# ANTIMICROBIAL EFFECTS OF METHANOLIC EXTRACT OF BROWN MUSTARD SEED ON SELECTED CLINICAL ISOLATE

**BY;**

**AKELE, SOPHIA**

**(BMS2001148)**


**DEPARTMENT OF MEDICAL LABORATORY SCIENCE,**

**SCHOOL OF BASIC MEDICAL SCIENCES,**

**COLLEGE OF MEDICAL SCIENCES,**

**UNIVERSITY OF BENIN,**

**BENIN CITY.**

**SEPTEMBER, 2025.**

# ANTIMICROBIAL EFFECTS OF METHANOLIC EXTRACT OF BROWN MUSTARD SEED ON SELECTED CLINICAL ISOLATE

**BY;**

**AKELE, SOPHIA**

**(BMS2001148)**

**SUPERVISED BY;**

**DR (MRS). N.A. OLISE**

**DEPARTMENT OF MEDICAL LABORATORY SCIENCE,**

**SCHOOL OF BASIC MEDICAL SCIENCES,**

**COLLEGE OF MEDICAL SCIENCES,**

**UNIVERSITY OF BENIN,**

**BENIN CITY.**

**SEPTEMBER, 2025.**

## CERTIFICATION

This to certify that this project work was carried out by AKELE, SOPHIA with matriculation number BMS2001148 in partial fulfillment of the requirements for the award of Bachelor of Medical laboratory science (BMLS) from the University of Benin, Benin city, Edo state, Nigeria.

_____ _____

**DR. (MRS) N.A. OLISE.** **DATE**

**(SUPERVISOR)**

_____ _____

**DR. (MRS) Z. OMORUYI.** **DATE**

**(HEAD OF DEPARTMENT)**

_____ _____

**EXTERNAL EXAMINER** **DATE**

## DEDICATION

This work is dedicated to Almighty God for His divine guidance and strength throughout the course of this project. It is also lovingly dedicated to my parents and my dear Uncle, Dr. (Mr) Sunday Eyaru, for their unwavering love and support.

## ACKNOWLEDGEMENTS

I sincerely thank Almighty God for His guidance, wisdom, and strength throughout the completion of this project. I acknowledge the professional supervision given to me by my supervisor, Dr. (Mrs) N.A. Olise during the period of the work. Special gratitude goes to the Head of Department, Dr. (Mrs) Z. Omoruyi for creating a conducive environment for learning. To all my Lecturers it was indeed a privilege to have learnt under your supervision. I sincerely appreciate the school of medical sciences for the approval to carry out this research work. I appreciate Mr John Uwadia, who guided me during this research work in the laboratory. I want to specially appreciate my Parents, Dr .(Mr) Sunday Eyaru, Late Evangelist Barrister Edna Adewoye, my siblings, Mr Bode Akande, Stanley Eyaru, Prophetess Mary Osadolor, Kenneth Osamudiamen , Samuel Etinosa and colleagues for their support during this period.

## TABLE OF CONTENTS


| | |
|---|---|
| **COVER PAGE** | i |
| TITLE PAGE | ii |
| CERTIFICATION. | iii |
| DEDICATION. | iv |
| ACKNOWLEDGEMENTS. | v |
| TABLE OF CONTENTS. | vi |
| LIST OF TABLES. | ix |
| LIST OF FIGURES | x |
| ABSTRACT | x |
| CHAPTER ONE | 1 |
| 1.0 INTRODUCTION | 1 |
| 1.1 Background of Study | 1 |
| 1.2 Statement of the problem | 2 |
| 1.3 Significance of study | 3 |
| 1.4 Aim of Study | 4 |
| 1.5 Objectives of study | 4 |
| 1.6 Research Questions | 4 |
| 1.7 Research Hypothesis | 5 |
| CHAPTER TWO | 6 |
| 2.0 LITERATURE REVIEW | 6 |
| 2.1 Brown mustard seed | 6 |
| 2.2 Overview of Antimicrobial Resistance | 13 |
| 2.3 Antimicrobial properties of *Brassica juncea* | 13 |

