A LEGACY OF LEADERSHIP: A SPECIAL ISSUE HONOURING THE TENURE OF OUR VICE CHANCELLOR, PROFESSOR ARMAYA'U HAMISU BICHI, *OON, FASN, FFS, FNSAP*

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EVALUATION OF *Pseudomonas aeruginosa* ACTIVITIES OF MUNG BEAN (*Vigna radiata*) SEED EXTRACT

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ABSTRACT

This research focused on exploring of mung bean seed extracts and its potential as antibacterial agents. Mung bean (*Vigna radiata*) is a warm-season legume originally from the Indian subcontinent, where it was domesticated around 1500 BC. It is now cultivated widely across Asia, Africa, and the Americas due to its adaptability to diverse climates and soils. They are widely recognized for their high nutritional value and medicinal properties. The study was motivated by the increasing global concern over antimicrobial resistance and the need for natural alternatives to synthetic antibacterial agents. The research began with the collection of mung bean seeds from northern Nigeria, followed by their extraction using polar (ethanol) solvent. The extraction process aimed to evaluate the antibacterial efficacy of the mung bean extracts, antibacterial assays was conducted using the agar-well diffusion method against Pseudomonas aeruginosa. The results showed that the mung bean extracts exhibited significant inhibitory effects on the bacterial pathogens, with the largest zones of inhibition observed at higher extract concentrations. The study further analyzed the statistical significance of these results using an ANOVA, which confirmed that the antibacterial activity was consistent across different concentrations, although the differences between the concentrations were not statistically significant. The study also highlighted the potential of mung beans in food preservation, given their consistent antibacterial activity across different concentrations.

Keywords: Anti-bacterial, Extraction, Mung bean seed, *Pseudomonas aeruginosa*

INTRODUCTION

The mung bean is thought to have originated from the Indian subcontinent where it was domesticated as early as 1500 BC. Cultivated mung beans were introduced to southern and eastern Asia, Africa, Austronesia, the Americas and the West Indies. It is now widespread throughout the Tropics and is found from sea level up to an altitude of 1850 m in the Himalayas (Lambrides *et al*., 2006; Mogotsi, 2006). The mung bean is a fast-growing, warm-season legume. It reaches maturity very quickly under tropical and subtropical conditions where optimal temperatures are about 28-30°C and always above 15°C. It can be sown during summer and autumn. It does not require large amounts of water (600-1000 mm rainfall/year) and is tolerant of drought. It is sensitive to water logging. High moisture at maturity tends to spoil the seeds that may sprout before being harvested. The mung bean grows on a wide range of soils but prefers well-drained loams or sandy loams, with a pH ranging from 5 to 8. It is somewhat tolerant to saline soils (Mogotsi, 2006). Mung bean production is mainly (90%) situated in Asia: India is the largest producer with more than 50% of world production but consumes almost its entire production. Biocides or phytochemicals with antimicrobial activity are gaining popularity because of their broad-spectrum activity with no known side effects. Several reports have been published showing the potential of mung bean as an antimicrobial agent (Tang *et al*., 2014; Ganesan and Xu, 2018; Shen *et al*., 2018; Mehta *et al*., 2021). A nonspecific lipid transfer peptide (nsLTP; Molecular weight: 9.03 kDa) that shows broad-spectrum antibacterial and antifungal activity has been isolated from mung bean (Shen *et al*., 2018). It is active against the bacterial species such as *Staphylococcus aureus*. The polyphenol extract from the sprouts exhibited antibacterial properties against *Helicobacter pylori,* which causes gastro-duodenal diseases in humans (Tang *et al*., 2014). Recently, methanolic extracts of mung bean seed coat have been shown to exhibit broad-spectrum antibacterial activity against *Bacillus cereus, Bacillus subtilis Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, and S. aureus* (Mehta *et al*., 2021). _Garlic (Allium sativum) Garlic extract inhibits *P. aureofaciens* growth and biofilm formation. Zhang *et al*. (2020). Neem (Azadirachta indica): Neem leaf extract exhibits antibacterial activity against *P. aureofaciens.* Singh *et al*. (2019). Turmeric (Curcuma longa) Curcumin inhibits *P. aureofaciens* growth and quorum sensing. Kumar *et al*. (2018). Cinnamon (Cinnamomum verum): Cinnamon oil exhibits antibacterial activity against *P. aureofaciens*. Liu *et al*. (2020). Eucalyptus (*Eucalyptus globulus*) Eucalyptus oil exhibits antibacterial activity against *P. aureofaciens*. Dos Santos *et al*. (2019). Ginger (*Zingiber officinale*). Ginger extract inhibits *P. aureofaciens* growth and reduces disease severity. Kim *et al.* (2019). Thyme (*Thymus vulgaris*). Thyme essential oil exhibits antibacterial activity against *P. aureofaciens*. Sienkiewicz *et al*. (2020). Essential oils from various aromatic plants, such as thyme (*Thymus vulgaris*) and oregano (*Origanum vulgare*), exhibit antimicrobial activity against *P. aeruginosa*. Nostro, *et al*., (2004). Flavonoids such as baicalein have been shown to possess significant antimicrobial activity against *P. aeruginosa*. Xie *et al*., (2015). Aloe vera has demonstrated antibacterial properties against *P. aeruginosa,* particularly its gel extract. Kumar *et al*., (2019). Eucalyptus species have shown potential antimicrobial activity against *P. aeruginosa* due to their phenolic compounds. Kadir *et al*., (2015). Curcumin, a component of turmeric (Curcuma longa), has shown strong antibacterial effects against *P. aeruginosa.*

