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Xeranthemum cylindraceum: A potential broad-spectrum antimicrobial

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Abstract
Novel plant ingredients continue to be key antibacterial, antifungal and antiviral agents in the fight against novel pathogens. Here, we have shown the antibacterial properties of *Xeranthemum cylindraceum* against gram-negative and gram-positive bacteria, and also C. *albicans*. Homogenates of X. *cylindraceum* were extracted with 95% ethanol and the extracts tested against various bacterial species. Blanks discs were soaked in extraction solvents as controls. X. *cylindraceum* demonstrated antimicrobial activity indicated by zones of inhibition against the following gram-negative and gram-positive bacteria: *E. coli*, *P. vulgaris*, *E. cloacae*, and *K. pneumoniae*, *S. aureus*, *S. agalactiae*, and *S. pyogenes*. X. *cylindraceum* did not demonstrate antifungal activity. By these data we conclude X. *cylindraceum* displayed broad-spectrum antibacterial activity against clinically relevant bacteria and has the potential for future development as a broad-spectrum antibiotic.

Keywords: antimicrobial, bacteria, antifungal, gram-positive, gram-negative, drug resistance, microbiology

Introduction
Microbial resistance to current antibiotics has quickly become a concern to clinicians and scientists across the globe. Bacteria have continued to rapidly adapt to antibiotics through a variety of unique mechanisms [1]. This rapid adaptation of microbes to antibiotics has already created substantial clinical and financial burden to patients and hospitals and poses a major threat to the global public health system [2, 3]. The search for new antimicrobial agents has rapidly gained traction in order to overcome the growing global crisis of antibiotic resistance. In an effort to further explore new antimicrobial agents, this experiment investigates the potential of *X. cylindraceum* to serve as an antimicrobial agent.

X. cylindraceum is a plant native to Europe, with a distribution across Europe and the Middle East [4]. Very little research has been done in the exploration of *X. cylindraceum* as an antimicrobial agent. However, *X. cylindraceum* has previously demonstrated significant antibacterial activity against *Bacillus cereus* and *Staphylococcus aureus* [5]. There is still much unknown about *X. cylindraceum* and its potential medicinal properties. In search of the antimicrobial properties of *X. cylindraceum*, this experiment tests gram-negative bacteria (*E. coli, P. vulgaris, E. cloacae, and K. pneumoniae)*, and gram-positive bacteria (*S. aureus, S. agalactiae, and S. pyogenes),* and the fungus *C. albicans* for their susceptibility to *X. cylindraceum.*

Gram-negative bacteria are one of the largest public health problems in the world due to high levels of antibiotic resistance [6]. Gram-negative bacteria display a wide range of mechanisms to induce antibiotic resistance, such as an extensive internal and external membrane [6]. The external membrane of gram-negative bacteria is composed of lipopolysaccharide, which can create an intense immune response, and potentially induce septic shock [6, 7]. This experiment tests the following gram-negative bacteria: *E. coli, P. vulgaris, E. cloacae, and K. pneumoniae.*

The first gram-negative bacteria tested in this experiment is *Escherichia coli (E. coli). E. coli* is a lactose fermenting gram-negative rod that colonizes the human gastrointestinal tract [8]. *E. coli* is one of the most common sources of genitourinary infections, gastrointestinal tract infections, and meningitis [8].

