antibacterial activity against *methicillin-resistant Staphylococcus aureus* (MRSA), a major pathogen of concern [9, 10]. This experiment tests the activity of *Ulmus pumila* against the following pathogenic gram-negative organisms: *E. coli, P. vulgaris, K, pneumoniae, and E. cloacae.*

While gram-positive bacterial resistance is of significant concern, gram-negative bacterial resistance is of greater concern due to their rapid nature of development and deleterious nature of their systemic infections implicated in major causes of mortality across the globe [11, 12]. These resistant gram-negative bacteria strains place significant strain on healthcare systems, and particularly high intensity care units [13]. Antibiotic resistant gram-negative bacterial strains are responsible for a variety of nosocomial infections, with high levels of mortality [11]. Continuous evolution of gram-negative bacteria has allowed them to develop antibiotic resistance through the use of a variety of antibiotic resistance mechanisms [12]. In order to prevent a global health crisis, development of novel antimicrobial compounds is necessary to protect against these dangerous gram-negative microorganisms [4, 5, 11, 12].

This research aims to test *Ulmus pumila* against the gram-negative pathogens of concern. *Escherichia coli* (*E. coli*) a common gram-negative pathogen native to the gastrointestinal tract, produces a variety of infections in humans [14, 15]. Some infections of concern in humans are systemic bacteremia infections, meningitis, prostatitis, and genitourinary infections [14, 15].

E. coli has several pathogenic strains that can cause diarrheal illness and vomiting [14, 15]. Additional infections of concern are watery and bloody diarrheal illnesses [14, 15]. A bloody diarrheal illness of concern in children is due to a specific strain of E. *coli* that causes Hemolytic Uremic Syndrome, a condition implicated in child mortality [14-16]. Antibiotic resistance of *E. coli* is becoming a rapid problem, as E. *coli* has demonstrated resistance against a wide variety of common treatments [14]. Another gram-negative bacterium of concern is *Proteus vulgaris (P. vulgaris)*. *P. vulgaris* has demonstrated antibacterial resistance and possesses the ability to cause severe urinary tract infections in the hospital setting [17-20].

Two major pathogens that have been known for a wide spectrum of extreme antibiotic resistance and also an agent of severe nosocomial infections include *Klebsiella pneumoniae* (*K. pneumonaie*) and *Enterobacter cloacae* (*E. cloacae*) [21-25]. Both bacteria are native to the gastrointestinal tracts [21, 24]. Both bacteria are common causes of nosocomial bacteremia. *K. pneumoniae* can also be a cause of urinary tract infections and pneumonia [21, 22]. Bacteremia due to *E. cloacae*, is a known cause of septic arthritis and endocarditis [24]. Both bacteria are major pathogens of concern as the options for treatment are rapidly fading [22-25].

This study aims to investigate *Ulmus pumila* as a potential novel antimicrobial against gram-negative pathogens of concern. Based upon previous activity of *Ulmus pumila* against gram-positive bacteria, we hypothesize that the extracts of *Ulmus pumila* has antibacterial activity against gram-negative pathogens.

Method

Sample preparation and plating

Fresh leaves (3 grams) of *Ulmus pumila* were homogenized and extracted with 15 ml of 95% ethanol [26]. The filtrate from the extracted samples were impregnated into sterile blank paper disc by soaking for 20 minutes, followed by air-drying [26]. Sterile blank paper discs were also soaked in 95% ethanol extraction solvent only, to serve as control for the effect of extraction solvent residue on bacterial growth. Bacterial stocks maintained in glycerol stocks were scaled in 25 ml broths and agar plates were also prepared as previously described [26]. 9 ml of 1% saline solution was used to dilute 1 ml of the scaled bacteria. The previously prepared extract-soaked discs or extraction solvent-soaked discs (control discs) were placed on bacterial plated plates and the bacterial plates incubated overnight at 37 degree Celsius. The zones at which bacterial growth were inhibited were measured as indicators of bacterial susceptibility to the extract under investigation.

Result

P. vulgaris, *E. coli*, *K. pneumoniae*, and *E. cloacae*, all demonstrated a significant degree of susceptibility to the extract of *Ulmus Pumila*, with zones of inhibition averaging 15 mm, 16 mm, 16 mm and 20.5 mm respectively (Table 1).

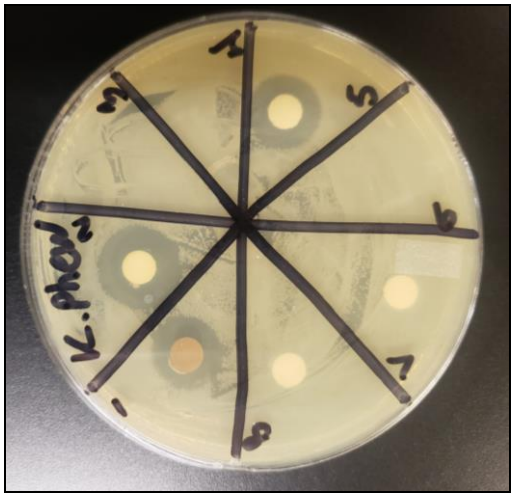

Fig 1: Blank disc only [Position 7], Blank disc infused with extraction solvent only [Position 8] shows no zone of inhibition. Blank disc infused with *Ulmus pumila* extract [Position 2] induced zones of inhibition against *K. pneumoniae.*

Fig 2: Blank disc only [Position 7], Blank disc infused with extraction solvent only [Position 8] shows no zone of inhibition. Blank disc infused with *Ulmus pumila* extract [Position 2] induced zones of inhibition against *E. Coli.*

Table 1: Zone of inhibition induced by *Ulmus Pumila* extract against Gram-negative bacteria.

	E. coli **[Inhibition zone, mm]**	***P. vulgaris*** **[Inhibition zone, mm]**	***E. cloacae*** **[Inhibition zone, mm]**	***K. pneumoniae*** **[Inhibition zone, mm]**
Disc [Blank]	0	0	0	0
Disc [*Ulmus pumila*]	16	15	20.5	16

Discussion

This study demonstrated that *Ulmus pumila* has antibacterial activity against several gram-negative bacteria including *E. coli*, *P. vulgaris*, *K. pneumoniae*, and *E. cloacae*. The gram-negative bacteria in this study present a major challenge and concern to our healthcare systems due to their ability to obtain antibiotic resistance and causing severe levels of morbidity and mortality [14-25]. Antibiotic resistance by gram-negative bacteria is rapidly becoming a global health crisis as it continues to place strain on our healthcare systems [1-3, 11]. Previous studies have shown *Ulmus pumila* to have chemotherapeutic properties, and antibacterial activity against gram-positive pathogens of major concern [9, 10]. The results of this study, in combination with previous studies of *Ulmus pumila* against gram-positive pathogens provide adequate support for continued development of this plant as an antimicrobial agent. We can conclude that there is significant potential for *Ulmus pumila* to be considered for development as a broad-spectrum antibiotic.

Limitations of the study

This study was limited due to the inability to test antibiotic resistant gram-negative bacteria strains due to safety concerns of the facility. Further research should be undertaken in a biohazard secure facility to test these antibiotic resistant gram-negative strains of bacteria. This research is a first step for the potential development of *U. pumila* as a novel antibiotic.

Conflicts of Interest

The authors declare no conflicts of interest.

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