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Broad-spectrum antimicrobial potential of *Crassula ovata*

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Abstract
Due to the rampant microbial resistance to current antibiotics, there must be a necessary search for novel compounds with antimicrobial properties. In effort of this search in plants, this investigation demonstrates the antibacterial characteristics of *Crassula ovata* (*C. ovata*) against gram-positive bacteria and gram-negative bacteria. 1-3g samples of *C. Ovata* leaf were extracted using 95% ethanol and the extract were infused into paper discs. The discs were incubated on previously plated bacterial plates and the zones of bacterial inhibition attributable to the infused agents were measured. Control disc were infused with extraction solvents. By the measured zones of clearing, *C. ovata* demonstrated antimicrobial activity against the following bacteria: *K. pneumoniae, E. cloacae, E. coli*, *P. vulgaris*, *S. agalactiae* and *S. aureus*. As a result of its antibacterial activity against various gram-negative and gram-positive bacteria, *C. ovata* showed broad-spectrum antibacterial activity, hence, could be investigated further for potential development as a broad-spectrum antimicrobial.

Keywords: Broad-spectrum, antimicrobial activity, zone of clearing, zone of inhibition, susceptibility, gram-negative bacteria, gram-positive bacteria

Introduction
Plants are endowed with phytochemicals that contribute to their antimicrobial properties, hence, have largely been used world-wide for medicinal purposes. As a result of the rapid increase in the development of microbial resistance to the relatively few available antibiotics, plants have become the key sources of active ingredients for the latest antimicrobial development. Bacteria are developing rapid resistance to many antibiotics via complex mechanisms, including inactivating antibiotics, altering their cellular structure to resist the permeability of antibiotics or acquiring plasmids bearing resistant genes from neighboring bacteria [1]. Microbial resistance is quickly becoming a global public health crisis, causing an increase in mortality due to drug-resistant strains of bacteria [2-4]. Antibiotic resistance currently costs the United States billions of dollars annually [2-4]. With the alarming projected rise in mortality and costs of healthcare due to antimicrobial resistance, it is imperative that research is dedicated to the comprehensive screening of potential antimicrobial agents of plant origin to help mitigate the looming global public health crisis of antimicrobial resistance.
C. ovata is a plant native to South Africa [5]. It is also a relatively common house plant in most North American homes [5]. C. *ovata* belongs to the kingdom Plantae, class Dicotyledonae, and family Crassulaceae [6]. C. *ovata* is typically a round evergreen shrub that is roughly 1-1.5 m tall [6]. Its stems are usually upright and approximately 30-50 cm in length, with gray short branches [6]. Its leaves are sessile and has rounded apex with a sharp pointed tip [6]. It is sometimes covered with white star-shaped flowers during the months of winter [6].
C. ovata has long been used as a key medicinal plant in both South Africa and China [7]. It has mainly been used to disinfect wounds and to help cure diarrheal illness [7]. However, there is much uncertainty about the efficacy of using *C. ovata* as an herbal remedy in the literature [7]. With the potential *of C. ovata* serving *as* a medicinal plant, this experiment investigates its antibacterial activity against gram-positive and gram-negative bacteria. The following gram-positive bacteria were tested: *S. aureus*, *S. agalactiae, and S. pyogenes*. Along with the gram-positive bacteria, the following gram-negative bacteria were tested as well: *E. coli*, *P. vulgaris*, *E. cloacae*, and *K. pneumoniae.*
In previous studies, *C. ovata* has demonstrated antibacterial activity against *E. coli* [7]. *E. coli* is a gram-negative rod found as part of the normal enteric flora [8]. It causes both genitourinary and gastrointestinal tract infections, watery and bloody diarrheal illnesses and septicemia [8, 9].