EHEC (Enterohaemorrhagic *E. coli)* is a particular strain of *E. coli* that is known to cause bloody diarrheal illness [8]. A specific EHEC serotype, *E. coli* O157:H7, is known to cause a potentially lethal condition called Hemolytic Uremic Syndrome in children [8]. *E. coli* displays a wide variety of virulence factors that help it evade host immune defenses and antibiotic therapies [8]. The second gram-negative bacteria tested in this experiment is *Proteus vulgaris. Proteus vulgaris (P. vulgaris)* is gram-negative bacteria found in soil and water habitats [9]. *P. vulgaris* is of concern due to its ability to cause drug-resistant infections [9]. The third gram-negative bacteria tested is *Klebsiella pneumoniae* (*K. pneumoniae*). *Klebsiella pneumoniae* is a gram-negative bacterium that is a common cause of catheter-associated urinary-tract infections and nosocomial infections [10]. *K. pneumoniae* is a pathogen with increasing morbidity due to multi-drug resistant strains that can lead to treatment failure [10]. The final pathogenic bacteria tested in this experiment is *Enterobacter cloacae* (*E. cloacae*). *E. cloacae* is gram-negative bacteria known for causing nosocomial bloodstream infections [11]. *E. cloacae*, like the other gram-negative bacteria in this experiment, are of concern due to increasing rates of antibiotic resistance [11].

Gram-positive bacteria remain clinically relevant due to their infectious nature and continued antimicrobial resistance [12]. Gram-positive bacteria are a prevalent source of bloodstream infections [13]. Gram-positive bacteria are known for their thick peptidoglycan cell wall that holds crystal violet stain [13]. This cell wall has been shown to be a predominant driver in creating antibiotic resistance [14]. This experiment tests the following clinically relevant bacteria: *Staphylococcus aureus* (*S. aureus*), *Streptococcus pyogenes* (Group A Streptococcus), and *Streptococcus agalactiae* (Group B Streptococcus).

The first gram-positive bacteria tested in this experiment is *Staphylococcus aureus* (*S. aureus*). *S. aureus* is a gram-positive bacterium that is found in the normal flora of skin and mucous membranes and causes a variety of illnesses [13, 15]. *S. aureus* is a common cause of skin abscesses, endocarditis, food poisoning, toxic-shock syndrome, septic arthritis, and osteomyelitis [13, 15]. A specific strain of concern, due to its multi-drug resistant characteristics, is *Methicillin-resistant Staphylococcus aureus* (MRSA) [15]. *Methicillin-resistant Staphylococcus aureus* (MRSA) is a concern in hospital and community settings because its treatment has become a serious clinical challenge due to its resistance against many antibiotics [15]. The second gram-positive bacteria tested in this experiment is *Streptococcus pyogenes* (Group A Streptococcus). *Streptococcus pyogenes* can cause a wide range of mild to severe infections [16]. *S. pyogenes* is a classic cause of skin infections and pharyngitis [16]. If improperly treated, *S. pyogenes* can result in potentially fatal conditions such as rheumatic fever and post-streptococcal glomerulonephritis [16]. *S. pyogenes* is also of concern for its high mortality with necrotizing fasciitis and toxic shock syndrome [16]. The final gram-positive bacteria tested in this experiment is *Streptococcus agalactiae* (Group B Streptococcus). *S. agalactiae is a common* cause of morbidity and mortality in neonates, pregnant women, and the elderly [17]. *S. agalactiae* can cause soft tissue infections, bacteremia, and meningitis [17]. *Streptococcus agalactiae* has shown some reduced susceptibility to Penicillin G [17].

Fungal infections are rapidly increasing in prevalence and are becoming a clinical issue of concern [18]. Fungal infections typically affect the immunocompromised, such as those receiving chemotherapy, immunosuppressive drugs, and those with diseases that cause an immunocompromised state [18]. *Candida albicans (C. albicans),* the fungus tested in this experiment, is one such opportunistic infectious agent amongst the immunocompromised [18]. *C. albicans* can infect the skin, mucous membranes, and internal organs in immunocompromised individuals [19].

