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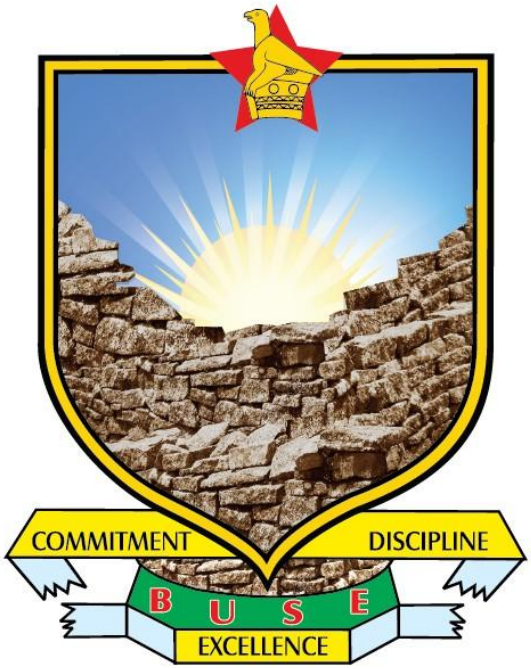

The antimicrobial activity of *Jatropha curcas* seeds extracts against *Staphylococcus aureus* and *Escherichia coli*

By

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A research project submitted in partial fulfilment of the requirements for the Bachelor of Science Honors Degree in Biological sciences

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APPROVAL FORM

The undersigned certify that they have read the dissertation titled: The antimicrobial activity of *Jatropha curcas* seed extracts against *E. coli* and *S. aureus* and confirm that it is suitable for submission to the Biological Sciences Department, Faculty of Science and Engineering, for assessment.

Signature of student:  Date: 02/06/2025

Signature of Supervisor:  Date: 02/06/2025

Signature of Chairperson of Department:  Date: 09/09/25

DECLARATION

I, Mercy Mutsetse (B212228B) declare that this research herein is my own work and has not been plagiarized from another source(s) without acknowledgement of the concerned author(s) either electronically or otherwise.

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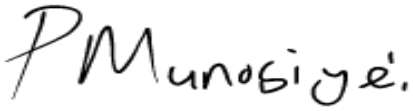
I, P. Munosiyei, declare that I have supervised this thesis and I am satisfied that it can be submitted to the Biological Sciences Department, Faculty of Science and Engineering, at Bindura University of Science Education.

Signature:  Date: 02/06/2025

DEDICATION

I dedicate this dissertation to my mother, whose unrelenting support and steadfast encouragement have been instrumental throughout this academic endeavor**.**

ACKNOWLEDGEMENTS

I extend my sincerest gratitude to my supervisor, Mr. P. Munosiyei, for his expert guidance, unwavering support, and constructive feedback throughout this research project. His insightful critiques and suggestions significantly enhanced the quality of this work. I also wish to express my appreciation to Mr. D. Katsande and Mrs. M. Goredema for their technical assistance and expertise in the laboratory, which were valuable to the success of this project. Furthermore, I am grateful to my friends for their encouragement and support, which helped to alleviate the challenges encountered during this research. Their collective contributions are deeply appreciated, and I am honored to have had the opportunity to work with such a dedicated and supportive team.

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ABSTRACT

The emergence of foodborne bacteria that are resistant to antibiotics presents a serious threat to food safety and human health. This study used the Kirby-Bauer disc diffusion method in triplicate to assess the antibacterial activity of ethanol extracts from *Jatropha curcas* seeds collected in Banket, Mashonaland West District, against two significant foodborne pathogens, *Escherichia coli* and *Staphylococcus aureus*. Due to the serious health dangers posed by these bacteria worldwide, particularly the emergence of antibiotic-resistant strains, natural alternatives are essential. The extracts were examined at 100 and 200 mg/mL concentrations. Clear concentration-dependent suppression of bacterial growth was demonstrated by the results. The inhibition zones against *E. coli* varied from 9 to 20 mm, with mean zones of 18.67 ± 1.53 mm at 200 mg/mL and 12.00 ± 3.00 mm at 100 mg/mL. The minimal inhibitory concentration (MIC) was 6.67 mg/ml. Inhibition zones for *S. aureus* varied from 6 to 11 mm, with mean zones of 8.00 ± 2.00 mm at 100 mg/mL and 10.00 ± 1.00 mm at 200 mg/mL. The MIC was 10 mg/mL. These findings suggest that antibacterial activity is stronger against Gram-negative *E. coli* than Gram-positive *S. aureus*. Statistical analysis with One-way ANOVA confirmed the significance of these findings. The p-value was less than 0.05 therefore the null hypothesis was rejected. This demonstrates that the antibacterial effects were due to bioactive chemicals rather than chance. While this work reveals the antibacterial activity of *J. curcas* seed extracts, more research is needed to identify whether the reported effects are bacteriostatic, suppressing bacterial growth, or bactericidal, resulting in bacterial death.

CONTENTS

CHAPTER 1

1.0. INTRODUCTION

1.1. Background of the study

E. coli and *S. aureus* are important pathogens with a great impact on the global heterogeneity of health. The *E. coli* is Gram-negative bacterium, which is commonly related to food poisoning, and the *S. aureus* is a Gram-positive bacterium which causes skin infection, pneumonia and other devastating diseases like sepsis. The fact of growing antibiotic-resistant strain is a proof of great necessity of new agents of antimicrobials which could restrain those living organisms. In the food industry, biological contamination which occurs mainly through the growth of molds on the surfaces of foods, in container vessels and equipment in the kitchen is a significant setback.

The *Jatropha curcas* species belonging to Euphorbiaceae family holds the significant possibility of the biosynthesis of bioactive chemical compounds. Long grown in warm climates of America, Africa and Asia, *J. curcas* is commonly known due to its use as a source of biofuel given that the seed kernels contained high quantity of oil (5860%) (Fairless 2007). *J. curcas* contains the phytochemicals flavonoids, tannins and saponins, which bear a correlation to high antimicrobial properties. It is postulated that these compounds undermine cell walls of microbes, interfere with the activity of enzymes, and impair the uptake of nutrients, preventing the growth of bacteria (Dada *et al*. 2014). These bioactive constituents are found across various parts of the plant like leaves, stems, and seeds which further indicates that the extraction of the antimicrobial agents can be done many ways.