E. coli O157:H7 especially is a lethal strain which cause Hemolytic Uremic Syndrome in both children and adults [8, 9]. Certain *E. coli* strains are also shown to have genes that confer antibiotic resistance [8, 9]. *Proteus vulgaris* is another gram-negative bacterium with the potential to increase morbidity and mortality in immunocompromised individuals. *Proteus spp*. have also shown the ability to confer some drug resistance [10]. *Klebsiella pneumoniae* (*K. pneumoniae*) is a gram-negative encapsulated bacterium that is often a common cause of nosocomial infections and pneumonia in immunocompromised patients [11]. *K. pneumoniae* is a common cause of nosocomial infection and has also been shown to gain antibiotic resistance [12, 13]. K. *pneumoniae* is of particular concern as it has gained resistance against many of the most effective broad-spectrum antibiotics in clinical practice [13]. *Enterobacter cloacae* (*E. cloacae*) is another gram-negative rod of concern due to its high levels of drug resistance against many of the most effective antibiotics available, such as carbapenem [13]. *E. cloacae* has been linked to many large clinical outbreaks of gastrointestinal infection [14].

While gram-positive bacteria are typically considered of less concern than gram-negative bacteria, there are several important pathogens gaining antibiotic resistance. Of significant concern is *Staphylococcus aureus* (*S. aureus*), a gram-positive bacterium found in the normal flora of skin and mucous membranes. *S. aureus* cause skin abscesses, food poisoning, infective endocarditis, toxic-shock syndrome, and osteomyelitis [15]. One particular strain of concern is Methicillin-resistant Staphylococcus aureus (MRSA) as a result of its severe resistance against antibiotics [15, 16]. MRSA can be implicated in both nosocomial and community acquired infections. Also, of concern is *Streptococcus agalactiae* (Group B Streptococcus). It is a gram-positive coccus especially deleterious to high-risk groups including neonates, pregnant women, and the elderly because of its ability to cause sepsis and shock [17]. Certain strains of *S. agalactiae* have shown resistance to both penicillin (a beta-lactam) and non-beta lactam antibiotics [17]. An additional gram-positive bacterium of concern is *Streptococcus pyogenes* (Group a Streptococcus). S. *pyogenes* cause necrotizing fasciitis, pharyngitis, and skin infections, with long-term sequelae, such as post-infectious rheumatic fever and post-streptococcal glomerulonephritis [18].

Antibiotic resistance is rapidly growing throughout the world and is a key public health crisis. Further investigation of antimicrobials is necessary to mitigate the rapidly climbing rates of antibiotic resistance. Due to the usage of *C. ovata* as a medicinal herb in some countries of the world for the treatment of wounds and diarrheal illness, we hypothesize in this study that *Crassula ovata* will demonstrate broad-spectrum antibacterial activity towards gram-negative and gram-positive bacteria.

Materials and Methods

Sample extraction and disc preparation

1-3g samples of C. Ovata were homogenized using mortar and pestle to achieve a medium fine paste. 3-4mls of 95% ethanol was added to the paste and swirled for 5 minutes to extract. The homogenate/ethanol mixture was filtered through a thin filter paper and the filtrate was collected into a 50 ml beaker. Blank disks were soaked into the filtrate and al-lowed to sit for 10 minutes. The disks were removed using tweezers and allowed to dry on blotting paper. Other blank discs were soaked in 95% ethanol and allowed to sit for 10 minutes. The discs were removed and allowed to dry on a blotting paper for 30 minutes. During this wait time, bacterial plates were prepared from previously scaled bacterial stocks of the bacterial strains.

Scaling up of bacterial from glycerol stocks

Bacterial stocks made from single colonies (500 microliters) were scaled-up in 1ml of Luria Bethani broth by shaking at 145 rpm and 37 degrees Celsius for 20 hours. A 100ul volume of the scaled-up stock was resuspended in 8ml of 1% saline solution.

Preparing plates

Mueller-Hinton agar plates were prepared by dissolving 38g of nutrient agar in 1 liter of water. The mixture was autoclaved for 15 minutes at a temperature of 121 C. The media was then cooled and plates were poured, using approximately 20 ml volumes of the mol-ten/autoclaved agar and allowed to set.