The purpose of this experiment is to determine if *Xeranthemum cylindraceum* demonstrates antimicrobial properties against a variety of gram-positive bacteria, gram-negative bacteria and *C. albicans.* We hypothesize that *Xeranthemum cylindraceum* will demonstrate broad-spectrum antimicrobial activity against gram-positive bacteria, gram-negative bacteria, and the fungus *C. albicans.*

Materials and Methods

2g samples of *Xeranthemum cylindraceum* were extracted using 95% ethanol as described previously (Puga *et al*., 2022) [20]. The filtrate from extract suspension was infused into sterile discs as previously described (Puga *et al*., 2022) [20]. Blanks discs were also infused with extraction solvents as vehicle controls. Approximately 1ml of previously prepared glycerol stocks of bacteria were suspended in LB broth and subject to shaking overnight. The new culture was diluted in 1% saline solution and plated on Muller Hinton agar plates. Paper discs that were previously soaked in plant homogenate extracts or solvent control (95% ethanol) were carefully positioned on previously plated bacterial plates and incubated overnight at 37 degree Celsius (Puga *et al*., 2022) [20].

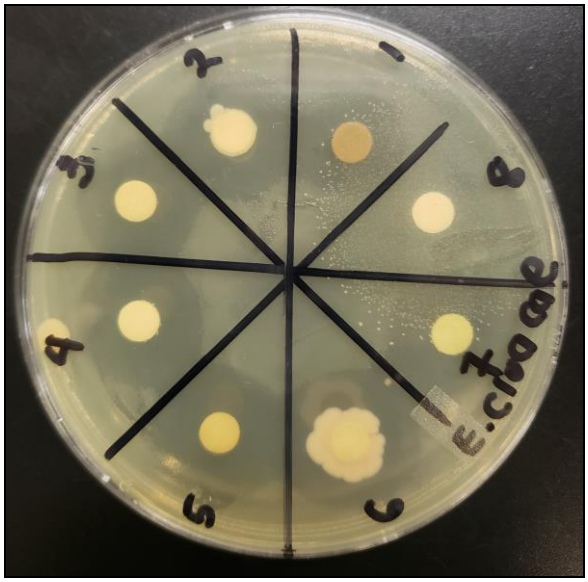

Fig 1: *Xeranthemum cylindraceum* (Position 4) demonstrates inhibition zone against *E. cloacae*. Blank disk (Position 8) with no agent (solvent only) shows no inhibition zone.

Results: *Xeranthemum cylindraceum* showed antimicrobial activity against the following gram-negative bacteria: *E. coli* (11 mm mean zone of inhibition), *P. vulgaris* (25 mm mean zone of inhibition), *E. cloacae* (20 mm mean zone of inhibition), and *K. pneumoniae* (20 mm mean zone of inhibition). *Xeranthemum cylindraceum* showed antimicrobial activity against the following gram-positive bacteria: *S. aureus* (24 mm mean zone of inhibition), *S. agalactiae* (15 mm mean zone of inhibition), and *S. pyogenes* (11 mm mean zone of inhibition). *Xeranthemum cylindraceum* did not show antifungal activity against *C. albicans.*

Table 1: Gram-positive bacteria mean zone of inhibition zone (in mm) against a blank disk and *Xeranthemum cylindraceum*.

	***S. aureus* (inhibition zone in mm)**	***S. agalactiae* (inhibition zone in mm)**	***S. pyogenes* (inhibition zone in mm)**
Blank Disk	0 mm	0 mm	0 mm
Xeranthemum cylindraceum	24 mm	15 mm	11 mm

Table 2: Gram-negative bacteria mean zone of inhibition zone (in mm) against a blank disk and *Xeranthemum cylindraceum*.

	***E. coli* (inhibition zone in mm)**	***P. vulgaris* (inhibition zone in mm)**	***E. cloacae* (inhibition zone in mm)**	***K. pneumoniae* (inhibition zone in mm)**
Blank Disk	0 mm	0 mm	0 mm	0 mm
Xeranthemum cylindraceum	11 mm	25 mm	20 mm	20 mm

Table 3: *C. Albicans* zone of inhibition zone (in mm) against a blank disk and *Xeranthemum cylindraceum.*