Furthermore, it has also been indicated that $AgNO_3$ nanomaterials produced using *J. curcas* leaf extracts, had a higher degree of antimicrobial activity against foodborne pathogens. These nanoparticles have strong inhibition properties to the bacteria such as *E. coli*, and *S. aureus*, and it is noted that there is a synergistic effect with the plant compounds with the silver ions (Kalimuthu *et al.,* 2010). More empirical and theoretical studies are supposed to be undertaken to expound the mode of action of *J. curcas* in completing the effect exerted against the antimicrobial effect. *J. curcas* as a source of antimicrobials in the food safety process might be profitable since it can curb the wastage and food spoilage among the producers and consumers (Rios & Recio, 2005). Also, its antimicrobial production and processing potential of *J. curcas*

would provide new business opportunities in the bio-technology and agricultural sectors (Nwokocha *et al*., 2011).

To conclude, *J. curcas* can be of massive potential as a natural antibacterial component that can kill foodborne infections (Kalimuthu *et al.*, 2010). Addressing such research gaps will introduce innovative all-natural methods of enhancing the national health and food safety.

1.2.Problem Statement

Antibiotic-resistant bacteria pose a health threat to veterinary that weakens the existing antimicrobial practices. This condition demands that research work on other forms of fighting the pathogens be conducted. Although *J. curcas* has a long history in herbal medicine, little scientific evidence is available on its action against clinically relevant bacteria. Shortage of empirical evidence on efficacy of *J. curcas* seeds against *S. aureus* and *E. coli* in particular, is the gap in knowledge which this research is intended to close. The broad-spectrum antibacterial activity of the plant has been demonstrated as the studies reported that J. curcas had an inhibitory effect on both gram-positive and gram-negative bacteria, including *E. coli* and *S. aureus* (Prastiyanto *et al*., 2020). The study investigated antibacterial effect of the *J. curcas* seed ethanolic extract on gram negative bacteria *Escherichia coli* and *Staphylococcus* aureus using Kirby-Bauer disc diffusion technique.

1.3. Significance of the Study

This study has implications for a number of fields, including food safety, public health, and biotechnology. *J. curcas* extracts may be used to fight antibiotic-resistant bacteria. The development of natural antimicrobial agents is essential for tackling public health issues. *J. curcas*-based antimicrobials may be used to treat a variety of infections and diseases. The use of natural antimicrobial agents, such as *J. curcas* extracts, may lessen the need for synthetic antibiotics. The findings of this study may guide the creation of new public health policies.

Additionally, this study helps the biotechnology and agriculture industries financially. The creation of antimicrobials derived from *J. curcas* may open up new markets for producers and farmers. The price of synthetic antibiotics may be lowered by using natural antibacterial agents. The findings of this research may influence creation of new medicine and technology.

1.4. Aim of the study

To evaluate the antibacterial activity of *J. curcas* seeds extracts against *E. coli* and *S. aureus* invitro.

1.5. Objectives

- To determine the efficacy of *J. curcas* seed extracts against *E. coli* and *S. aureus.*
- To compare the efficacy of *J. curcas* seed extracts between *E. coli* and *S. aureus*.
- To evaluate the efficacy of different concentrations of *J. curcas* seed extracts against *E. coli* and *S. aureus.*

1.6. Research questions

- Do *J. curcas* seed extracts have a significant antibacterial effect on *E. coli* and *S. aureus*?
- Is there a statistically significant difference in efficacy of *J. curcas* seed extracts on *E. coli* and *S. aureus*?
- How does the concentration of *J. curcas* seed extracts influence their antimicrobial effectiveness against these bacterial strains?

1.7. Hypotheses

- H_0: *J. curcas* seeds have no effect on *E. coli* and *S. aureus*.
- H_1: The *J. curcas* seed extracts have an effect on *E. coli* and *S. aureus*.

1.8. Limitations of the study

A major limit of this research is that it is confined to in vitro analysis, meaning the antibacterial activity was tested in a controlled laboratory setting rather than in living organisms. While in vitro results are useful for initial screening, they may not accurately reflect real-life effectiveness inside a living system

CHAPTER 2

2.0. LITERATURE REVIEW

2.1. *Jatropha curcas*

The tropical plant *J. curcas* is a member of the Euphorbiaceous family. Because of its phytochemical makeup and possible antimicrobial qualities, it has drawn a lot of interest for its therapeutic and financial worth. This review analyzes the antibacterial activity of *J. curcas* seeds against two pathogens: *E. coli* and *S. aureus*. These bacteria are connected to a number of ailments that include foodborne illnesses, UTIs, as well as skin infections making this research of natural antimicrobial agents like *J. curcas* seeds particularly significant.

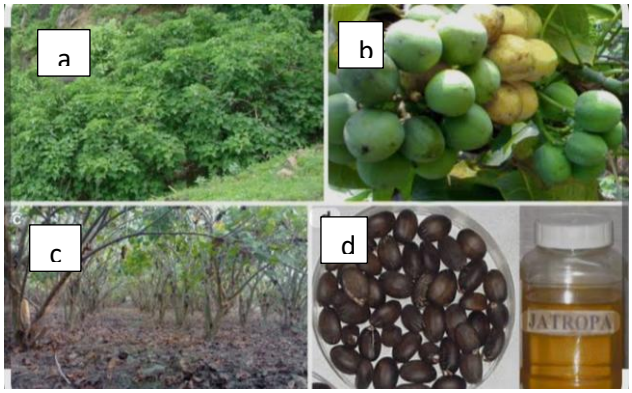

Figure 1: Jatropha curcas (a) shrubs (b) fruit (c) forest (d) dried seeds.