Plating

100 microliters of the saline bacterial suspension were spread on Mueller Hinton plates. The discs with extracts (previously bathed in extracts) were placed on the plates; 4-7 discs/ per plate along with a blank disc (with no active ingredient impregnated, as negative control) or blank disc infused with the solvent used for plant extraction (control for solvent effect). The plates were incubated at 37 degrees Celsius for 24-48 hours and monitored for zones of inhibition.

Results

C. ovata demonstrated antibacterial activity against these gram-positive bacteria: *S. aureus* (22 mm mean zone of clearing) and *S. agalactiae* (8 mm mean zone of clearing) and these gram-negative bacteria: *E. coli* (14 mm mean zone of clearing), *P. vulgaris* (13 mm mean zone of clearing), *E. cloacae* (16 mm mean zone of clearing), and *K. pneumoniae* (13 mm mean zone of clearing) but not against *S. pyogenes.*

Figures and Tables

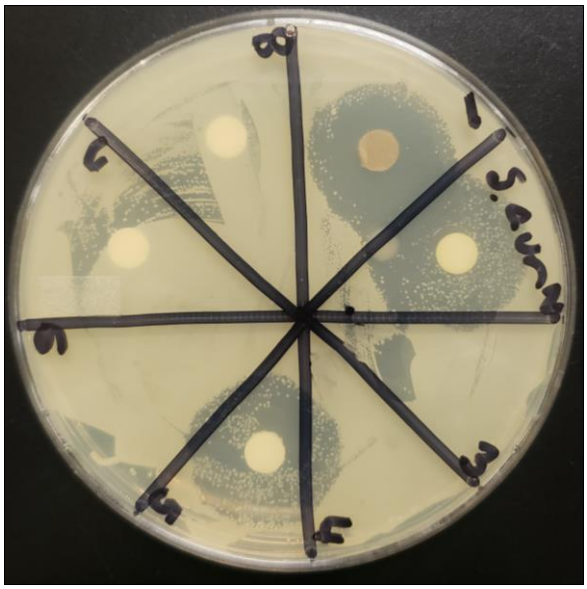

Fig 1: Blank disk only [position 7], Blank disk infused with extraction solvent [Position 8], Blank disk infused with *C. ovata* extract [Position 1]. Zone of clearing/inhibition against *S. aureus* indicated as a clear zone around disk

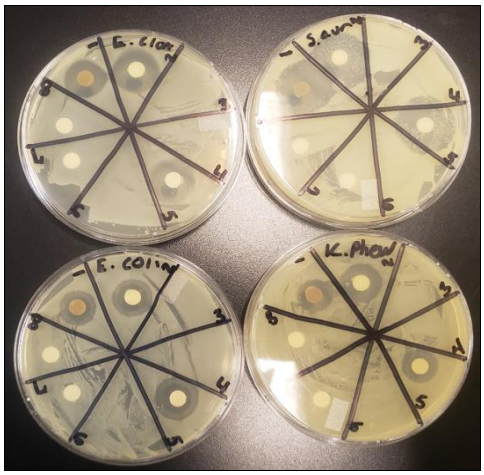

Fig 2: Blank disk only [position 7], Blank disk infused with extraction solvent [Position 8], Blank disk infused with *C. ovata* extract [Position 1]. Zone of clearing/inhibition against *S. aureus, E. cloacae, E. coli, and K. pneumoniae*. Zones of indicated as clear zone around disk

Table 1: Antimicrobial zone of inhibition of Gram-positive bacteria, with mean zone of inhibition due to *C. ovata* extract, recorded in millimeters

	***S. aureus* [Inhibition zone, mm]**	***S. agalactiae* [Inhibition zone, mm]**	***S. pyogenes* [Inhibition zone, mm]**
Blank Disk	0	0	0
C. ovata	22	8	0

Table 2: Antimicrobial zone of inhibition of Gram-negative bacteria, with mean zone of inhibition due to *C. ovata* extract, recorded in millimeters