***C. Albicans* (zone of inhibition in mm)**	
Blank Disk	0 mm
Xeranthemum cylindraceum	0 mm

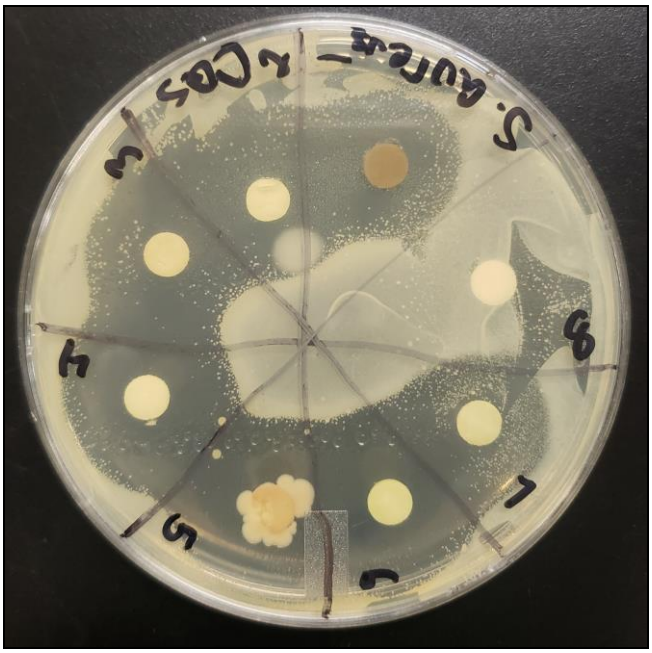

Fig 2: *Xeranthemum cylindraceum* (Position 4) and blank disk (Position 8) against *S. aureus.*

Discussion

In this experiment, *Xeranthemum cylindraceum* has demonstrated extended coverage against gram-positive and gram-negative organisms, which proves that the extract has the potential to serve as a broad-spectrum antimicrobial.

X. cylindraceum did not display antifungal activity against *C. albicans*, however, this does not rule out the possibility that *X. cylindraceum* has antifungal properties against other fungi. *X. cylindraceum* must be tested against different fungi to see if antifungal properties are present.

Antibiotic resistance is rapidly becoming a threat to the global public health system [3]. Antibiotic resistance has already demonstrated significant clinical and financial burden for patients, clinicians, and health systems in the United States and across the globe [2, 3]. Therefore, the search and development of new antimicrobial compounds is becoming vital in the future fight against pathogens. This experiment demonstrated that *Xeranthemum cylindraceum* has shown activity against gram-positive and gram-negative bacteria. We can conclude that *Xeranthemum cylindraceum* has antimicrobial properties against a variety of gram-positive and gram-negative bacteria, and may have the potential for development as a future antibiotic.

This study presents a good base of support for further experimentation of *Xeranthemum cylindraceum. X. cylindraceum* must be tested for potential toxicities and side-effect profiles in humans, and be tested against specific drug resistant strains of bacteria. We acknowledge this as a limitation of this study. However, due to facility safety concerns, drug resistant strains of bacteria could not be tested. We encourage researchers to test *X. cylindraceum* against drug resistant strains of bacteria in facilities with a sufficient level of biohazard security. Finally, further testing of *X. cylindraceum* against different fungi must be conducted to see if any antifungal properties exist.

Limitations of the Study: This study was limited in the usage of drug resistant strains of bacteria due to budget and biohazardous concerns. Further work must be done in a secure facility with sufficient biohazard protocols, in order to test the antimicrobial properties of *Xeranthemum cylindraceum* against multi-drug resistant strains of bacteria. The study was also limited in the extent of fungi testing as it only tested *C. Albicans*. Testing of different species of fungi should be conducted in order to see if antifungal properties of *Xeranthemum cylindraceum* exist.

Conflicts of Interest: The authors report no conflict of interest regarding this work.

Author Contributions

Conceptualization, PNA; Data presentation, TP; Experimentation, KC, PLT and PNA; Experimental design and execution of protocols, PNA; Writing the paper – TP and JS; Paper review & editing, JS and PNA

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