2.2. History of *Jatropha curcas* in Zimbabwe.

Due to its agricultural, medicinal, and bioenergy prospects, *J. curcas*, a drought tolerant shrub, has received importance in Zimbabwe. In Zimbabwe, traditional medicine has utilized the plant and live fence in the rural and per-urban villages because of its toxicity coupled with resistance in the marginal soils (Mujeyi *et al*., 2010).

The interest in biofuels started to peak and spread all over the world in the early 2000s, giving a boost to the *J. curcas* propagation in Zimbabwe. Government and development bodies promoted the *J. curcas* as a source of biodiesel production as a part of rural development and renewable energy strategies (Musiyiwa *et al*., 2014). This has contributed to the wide spread of the plant throughout the country and in particular arid and semi-arid regions such as Masvingo, Midlands and Matabeleland provinces (Chikova *et al.*, 2020). Although, biodiesel mega projects have largely remained at a standstill due to log and financial challenges, the plant continues to be widely cultivated and possess unexploited potential in other sectors.

The recent study of the antimicrobial properties of *J. curcas* conducted in Zimbabwe has also been matched with an increasing interest in plant-based and alternative antimicrobials in the context of increasing antibiotic resistance. Phytochemicals which are present in most plant parts especially the leaves, seeds, and latex include tannins, flavonoids, saponins, and alkaloids that have been demonstrated to possess antibacterial effects (Idu & Ovuakporie-Uvo, 2018). As an example, according to Aboh *et al*. (2014), strong inhibitory effect on the bacteria strains like *S. aureus* and *E. coli* was observed in the leaf extracts of *J. curcas*. In the same way, Ezeigbo *et al*. (2016) confirmed that ethanolic and water-based leaf extracts were effective against common infections in regard to antibacterial effects.

J. curcas has been used to treat various ailments in rural people of Zimbabwe, including wounds, gastrointestinal and skin infections, and thus its antibacterial properties were historically empirically known not only by the rural Zimbabwean people but also by traditional healers there (Mawere, 2011). The latex of the plant is commonly put up on the cuts and other wounds with the effort to accelerate the process of healing and prevent the occurrence of bacterial infections. Although the scientific evidence of such applications is mainly anecdotal, they are slowly being scientifically confirmed, explaining the need to conduct further pharmacological research (Fayemi *et al*., 2020).

The Inherent toxic nature of *J. curcas* especially in the seeds and oil, which harbor phorbol esters, continues to limit its traditional and official usage into the healthcare systems of Zimbabwe, although its potential bioactivity poses such an opportunity (Gubitz *et al*., 1999). However, detoxification and suitable formulation methods can pave the way towards safe antibacterial therapy prepared using the plant.

The potential of *J. curcas* to become a sustainable source of locally available antibacterial chemicals is huge in Zimbabwe. Provided its cultural and historical context as well as the scientific evidence of its bioactive properties, *J. curcas* can be regarded as a necessity in the struggle against microbial resistance and extension of the phytomedicine arsenal in the country.

2.3. Phytochemical Composition of *J. curcas* seeds

J. curcas seeds possess antibacterial properties that are directly associated with their characteristically different chemical composition that consists of diverse bioactive compounds, including flavonoids, tannins, terpenoids, phenolic compounds, and phorbol esters. They are

extensively studied as the potential agents able to suppress the growth of *E. coli* and *S. aureus* and other harmful bacteria (Kumar *et al,* 2015; Sahu *et al,*2017). Flavonoids are also known to be antibacterial and belong to a type of polyphenolic chemical that disrupts the works of cells such as energy metabolism, as well as nucleic acid production and disrupts cell membranes of bacteria (Bhalodia & Gorham, 2011). The *J. curcas* seeds contain predominant compounds, flavonoids, that inhibit the growth of *E. coli* and *S. aureus* (Akinmoladun *et al,* 2019). The other polyphenolic compounds are tannins which have a strong antibacterial effect due to the attachment of the proteins against cell wall of the bacterium, which causes a structural damage and blocks the enzyme activity (Kumar *et al,* 2011). Research has revealed that the *J. curcas* seeds are especially helpful against S. aureus due to their presence of tannins in the seeds (Shukla *et al,* 2021).

Sahu, Mishra, and Pradhan (2017) also state that the antimicrobial effect of *J. curcas* seeds is promoted by terpenoids, which is a diverse and large group of organic substances, rupturing the bacteria cell membranes and leading to cell lysis and death. Prajapati, Patel and Joshi (2020) assert that such substances have been shown to be effective against Gram-positive and Gram-negative bacteria. Bharati and Gorham (2011) assert that phenolic chemicals, which are widely known due to their antibacterial and antioxidant properties, inhibit growth of bacteria by disrupting enzyme activity, as well as breaking the integrity of the cell membrane. The total antibacterial potential of the *J. curcas* plant is enhanced by phenolic chemicals in the seeds (Makkar and Becker 1999). Additionally, phorbol esters, although primarily known for their toxic effects, may also contribute to antimicrobial activity by exhibiting cytotoxic effects on bacterial cells, particularly at higher concentrations (Omar, Abdullah, & Hassan, 2017). However, their toxicity limits their direct therapeutic use without further purification or modification.

The combined action of these bioactive compounds makes *J. curcas* seeds a potent natural antimicrobial agent. Flavonoids and tannins target bacterial cell walls and metabolic processes, while terpenoids and phenolic compounds disrupt membrane integrity and enzymatic functions. Even phorbol esters, despite their toxicity, play a role in the seed antibacterial activity. This multifaceted mechanism of action highlights the potential of *J. curcas* seeds as a natural alternative to conventional antibiotics, particularly in the context of increasing antibiotic resistance. However, further research is needed to fully understand the interactions between these compounds and their potential applications in therapeutic settings.