	***E. coli* [Inhibition zone, mm]**	***P. vulgaris* [Inhibition zone, mm]**	***E. cloacae* [Inhibition zone, mm]**	***K. pneumoniae* [Inhibition zone, mm]**
Blank Disk	0	0	0	0
C. ovata	14	13	16	13

Discussion

Based on our microbial susceptibility testing, the extracts from *C. ovata* has shown broad-spectrum antimicrobial activity against 3 strains of gram-positive bacteria and 4 strains of gram-negative bacteria (Table 1 and Table 2, respectively). The bacteria susceptible to *C. ovata* extracts include: S. *aureus*, S. *agalactiae*, E. *coli*, K. *pneumoniae*, E. *cloacae* and P. *vulgaris.* Although the zone of inhibition of *S. aureus* (Figure 2) by *C. Ovata* was greater in magnitude compared to the zone of inhibition of *K. pneumoniae, E. cloacae* and *E. coli* (Figure 2) by *C. ovata*, this only suggest higher susceptibility and not higher potency [19]. Its potency in the inhibition of S. *aureus*, S. *agalactiae*, E. *coli*, E. *cloacae*, K. *pneumoniae*, and P. *vulgaris* need to be assessed by in-vivo treatments [19].

Antibiotic resistance is a major global public health crisis that is rapidly unfolding. Antibiotic resistance is causing increased mortality in humans and billions of dollars to health care systems [2-4]. The exploration for potential antimicrobials is vital for the future existence of our species. Plants have been used for medicinal purposes throughout history and have played a vital role in the search for antimicrobial agents. However, there are a lot more plants available to be screened for antimicrobial properties. The plant C. *ovata*, a member of the Dicotyledonae class and the Crassulaceae family, has previously been used as a medicinal plant in South Africa and China to treat wounds and diarrheal illness, However, there were significant concerns about the efficacy of C. *ovata* for usage in these treatments [5-7]. In this experiment, C. *ovata* demonstrated activity against several bacteria of major clinical concern such as: S. *aureus*, E. *coli*, E. *cloacae*, and K. *pneumoniae.* Antibiotic resistance in these pathogens is becoming a major concern as these pathogens are demonstrating resistance some of the most effective broad-spectrum antibiotics available [8, 9, 12-16]. *C. ovata* demonstrated activity against E. *cloacae* and K. *pneumoniae*, which are two bacteria of major concern for their ability to cause large nosocomial outbreaks; and with drug resistance against the most effective antibiotics [12-14]. The activity of C. *ovata* against these bacteria of concern are the reason the novel results of this research are of absolute need to the field of medicine. These results show that *C. ovata* has broad-spectrum antibacterial activity against gram-negative and gram-positive bacteria, and further investigation must be undertaken to further enlighten the active ingredients of *C. ovata* and its subsequent development as broad-spectrum antibiotic against these species of concern.

Further investigation is necessary to determine the full extent of antimicrobial ability of *C. ovata* in terms of its physicochemical properties and the pharmacokinetics of its active ingredients. We do acknowledge that this study does not include specific drug-resistant strains, and does not include testing for toxicities and side effects. However, we encourage further evaluation of these extracts in facilities that have the necessary biohazard safety standards that are currently not available at our facilities. Finally, further investigation must also be carried out to explore the mechanism through which C. *ovata* exerts its effects. This research serves as a novel beginning into the potential development of C. *ovata* as a broad-spectrum antimicrobial. We encourage our colleagues in the field to advance the studies of this extract.

Conclusions

Based on our results, we have shown that *C. Ovata* has antibacterial activity against various gram-negative and gram-positive bacteria, indicated by significantly clear zones of inhibition, hence, qualifying *C. Ovata* as a potential broad-spectrum antibacterial agent which could be developed to mitigate antibiotic resistance.

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Contributions of Authors: Conception - PNA; Curation of data - TP; Experimentation - PNA and AB; Method- PNA; Manuscript - TP and JS; Data presentation - JS; Editing - JS and PNA.

Statement of ethics approval

The study does not require ethics approval.

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