Moustafa, *et al*., (2017). Ginger (*Zingiber officinale*) extract has been reported to exhibit activity against P. aeruginosa. Adetutu *et al*., (2018). Black cumin seed oil (*Nigella sativa*) has been shown to possess antimicrobial properties against *P. aeruginosa*. Tarkhan *et al.,* (2015). The research aim to investigate the Mung bean seed extracts for antibacterial activities against *Pseudomonas aeruginosa* microorganisms.

MATERIALS AND METHODS

Study Area

Mung bean seeds were obtained from the Department of Crop Production, Faculty of Agriculture, University of Maiduguri, Borno State. And then transported the samples to the Department of Biological Science Laboratory, Faculty of Life Science, Federal University Dutsin-Ma, Katsina State. Where the research was carried out.

Dutsin-Ma LGA lies on latitude 12°26'N and longitude 07°29'E. It is bounded by Kurfi and Charanchi LGAs to the north, Kankia LGA to the east, Safana and Dan-Musa LGAs to the west, and Matazu LGA to the southeast. Dutsin-Ma LGA has a land size of about 552.323 km2 with a population of 169 829 as at 2006 national census (Federal Republic of Nigeria, 2012). The people are predominantly farmers, cattle rearers and traders.

Materials

Mung bean seeds, organic solvent (ethanol), media or culture plates for microbial growth, bacteria strain (*Pseudomonas aeruginosa*) for antibacterial testing, chemical reagents for extraction and analysis (buffers), antibiotics for comparison, distilled water, vial bottles, Mortar and pestles, and foil paper.

Equipment

Vortex mixer for sample mixing, rotary evaporator for solvent removal, centrifuge for separating solids and liquids, incubator for microbiological culture, spectrophotometer for antimicrobial testing (turbidity), autoclave, laminar flow hood, wire loop, and scale (analytical weighing balance).

Samples Collection

Mung bean seeds were obtained from the Department of Crop Production, Faculty of Agriculture, University of Maiduguri, Borno State. A sterile bag was used to transport the samples to the Department of Biological Science Laboratory, Faculty of Life Science, Federal University Dutsin-Ma, Katsina State.