2.4. Antimicrobial activity against *Escherichia coli*

Gram-negative *E. coli* is an essential point of antimicrobial studies because it is related to food-related diseases and quarantines in the urinary tract. *J. curcas* seeds have been found to have potent inhibitory effects against the growth of *E. coli* with variations in MIC values depending on the concentration of the methanolic extracts mainly due to flavonoid and tannins (Akinmoladun, Olaleye, & Farombi, 2019). The extraction methods matter in terms of antimicrobial activity; hydro-distillation yields extract of higher certified concentrations of the volatile compounds which exhibit better activity as antimicrobial agents against *E. coli* than cold extracted substances (Prajapati, Patel, & Joshi, 2020). The presence of antimicrobial activity of aqueous leaf extracts indicates that different plant parts contain bioactive compounds active against *E. coli* (Mohite, Shah, & Patel, 2018). Extracts of *J. curcas* roots were also shown to be effective because there were *E. coli* clinical isolates that had antibacterial activity using Zeikus Index (ZI) values which showed high activity (Yau, 2011). Multi-mechanisms involve membrane degradation of bacteria, blockage of metabolic enzymes and toxicity to cells. The results of in vitro experiments have proven that the methanolic extracts of defatted seeds suppress *E. coli* at lower concentrations, which may indicate a high level of antimicrobial properties (Haq *et al*., 2016). The observations indicate the possibility of *J. curcas* seed extracts as natural substitutes or supplements to traditional antibiotics to treat *E. coli* infections.

2.5. Antimicrobial activity on *Staphylococcus aureus*

S. aureus is a Gram-positive bacterium which also happens to be the common cause of skin and soft tissue diseases, and it may be exacerbated by drug resistance increasing (Lowy, 1998). Recently, the antibacterial activity of *J. curcas* seed extracts against *S. aureus* have been studied, which showed its potential subjects as adjunct or alternative therapeutic agents. According to Shukla, Pandey and Singh (2021), Hexane extract of *J. curcas* seed exhibited robust antibacterial activities against *S. aureus*. It is postulated that the bacteria cell membranes undergo disruption by the lipophilic chemicals in the hexane extract that lead into cell lysis and inhibition of growth. This membrane-permeant approach will be especially helpful because the occurrence of antibiotic-resistant S. aureus strain is on the rise (Shukla *et al*., 2021).

Moreover, Rahman, Islam and Hossain (2022) also investigated the role of *J. curcas* seed extracts in working together with conventional antibiotics. Based on their finding's obliteration of bacteria *S. aureus*, the co-administration of some antibiotics such as amoxicillin and vancomycin enhanced the activity of antibiotics. This implies that the compounds in *J. curcas*

might act as adjuvants to boost the efficiencies of antibiotics and even reduce the amount required to kill infection (Rahman *et al*., 2022). *J. curcas* seed extracts exposed *S. aureus* and clinical isolates including those known to be methicillin resistant (MRSA) to methanol which exhibited bactericidal activity, which aligns with the findings in a study by Adewole *et al*. (2020). The scientists attributed this activity to phytochemicals such as flavonoids and phenolic acids, which form oxidative stress and inhibit bacterial enzymes indispensable to cell survival (Adewole *et al.*, 2020).

Another research by Zhang *et al*. (2019) found that *J. curcas* seed oil contains diterpenoid chemicals that inhibit biofilm development in *S. aureus*. Biofilms contribute to chronic infections and antibiotic resistance, and the potential of *J. curcas* extracts to suppress biofilm growth presents a substantial therapeutic advantage (Zhang *et al*., 2019). When taken as a whole, these studies demonstrate that *J. curcas* seeds have antimicrobial properties against *S. aureus* through mechanisms including membrane rupture, enhanced antibiotic activity, oxidative stress induction, and biofilm suppression. This plant's diversity of activity highlights its potential as a source of natural antibiotics, particularly in light of the worldwide problem of antibiotic resistance (Rahman *et al*., 2022; Adewole *et al*., 2020).

2.6. Traditional and therapeutic uses of *Jatropha curcas*

Due to its medicinal properties *J. curcas* has played a significant role in the traditional medicine. Kumar, Sharma, and Singh (2011) hold that ailments such as inflammation, skin infections, and arthritis have been treated using the seeds and the leaves. Latex of the plant has been exploited to cure infectious conditions in the mouth of children and also due to its claimed antibacterial and healing properties, the seed oil has been applied topically in wounds and other skin inflammations (Srivastava, Srivastava, & Singh, 2013). Moreover, the seeds also possess chemicals like jatrophone which are effective against diverse microbes due to their antibacterial abilities (Omar, Abdullah, & Hassan, 2017). These are traditional applications in line with scientific studies of the antibacterial properties of the herb.

Table 1: Summary of traditional uses of *Jatropha curcas* seeds

Traditional Use	Plant Part Used	Purpose/Condition Treated	Reference (APA Style)

Treatment of arthritis, skin infections, and inflammation	Seeds and leaves	Anti-inflammatory, antimicrobial	Kumar, A., Sharma, S., & Singh, R. (2011)
Disinfectant for infectious diseases in children's mouths	Latex	Antiseptic/disinfectant	Srivastava, S., Srivastava, R., & Singh, S. (2013)
Topical treatment of wounds and skin irritations	Seed oil	Antibacterial, wound healing	Srivastava, S., Srivastava, R., & Singh, S. (2013)
Antibacterial activity against various microorganisms due to compound jatrophone	Seeds (compound: jatrophone)	Antimicrobial effect	Omar, M. N., Abdullah, N., & Hassan, N. M. (2017)

CHAPTER 3

3.0. MATERIALS AND METHODS

3.1. Study site

This study was carried out at Bindura University Laboratory, located in Bindura, Zimbabwe. The laboratory provided a controlled environment for conducting the experiments. The Jatropha curcas seeds used in the study were sourced from Banket, a local area known for its agricultural activities.

3.2. Sample Preparation and Collection

3.2.1 Seed Preparation

Dried *Jatropha curcas* seeds were collected from Banket and thoroughly cleaned to remove any impurities (Kumar *et al*., 2011). The outer shells were carefully removed to get the kernels. They were ground using motor and pestle into a powder.

