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Novel *Coleus* antimicrobial activity against *S. agalactiae* and *S. pyogenes*

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Abstract
This paper highlights the novel antibacterial properties of *Coleus scutellarioides* against certain species of the domain bacteria, of which no *Coleus* antimicrobial activity was previously demonstrated. *Coleus* has demonstrated antimicrobial properties against many species of bacteria and fungi. However, the search for novel antimicrobial compounds is vital in this age of growing microbial resistance. Samples of *Coleus* extraction were carried out with 95% ethanol and the filtrate was impregnated into sterile discs. The extraction solvent (95% ethanol) alone was also impregnated into sterile disc as vehicle control. Discs of *Coleus* extracts or control were placed on bacterial and fungi plates and the zones of inhibition were measured. *Coleus* leaves displayed novel antimicrobial activity against *S. agalactiae and S. pyogenes. Coleus* stems displayed antimicrobial activity against *S. pyogenes*. This research demonstrated novel *Coleus* activity against *S. pyogenes and S. agalactiae,* which had not been previously demonstrated.

Keywords: Drug resistance, antimicrobial, gram-negative, gram-positive, bacteria

Introduction
Since the development of antibiotics, bacteria have steadily evolved to evade the effects of various antibiotics. Bacteria have used a wide variety of mechanisms to gain antibiotic resistance [1, 2]. The resistance of these evolving bacteria to antimicrobial agents is quickly becoming a major global health crisis [2, 3]. Antimicrobial resistance is resulting in higher levels of mortality and large financial and physical strain on our healthcare systems [2-4]. Multidrug resistant bacteria that are resistant to all antibiotics will quickly become a reality unless there is further development of antimicrobials [5, 6]. The search for novel antimicrobial compounds is necessary to prevent large outbreaks and significant strain on our healthcare systems.
Plants remain a central source for novel antimicrobial agents [7, 8]. There have been tremendous successes in their use in the treatment of illnesses across the globe [9, 10]. The antimicrobial activity of many plants has yet to be fully explored. A potential plant with promise for development as an antimicrobial is *Coleus scutellarioides. Coleus scutellarioides* is a plant that is native to the tropics of Asia [11]. *Coleus* is well known for its foliage and wide variety of colors that it produces [11]. *Coleus* typically grows during the summer months and can reach up to three feet in height [12]. *Coleus spp*. has been shown to have antibacterial activity against *S. epidermidis, S. aureus, K. pneumoniae, B. subtilis, E. coli, S. pneumoniae, and P. aeruginosa* [13, 14]. *Coleus* has also shown antifungal properties against *C. albicans* [13]. *Coleus* leaves and stems have not yet been demonstrated to have antibacterial activity against *S. pyogenes or S. agalactiae* [15]. The gram-positive coccus, *Streptococcus agalactiae* (Group B) is pathogenic to the elderly, expectant mothers and neonates [16]. *S. agalactiae* has shown resistance to commonly used antibiotics for treatment [16, 17]. S. *agalactiae* is known to cause still birth and diseases in neonates [18]. Moreover, S. *agalactiae* is slowly becoming an infectious agent to healthy adults [19]. A second pathogenic gram-positive bacteria of concern of concern is *Streptococcus pyogenes* (Group A). S. *pyogenes* is a common cause of skin infections, pharyngitis and necrotizing fasciitis [20, 21]. *S. pyogenes* can cause severe secondary complications including post-streptococcal glomerulonephritis and rheumatic fever [20, 21]. S. *pyogenes* has demonstrated resistance to several antibiotics [22, 23]. In this experiment, we hypothesize that *Coleus* demonstrates novel antibacterial activity against S. *agalactiae* and S. *pyogenes*.

Methods
Sample preparation
3 grams of *Coleus scutellarioides* were extracted with 95% ethanol (Puga *et al*., 2022) and the filtrate impregnated into sterile discs as described previously (Puga *et al*., 2022).

The vehicle (95% ethanol) was also impregnated into blank discs to serve as controls for any effect from the solvent. Overnight culture of glycerol stocks of bacteria was diluted in 1 to 9 ratios using 1% saline solution and a 100 ul volumes of the resulting dilution were plated on Muller Hinton Agar plates as described earlier (Puga *et al*., 2022). Extract-infused or vehicle-infused discs were incubated on bacterial-plated plates overnight at 37 °C. Zones of inhibition were measured after 24 hours of incubation.

Results

Coleus leaves showed antimicrobial activity against both *S. pyogenes* (10 mm mean zone of inhibition) and *S. agalactiae* (8 mm mean zone of inhibition) and. *Coleus* stems showed antimicrobial activity against *S. pyogenes* (7 mm mean zone of inhibition). *Coleus* stems were unable to demonstrate activity against *S. agalactiae* (0 mm mean zone of inhibition).

Table 1: *Coleus* leaves induces zone of inhibition (in mm) in the presence of S. *agalactiae,* and S. *pyogenes. Coleus* stems induces zone of inhibition in the presence of S. pyogenes but not *S. agalactiae*. The control disc infused with only the extraction solvent did not induce inhibition zone.

	***S. agalactiae* (Inhibition zone in mm)**	***S. pyogenes* (Inhibition zone in mm)**
Blank Disk	0	0
Coleus leaves	8	10
Coleus stems	0	7

Discussion

In this experiment, *Coleus* leaves demonstrated antibacterial activity against *S. pyogenes* and *S. agalactiae. Coleus* stems did not demonstrate antibacterial activity against S. *agalactiae*. The antibacterial activity of *Coleus* against S. *agalactiae* and S. *pyogenes* has not been demonstrated in previous research [15]. Both S. *agalactiae* and S. *pyogenes* are bacteria of public health concern because they are able to cause severe clinical infections and complications [16-23]. In addition, they have demonstrated a degree of antibiotic resistance to commonly used antibiotics, making them a pathogen of concern [16, 17, 22, 23]. While these bacteria only have some resistance, the need for the development of novel antimicrobials is vital before these pathogens begin to display multi-drug resistance. The need for novel antimicrobials is vital to slow the global health emergency of antibiotic resistance [2-6]. Previous research of *Coleus* has shown antibacterial activity against gram-positive and gram-negative bacteria [13, 14]. The demonstration of an additional novel activity of *Coleus* against S. *agalactiae and* S. *pyogenes*, provides further support for the development of *Coleus* as an antimicrobial of the future.

Limitations of the Study

The study was unable to test antimicrobial resistant strains of S. *agalactiae* and S. *pyogenes* due to facility biohazard concerns. Further research should be conducted within the confines of a secure biohazard facility to further test *Coleus* against antimicrobial resistant strains of S. *agalactiae* and S. *pyogenes.*

Conflicts of Interest

The authors declare no conflicts of interest.

Author Contribution

Conceptualization- PNA; Data curation-TP; Investigation-CQS, NI and PNA; Methodology- PNA; Original draft- TP and JS; Visualization- JS; Review & editing- JS and PNA.

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Statement of ethics approval

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