Bacterial Isolates

A total of one bacterial isolate was collected from the Department of microbiology, federal university Dustin-ma, katsina state. The specific isolate was *Pseudomonas aeruginosa*. This isolate was carefully taken to the Department of Biological Science Laboratory, Faculty of Life Science, Federal University Dustin-ma, Katsina State, using sterile container to ensure the sample remained uncontaminated. Upon arrival at the laboratory, the bacterial isolates were stocked and preserved for further analysis.

Samples Preparation

The seed sample was thoroughly cleaned to remove any dirt and impurities by rinsing them with distilled water and allowing them to dry completely. The dried seeds were then ground into a fine powder using a laboratory mortar and pestle (Association of Official Analytical Chemist, 2005). A weighing balance was used to weigh the powder and about 750g of mung bean seed powder was produced. The mung bean seed powder sample was placed into a container for further analysis.

Media Preparations

Mueller-Hinton Agar was prepared according to standard protocols of Clinical and Laboratory Standards Institute, (2012). The agar medium was sterilized by autoclaving at 121 degrees Celsius for 15 minutes and then poured into sterile Petri dishes. The agar plates were allowed to solidify and were subsequently dried by opening and inverting the plates in a hot air oven until it's completely dry.

Mung Beans Seed Extraction

The extraction method used in this research was maceration in accordance to the descriptions of Azwanida (2015). The method is a widely recognized techniques for extracting bioactive compounds from plant materials.

A total amount of 500g of mung bean seed powder was weighed using a weighing balance. The weighed mung bean seed powder was placed into a glass extraction vessel. A sufficient amount of ethanol solvent was added to the extraction vessel to cover the mung bean seed powder completely, calculated using a solvent ratio of 5:1. The mixture was gently stirred to ensure adequate contact between the mung bean seed powder and the solvents. The mixture was allowed to sit for a period, of three days, to enable proper extraction. This process allowed the solvents to penetrate the plant material and dissolve the desired bioactive compounds (Handa *et al*., 2008). The mixture was occasionally stirred to enhance the extraction efficiency. After the extraction period, the supernatant containing the extract was separated from the solid residue using a separating funnel. The liquid phase was collected in a clean glass container, ensuring that no solid particles was carried along. This liquid phase contained the extracted compounds from the mung bean seeds. The ethanol solvent were then carefully evaporated from the liquid phase to obtain the dry mung bean extract. This evaporation was done using a rotary evaporator (Azwanida, 2015). The dried mung bean seed extract was stored in a clean, airtight container to prevent it from absorbing moisture or reacting with the environment.

Stock Sample Preparations

Stock solution of the mung bean extract was prepared at different concentrations to test for antimicrobial activity. The extract was dissolved in sterile distilled water to create solutions with concentrations of 1000 mg/L, 750 mg/L, 500 mg/L, 250 mg/L, and 0 mg/L (as control). These solutions were stored in sterile test tubes until use.

Anti-Bacterial Assay

Inoculation of Pathogen

The pure culture of *Pseudomonas aeruginosa* was obtained and cultured on nutrient agar plate. After 24 hours of incubation at 37 degrees Celsius, the broth cultures were used to prepare inoculums at a room temperature. The inoculum was prepared by suspending bacterial colonies in sterile normal saline to match the turbidity of a 0.5 McFarland standard. This standard represents an approximate bacterial concentration of 1.5×10^8 CFU/mL, ensuring uniformity in the subsequent antibacterial assays (Andrews, 2001).

Antibiotic Testing

The anti-bacterial activity of the mung bean extracts was tested using the agar-well diffusion method. Firstly, 100 µL of the bacterial inoculum was spread evenly on the surface of agar plates using a sterile cotton swab. The plate was allowed to dry for 5 minutes with the lid closed to prevent contamination. Wells were then bored into the agar using a sterile cork borer. Four wells were made on the plate, and 100 µL of each extract concentration (1000 mg/L, 750 mg/L, 500 mg/L, 250 mg/L, and 0 mg/L (as control)) was added to the respective wells. The plate was incubated at 37 degrees Celsius for 24 hours for the bacteria at a room temperature. After the incubation period, the plate was examined for zones of inhibition around the wells, indicating antibacterial activity. The diameter of each zone of inhibition was measured in millimetres.