Figure 2: Dried *J. curcas* seeds

3.2.2. Extraction Process

The powder was soaked in 100% ethanol (Shukla *et al*., 2021). The mixture was left to soak at room temperature (21–22 °C) for 24 hours. After soaking, the extract was filtered to remove solid residues using filter paper. Ethanol was evaporated using a Bunsen burner, leaving behind the active compounds of the extract. The extract was then prepared in two concentrations: 200mg/ml and 100mg/ml.

Figure 3: *J. curcas* seeds powder soaked in ethanol,

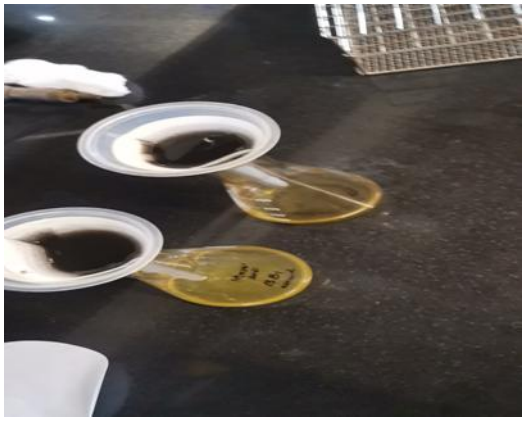

Figure 4: *J. curcas* filtered

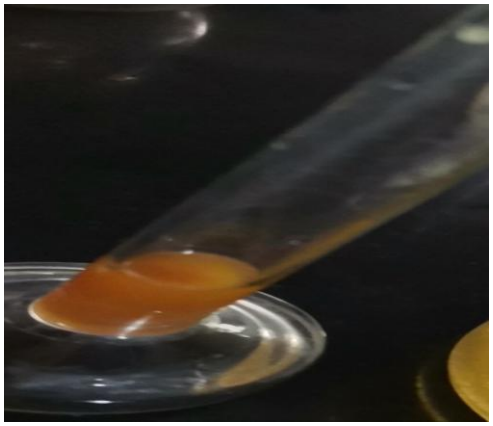

Figure 5: *J. curcas* extract without ethanol

Figure 6: *J. curcas*, 200mg/ml and 100mg/ml extracts.

3.3. Antimicrobial assay

3.3.1. Preparation of Mueller Hinton agar and Nutrient Broth

Mueller Hinton agar and nutrient broth were prepared as per the manufacturer's protocol (Akinmoladun *et al*., 2019). The media was autoclaved at 121 °C for 15 minutes.

3.3.2. Preparation of inoculum

E. coli and *S. aureus* cultures were prepared overnight in nutrient broth (Prajapati *et al*., 2020). The stock cultures were inoculated into individual tubes that had 10 mL of nutrient broth and incubated at 37 °C for 24 hrs. A bacterial concentration of 1 x 10^8 CFU/mL was prepared from the overnight cultures.

3.3.3. Inoculation of bacterial cultures onto Mueller Hinton agar Plates

The standardized cultures were then inoculated onto the prepared agar plates using a sterilized swab for even distribution.

3.3.4. Kirby Bauer disc diffusion assay

Discs of *J. curcas* extract at concentrations of 200 mg/mL and 100 mg/mL, respectively were aseptically placed in Mueller-Hinton agar plates using sterile forceps (Akinmoladun, Olaleye, & Farombi, 2019). Ciprofloxacin, was used as the positive control and water was used as the negative control. The plates were incubated at 37 °C for 24 hrs, and zones of inhibitions were measured in millimeters.

3.4. Statistical analysis

The data was analyzed by R statistical package, a statistical software, One-way ANOVA was used to determine the antimicrobial activity on *E. coli* and *S. aureus*.

3.5. Quantitative Analysis

Quantitative assessment of antimicrobial effectiveness of *J. curcas* seed extract was done through determination of MIC values against two clinically important strains; *E. coli* (Gram-negative) and *S. aureus* (Gram-positive). The MIC is the minimum dosage of an anti-microbial agent at which there is no observable growth of microbes and is also an important measure of potency.

CHAPTER 4

4.0. RESULTS

4.1. Antimicrobial activity of *Jatropha curcas* Extract

The antimicrobial potential of *Jatropha curcas* seed extract was evaluated against *Escherichia coli* and *Staphylococcus aureus*. The findings indicate varying degrees of inhibitory effects, which were concentration dependent.

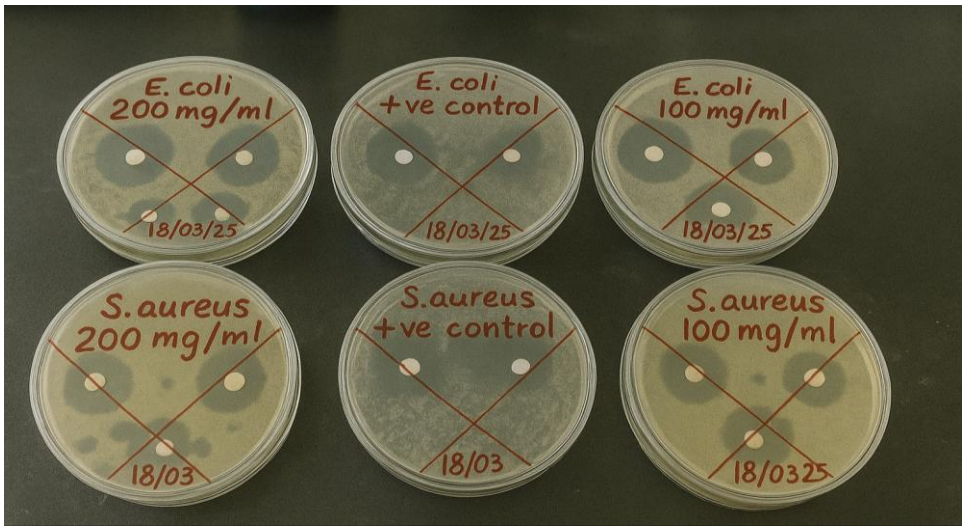

Figure 7: Kirby Bauer disc diffusion assay showing zones of inhibition.