Data Analysis

Data analysis was conducted to assess the significance of the variations in antibacterial activity of the isolate between the various concentrations of mung bean extracts. An Analysis of Variance (ANOVA) was performed using SPSS version 27 to determine whether there was statistically significant differences in the antibacterial efficacy among the different concentrations and bacterial strains. Results was considered statistically significant at a p-value of less than 0.05.

RESULTS AND DISCUSSION

Results

The results shows that mung bean extract exhibits significant activity against *Pseudomonas aeruginosa*. The highest zone of inhibition was observed at a concentration of 1000 mg/L, measuring 21.07 mm diameter. This was closely followed by a 20.84 mm diameter zone of inhibition at 750 mg/L. The zones of inhibition for the 500 mg/L and 250 mg/L concentrations were 20.56 mm and 20.13 mm diameter, respectively. No inhibitory activity was observed in the 0 mg/L (control), as presented in Table 1.

However, the differences in inhibition between the different concentrations are not statistically significant, as indicated by the F-value (0.0294) for concentrations, which is less than the critical F-value (7.7086), and a P-value greater than 0.05 (0.8721), suggesting no significant difference between the concentrations tested.

Table 1: Antimicrobial sensitivity of different concentrations of Mung-bean seeds extracts against *Pseudomonas aeruginosa*

Concentration of Mung-bean seeds extracts (mg/L)	*Zone of inhibition against Pseudomonas aeruginosa* (mm)	Level of significance
1000	21.07	
750	20.84	
500	20.56	0.87211
250	20.13	
0 (Control)	0	

p-value (p>0.05) no significant

Discussion

Mung beans (*Vigna radiata*) are a valuable legume widely cultivated for their nutritional benefits and potential therapeutic properties. The mung beans origins trace back to the Indian subcontinent, where it has been domesticated since around 1500 BC. Its adaptability to a variety of climates and soil conditions, particularly in tropical and subtropical regions, has made it an important crop worldwide, especially in Asia. Beyond its nutritional value, mung beans have garnered attention in recent years due to their antimicrobial properties, which have been explored for potential applications in medicine and food preservation (Shen *et al*., 2018; Mehta *et al*., 2021). This research aimed to investigate the antibacterial properties of mung bean extract against *Pseudomonas aeruginosa*, a pathogen of considerable concern due to its resistance to many antibiotics.

The study found that mung bean extract demonstrated significant antibacterial activity against *Pseudomonas aeruginosa*, as evidenced by the formation of inhibition zones around the wells in the agar diffusion test. The diameter of these zones was used as an indicator of the extract's antimicrobial efficacy. The results indicated that the highest concentration (1000 mg/L) of mung bean extract resulted in a zone of inhibition measuring 21.07 mm, followed by 20.84 mm at 750 mg/L, 20.56 mm at 500 mg/L, and 20.13 mm at 250 mg/L. No inhibition was observed in the control group (0 mg/L), confirming the specificity of the extract's activity. These findings align with previous studies that have shown broad-spectrum antimicrobial properties of mung bean extracts, particularly against Gram-negative bacteria such as *Pseudomonas aeruginosa* (Mehta *et al*., 2021).

The results of this study also support the earlier research by *Shen et al*. (2018), who isolated a nonspecific lipid transfer peptide from mung beans, which exhibited antibacterial activity. Similarly, the study by Tang *et al*. (2014) on polyphenol extracts from mung bean sprouts demonstrated antibacterial properties against other pathogens like Helicobacter pylori, reinforcing the idea that mung beans contain bioactive compounds capable of inhibiting a range of bacterial species.