Figure 7 presents the visual outcomes of the disc diffusion assay, illustrating the zones of inhibitions of *J. curcas* extract at concentrations of 100 and 200 mg/mL against *E. coli* and *S. aureus*. Distinct zones of inhibition are visible around the discs containing the extract, confirming the presence of bioactive compounds with antimicrobial properties.

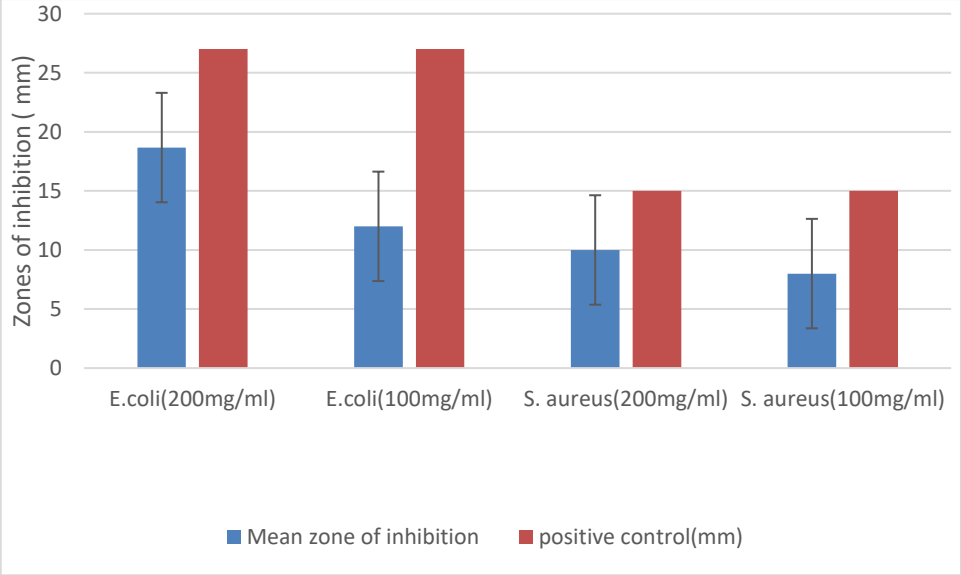

Figure 8: Graphical presentation of the antimicrobial effect of *J. curcas* against *E. coli* and *S. aureus*

The results demonstrate that the extract possess antimicrobial properties, with inhibition zones for *E. coli* ranging from 9–20 mm. The highest mean inhibition zone (18.67 ± 1.53 mm) was observed at 200 mg/ml, while the lowest (12.00 ± 3.00 mm), was recorded at 100 mg/ml. Positive control was 27mm. The extract also showed proved effective on *S. aureus*, with ZOI values ranging from 6–11 mm. The highest mean zone of inhibition (ZOI) (10.00 ± 1.00 mm) was observed at 200 mg/ml, while the lowest (8.00 ± 2.00 mm) was recorded at 100 mg/ml. The positive control of *S. aureus* (15mm).

4.2. Determining the MIC of *J. curcas* seed extract

In case of *E. coli*, minimum concentration of seed extract observed, accompanies inhibitory activity of 100 mg/mL used in the experiment with ZOI observed to be 15mm, 9mm and 12mm across replicates. On the basis of maximum values of ZOI at this concentration, MIC was determined to be 6.67 mg/mL. This MIC value indicates that *J. curcas* seed extract is especially effective against *E. coli*, thus the good antibacterial potential.

In the case of *S. aureus*, the extract also showed an activity at the same concentration minimum 100 mg/mL with ZOI of 10mm, 6mm and 8mm. The minimum inhibitory concentration of this organism was found to be 10 mg/mL that showed a relatively low level of susceptibility as compared to *E. coli*. In spite of it being an effective extract against *S. aureus*, increased MIC means a higher level is needed to exert the same degree of inhibition which will assume lower efficacy against Gram positive bacteria.

4.3. Statistical analysis

The significance of the occurrence of antibacterial activity was determined as one-way ANOVA. The difference between the groups was significantly high with an F of 20.99765 that is a much higher value than F critical of 4.256495. P-value (0.0004084) was much smaller than the standard value (0.05), and this indicates that the differences identified are unlikely to occur. So, the null hypothesis was dropped.

CHAPTER 5

5.0. DISCUSSION

The research proposed the identification of antibacterial activity of *J. curcas* seed extracts against two bacteria of medical importance that comprise *S. aureus* (Gram-positive bacteria) and *E. coli* (Gram-negative bacteria). All outcomes showed the observed inhibitory effect of the two bacteria in a concentration-dependent manner as *E. coli* was more susceptible to the inhibition process as compared to *S. aureus*. The highest inhibition of *E. coli* was 18.67 +/- 1.53 mm in 200 mg/ml extracting concentration and the zones of inhibition varied between 9 and 20 mm. Conversely the inhibition zones of *S. aureus* were narrower than that of *E.coli* at corresponding dose with its highest of 10.00 +/- 1.00 mm and a range of 6 to 11 mm. These findings help explain the high antibacterial bioactivity of the *J. curcas* seed extracts and that they fit the best-known pharmacological principle of dose-response where the more the concentration of the bio factors, the greater the effectiveness of the antimicrobial activity is attained until its saturation point (Kumar, Rai, & Mehta, 2019).

It is noteworthy that *E. coli* being Gram negative have been more tolerant because of the presence of an outer covering membrane given that it was susceptible to it. The antimicrobial activity of *J. curcas* against *E. coli* can be explained by the presence of phytochemicals that disrupt the outer membrane of Gram-negative bacteria. The phytochemicals present in *J. curcas* seeds are phenolic compounds, alkaloids, flavonoids, and tannins, since they are characterized as disruptors of membranes (Haq *et al*., 2016; Shukla, Pandey, & Singh, 2021). Silhavy, Kahne, and Walker (2010) have explained an amphiphilic nature of such compounds that allows them to penetrate outer membrane of Gram-negative bacteria. The coating layer of peptidoglycan caused by the presence of Gram-positive bacteria however, might be seen as acting like a barrier on the diffusion of these bioactive substances hence failing to penetrate and form smaller colonies of inhibition.

Moreover, effects of extraction techniques having serious implications on antibacterial action are noticed. Prajapati, Patel, and Joshi (2020) disclosed that hydro-distillation was found to be more effective in the activity against *E. coli* due to the increase in the volatile phytochemicals. It implies that the extraction parameters, which are preconditioned by time and temperature influence the concentration and release active chemicals. Similarly, with other phytochemical studies, we extracted the extract in our research study by soaking the extract in the 24-hour period (Akinmoladun, Olaleye, & Farombi, 2019; Haq *et al*., 2016). However, the antibacterial

activity can be increased by massive magnitude with prolonged incubation e.g., 72 hours) because of the consequent increase in the phytochemical solubility and yield (Rahman, Islam, and Hossain, 2020). It implies that the prolonged soaking of the extraction of *J. curcas* might enhance their antibacterial activity.

These results can be confirmed by other pieces of research since other research revealed that elements of *J. curcas* have antibacterial effects on various arrangements of it. Khan *et al*. (2021), have contended that the antibacterial activity of the methanol extract, ethanol extract, acetone extract and the water extract of the roots, stems, leaves, flowers and the seeds of *D. siphonophorum* against *E. coli*, *Klebsiella pneumoniae*, and *Agrobacterium tumefaciens* also varied. A report by Khan *et al*. (2021) noticed that the substances of the root such as extracts or roots water provided the best performance (35.52 3.53 mm) in a bigger diameter in respect of inhibitory activity as compared to the seed extracts that are less active (16 .41 mm). The findings indicate that the solvents polarity and the type of plant components utilized play a significant role on maximum antibacterial activity.

J. curcas leaves containing lipophilic substances also had antibacterial potential, which was also known to be high using hexane as the extractant, to the extent that they were more effective than antibiotics in certain parts many times (Ojo *et al*., 2025). N-hexane extract of *J. curcas* leaves up to 250 mg/ml. Moreover, Rahman, Islam and Hossain (2022) also established that *J.* curcas seeds extracts and common medicine such as amoxicillin. The findings indicate that *J. curcas* extracts have potential in minimizing resistance and enhancing the efficacy of commercially available antibiotics as well as help deal with drug resistance bacteria. All the above can be achieved by the use of combination therapy involving antibiotics and plant extracts in reducing adverse effects, delaying the development of resistance and reducing the amount of antibiotics required.

Based on MIC data, the antibacterial activity of *J. curcas* seed extracts points to potential uses in the creation of natural antimicrobial agents. Plant-based substitutes like *J. curcas* provide multi-targeted modes of action through a variety of phytochemicals, lowering the risk of resistance formation in light of the global increase in antibiotic resistance (Haq *et al.,* 2016; Akinmoladun, Olaleye, & Farombi, 2019). Also, the moderate inhibitory zones in comparison to common antibiotics such as ciprofloxacin suggest that *J. curcas* extracts would work better as topical or adjunctive treatments than as stand-alone systemic antibiotics.

Khan *et al.* (2021) argue that evidence exists regarding the insufficiency of extraction optimization in the case of varying antibacterial activities that a range of plant constituents and extraction solvents may display. Optimal extraction conditions should be attained by selecting the correct solvent polarity, the temperature of extraction, and the duration of extraction to maximize production of some specific bioactive chemicals. Complete plant extracts, or combination of these should be studied in future research, because water and ethanol extracts of roots and flowers in many cases have been shown to have stronger antibacterial effects than those done on seeds. Toxicological studies are necessary prior to clinical use. Although antibacterial effects of *J. curcas* extracts are promising, additional studies are required to determine the full safety profile, cytotoxicity, bioavailability, and the long-term effect of extracts of the plant (Azmir *et al*., 2013). This indicates the prospect of *J. curcas* as a natural antibacterial especially against resistant infections to most medications.

Apart from the seed extracts, *J. curcas* leaf and root extract had shown to exhibit broad-spectrum antibacterial action, that is, bioactivity chemicals of antibacterial action are not restricted to a particular part of the plant (Mohite, Shah, & Patel, 2018; Yau, 2011). A wide spread of action is supplemented by a set of phytochemicals, including flavonoids, saponins, alkaloids, and tannins (Haq *et al*., 2016).

In this study the MIC values of *E. coli* and *S. aureus* were 6.67 mg/ml and 10 mg/ml respectively. These MICs also confirm quantitatively the nature of the differential susceptibility exhibited in each of these cases in the inhibition zones testing since they indicate that *E. coli* will require a lower concentration of the extract to repress the growth. According to MIC values, *J. curcas* phytochemicals will disrupt cellular bacterial processes with greater success Gram-negative and likely due to characteristics of the bioactive constituent used and permeability through the membrane.

Haq *et al*. (2016) explain that the extracts of the seed of *J. curcas* in defatted methanol demonstrated strong antimicrobial effects that elevated with the concentration. This is in compliance with this case of the antibacterial activity that is dependent on concentration. Similar conclusions were made by Akinmoladun, Olaleye, and Farombi (2019), and the authors of the research described the different inhibitory activity of the different concentrations of methanolic extract against *E. coli* by flavonoids and tannins. These data are significant to point at the need to adjust the concentration of the extract to choose the least antibacterial activity and keep in mind its potential cytotoxicity.

Other parameters, besides their concentration, that influence the values of MIC are extraction parameters such as: pH, temperature, ionic strength, or a combination of the above parameters. The amount of secondary metabolite like alkaloids, flavonoids, and phenolic compounds also increased significantly with regard to increased soaking periods and directly related with antibacterial property according to Iqbal *et al*. (2018) (Shukla, Pandey, & Singh, 2021; Haq *et al*., 2016). Another allied method can be used to increase the potency of *J. curcas* extract as Rahman, Islam, and Hossain (2020) showed that when *Azadirachta indica* seeds were soaked in water for 72 hrs, there was a promotion of its antibacterial activity because of improved solubility of phytochemicals in water.