While the antibacterial effects of mung bean seed extracts were observed across all concentrations, the statistical analysis of the results revealed that the differences between the inhibition zones at various concentrations were not significant. The p-value (0.8721) was greater than the critical value of 0.05, indicating that there was no statistically significant difference in the antibacterial activity at the different concentrations of mung bean extracts tested. The F-value of 0.0294 was also much lower than the critical F-value (7.7086), further confirming that the variation in antibacterial activity across the concentrations was not statistically significant.

This finding may suggest that while the antibacterial activity of mung bean extract is evident, increasing the concentration of the extract beyond a certain point does not lead to a significant increase in its effectiveness against *Pseudomonas aeruginosa*. This could indicate that mung bean extract reaches a saturation point in its ability to inhibit bacterial growth, and higher concentrations may not provide any additional antimicrobial benefits. This is consistent with the principle of diminishing returns, where the effects of a compound or treatment plateau after reaching a certain threshold.

The findings from this research suggest that mung bean extract exhibits comparable, if not slightly superior, antibacterial properties compared to other natural antimicrobial agents. Studies on other plant extracts, such as garlic, neem, and ginger, have similarly shown inhibitory effects against *Pseudomonas aeruginosa* (Zhang *et al*., 2020; Singh *et al*., 2019; Adetutu *et al*., 2018). However, the broad-spectrum nature of mung bean extracts, as seen in previous research, distinguishes them from many other plant extracts that tend to exhibit selective antibacterial effects. For example, while turmeric and ginger extracts have demonstrated antibacterial properties, they may not be as effective against the diverse range of bacterial species that mung bean extracts can inhibit (Kumar *et al.*, 2018; Liu *et al*., 2020).

Moreover, the antibacterial efficacy of mung beans could be attributed to various bioactive compounds present in the seeds, including polyphenols, flavonoids, and nonspecific lipid transfer peptides. These compounds have been shown to exert antibacterial effects through mechanisms such as disrupting bacterial cell membranes, inhibiting protein synthesis, and interfering with bacterial metabolism (Mehta *et al*., 2021; Tang *et al*., 2014). Therefore, the broad-spectrum activity of mung beans may make them a more versatile candidate for developing natural antibacterial agents compared to more selective antimicrobial compounds found in other plants.

The results of this study also have practical implications for the food industry and medicine. Given the increasing concerns about antibiotic resistance and the potential side effects of synthetic antimicrobial agents, mung bean extract could serve as a natural alternative in food preservation. Its antibacterial properties could help extend the shelf life of food products by preventing bacterial growth, particularly in the context of foodborne pathogens like *Pseudomonas aeruginosa*. Furthermore, as the extract has shown effectiveness against a broad spectrum of bacteria, it could be explored for use as a natural preservative in various food products, reducing reliance on chemical preservatives.

Additionally, the potential of mung bean extract as a natural alternative to antibiotics in the treatment of infections warrants further investigation. As antibiotic resistance continues to pose a global health threat, exploring plant-based antimicrobial agents, such as mung bean extracts, could provide valuable solutions in combating bacterial infections. The lack of significant differences in antibacterial efficacy across various concentrations of mung bean extract also suggests that even lower concentrations could be effective, which may make it more economically viable and safer for use in therapeutic applications.

CONCLUSION

In conclusion, this research provides compelling evidence that mung bean extract possesses significant antibacterial activity against *Pseudomonas aeruginosa*. While the extract was effective at all tested concentrations, no statistically significant difference in inhibition was observed across the concentrations, suggesting a plateau effect in the antimicrobial efficacy of mung bean extracts. This study contributes to the growing body of research highlighting the antimicrobial potential of mung beans, which could be applied in food preservation, as a natural alternative to synthetic antibiotics, and in the development of novel therapeutic agents. However, further studies are needed to identify the specific bioactive compounds responsible for the antibacterial effects and to explore their mechanisms of action in greater detail. Additionally, research on the safety and long-term efficacy of mung bean extracts in clinical and food preservation settings is essential for advancing their practical applications.

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