The Soxhlet extraction, UAE, MAE and SFE are some of the advanced extraction techniques that have been demonstrated to be efficient in phytochemical recovery because of higher extraction and protection of the heat sensitive compounds (Azmir *et al*., 2013; Bimakr *et al*., 2011). *J. curcas* could be subjected to these methods and even better antibacterial activity may render the MIC values to be reduced. One of the extraction techniques which promotes the amount of bioactive chemicals which could have more superior antibacterial activity and smaller MICs is ultrasound-assisted extraction that also promotes better solvent solubility and penetration.

Moreover, Rahman, Islam, and Hossain (2022) identified that the seed extracts of *J. curcas* in combination with the commonest mediations such as amoxicillin and vancomycin was effective particularly on the resistant strains of *S. aureus*. These findings indicate the prospect of *J. curcas* extracts as resistance regulators capable of enhancing the performance of antibiotics available in the market, and helping curb drug-resistant bacteria. The combination of antibiotics and plant extracts can reduce unfavorable consequences, delay the development of resistance, and reduce required antibiotic Dose.

According to MIC, antibacterial properties of *J. curcas* seed extracts show its possible applications in developing natural antimicrobials agents. Multi-targeted modes of action of phytochemicals found in plant-based alternatives such as *J. curcas* reduces the chances of resistance development in the context of growing antibiotic resistance globally (Haq *et al*., 2016; Akinmoladun, Olaleye, & Farombi, 2019). The moderate inhibitory zones by comparison to regular antibiotics like ciprofloxacin also indicate that *J. curcas* extracts would be better topical or adjunctive therapies in the case systemic antibiotics but not as monotherapies.

Khan *et al*. (2021) believes that there is an important necessity to optimize extraction processes because antibacterial activity among different components of various plants and extraction solvents varies. In order to maximize specific bioactive chemical production, proper solvent polarity, and extraction temperature and time should be selected. Whole-plant extracts or mixtures of them also should be studied in further work, water and ethanol extracts of roots or flowers (example), often showing greater antibacterial influence than seed extracts. Toxicological tests are important before clinical use. Although the antibacterial activity of *J. curcas* extracts is promising, during additional studies, it is necessary to comprehend the safety of their use, as well as the dependence of their cytotoxicity, bioavailability, and chronic action (Azmir *et al.*, 2013). These two aspects of effectiveness and safety are achievable through determining the window of treatment and minimum bactericidal concentrations (MBCs).

Moreover, the addition of *J. curcas* extracts to pharmaceutical and cosmetic products, e.g., natural preservatives, wound healing formulas, can provide alternative, long-term replacements of synthetic antibiotics. In addition to antibacterial properties, *J. curcas* could also exhibit an antifungal property against microorganisms like *Aspergillus niger* and *Penicillium notatum* (Khan *et al.*, 2021).

CHAPTER 6

6.0. CONCLUSIONS AND RECOMENDATION

6.1. Conclusion

In this research work, the in vitro extracts of *J. curcas* seeds were evaluated as possible antibacterial agents against two foodborne pathogens, *E. coli* and *S. aureus*. The finding was that the seed extracts of *J. curcas* possess potent antibacterial properties and can well demonstrate clear concentration-dependent inhibitory effect against both bacteria. As compared to *S. aureus*, the extract had enhanced activity against *E. coli* with larger inhibitory areas and reduced minimum inhibition concentration (MIC). These results are in favor of the presence of bioactive phytochemicals such as flavonoids, tannins, terpenoids, and phenolic compounds. Such compounds are likely to contribute to the antibacterial effect in various ways which might include destabilization of bacterial cell walls and hampering their metabolism.

The potential of the plants-derived extracts of *J. curcas* use in the place of the common antibiotics is great as far as they seem to be equal as a natural replacement (or alternatively) to traditional medicines. To enhance the quantity and activity of bioactive chemicals, the use of optimized parameters of extraction, like the time of soaks and extractant used, was also an important focus of the inquiry.

Additional studies that include in vivo research, research on specific active compounds and formulation development are suggested so that all the therapeutic benefit of *J. curcas* can be realized and effective, safe, and sustainable antimicrobial products can be developed.

6.2. Recommendations

1. Expand Concentration Range: Future studies should test additional concentrations (e.g., 50, 150, 250 mg/ml) for a more comprehensive dose-response analysis.
2. Expand soaking time of *J. curcas* seeds in ethanol: Future studies should test various soaking durations to identify the optimal time for maximum efficacy.
3. Phytochemical Profiling: Further research should isolate and characterize the active compounds in *Jatropha curcas* seeds.

4. Mechanistic Studies: Investigating the exact mode of action (e.g., cell membrane disruption, enzyme inhibition) would enhance understanding of its antimicrobial potential.

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APPENDICES

Appendix A: Taxonomical classification of *J. curcas*

Kingdom: Plantae

Phylum: Streptophyta

Class: Equisetopsida

Subclass: Magnoliidae

Order: Malpighiales

Family: Eutrophorbiaceae

Genus: *Jatropha*

Species: *Jatropha curcas*

Appendix B: One-Way ANOVA statistical analysis of the antimicrobial efficacy of *J. curcas* seeds against *E. coli* and *S. aureus*

Summary

Groups	*Count*	*Sum*	*Average*	*Variance*
Treatment(mg/ml)	4	600	150	3333.333
Mean zone of inhibition(mm)	4	48.67	12.1675	21.45889
Positive control(mm)	4	84	21	48

ANOVA results

Source of variation	*SS*	*Df*	*MS*	*F*	*P-value*	*Fcrit*
Between Groups	47622.41	2	23811.21	20.99265	0.000408	4.256495
Within Groups	10208.38	9	1134.264			
Total	57830.79	11				

Appendix C: Determining the MIC for *E. coli*

MIC= (Lowest concentration exhibiting inhibition zones) / (Highest inhibition zone at that concentration)

MIC= 100mg/ml / 15mm

=6.67mg/ml

Appendix D: Determining the MIC for *S. aureus*

MIC= (Lowest concentration exhibiting inhibition zones) / (Highest inhibition zone at that concentration)

MIC= 100mg/ml / 10mm

= 10mg/ml