2.4 Methodologies for accessing antimicrobial activity 14

2.5 Clinical and therapeutic implications 15

2.6. Safety considerations and side effects 15

2.7 Challenges in translating Laboratory Findings to clinical practice 16

2.8. Extraction Techniques for *Brassica juncea* seeds 16

2.9 Mechanisms of Antimicrobial action 17

2.10 Pseudomonas Species 18

2.11 Escherichia coli 21

2.12. Staphylococcus aureus 22

2.13. Candida app. 23

CHAPTER THREE 29

3.0. METHODOLOGY 29

3.1 Study Area 29

3.2 Materials for phytochemical screening 30

3.3 Materials for Antimicrobial Studies 31

3.4 Methods 31

3.5 Antimicrobial Assay 32

3.6 Phytochemical screening 35

CHAPTER FOUR 37

RESULTS 37

4.1 RESULTS 37

4.2 percent yield of methanolic extract and properties 39

4.3 Zone of inhibition of Methanolic extract of Brassica juncea seeds 41

4.4 Zone of Inhibition of clinical Isolates at different extract concentration 45

4.5 Minimum Inhibitory Concentration 45

4.6 Minimum fungicidal concentration 46

4.7 Phytochemical constituents of methanolic extract of Brassica juncea seeds 48

CHAPTER FIVE

5.1 DISCUSSION 52

5.2 CONCLUSION 57

5.3 RECOMMENDATIONS 61

REFERENCES 62

APPENDIX I 71

## LIST OF TABLES

Table 4.1: Physical properties and percent yield of Brassica juncea seeds 32

Table 4.2: Zone of inhibition of Methanolic Extract of Brassica juncea seeds 40

Table 4.3: Zone of inhibition of clinical Isolates at different Extract concentrations 43

Table 4.4: Minimum inhibitory concentration (MIC) of methanolic Extract of Brassica juncea seeds 45

Table 4.5: Minimum bactericidal concentration of Brassica juncea seeds 47

Table 4.6: Minimum fungicidal concentration (MFC) of methanolic Extract of Brassica juncea seeds 49

Table 4.7: Phytochemical constituents of the Methanolic extract Brassica juncea seeds 51

## LIST OF FIGURES

Figure 2.1: Images of *Brassica juncea* seeds 8

## ABSTRACT

Antimicrobial resistance poses a major threat to global health, necessitating the exploration of alternative therapeutic options from natural sources. This study evaluated the antimicrobial activity of the methanolic extract of brown mustard seed against selected clinical isolates. The antimicrobial activity of the extract was tested at concentrations ranging from 50–600 mg/ml using agar well diffusion assay. The Minimum Inhibitory Concentration (MIC), minimum bactericidal concentration (MBC), and Minimum Fungicidal Concentration (MFC) were determined using broth dilution and subculture techniques. Phytochemical screening was conducted to identify the bioactive constituents of the extract. Data were analyzed using SPSS version XX, with chi-square tests applied to assess associations between extract concentrations and microbial growth responses. The results revealed that *Staphylococcus aureus* recorded the highest mean zone of inhibition (17.50 ± 2.49 mm), followed by *Candida albicans* (8.67 ± 4.26 mm), while *Escherichia coli* (6.50 ± 2.95 mm) and *Pseudomonas aeruginosa* (6.00 ± 4.10 mm) showed lower susceptibility. Post hoc analysis indicated concentration-dependent inhibition in *Candida albicans* and *Escherichia coli*, while inhibition of *Pseudomonas aeruginosa* was observed only at the highest concentration. MIC values were established at 100 mg for *Staphylococcus aureus*, 400 mg for *Escherichia coli* and *P. aeruginosa*, and 600 mg for *Candida . albicans*. MBC values confirmed bactericidal activity at 100 mg for *Staphylococcus aureus* and 600 mg for *Escherichia coli* and *Pseudomonas aeruginosa*, whereas the MFC for *Candida albicans* was 600 mg. Phytochemical analysis revealed the presence of alkaloids, flavonoids, terpenes, steroids, tannins, phenols, and resins, with saponins absent. These findings demonstrate that brown mustard seed possesses broad-spectrum antimicrobial properties, with *S. aureus* being the most sensitive organism, supporting its potential as a source of plant-based antimicrobial agents.

# CHAPTER ONE

# INTRODUCTION

## 1.1. Background of Study

Since the development and spread of drug-resistant bacteria with novel resistance mechanisms continue to impede the effective treatment of communicable illnesses, antimicrobial resistance (AMR) is a global issue (Tyrrell *et al*., 2024). With 338 genera and over 37000 species, the Brassicaceae family also known as crucifers stands out as one of the most widely grown and consumed globally. The Brassica (B.) genus is the most well-known and significant of all the genera in the Brassicaceae family (Ayadi *et al*., 2022). With its distinct fiery and spicy flavor, Brassica juncea seeds are a member of the Cruciferae (Brassicaceae) family. They include a variety of chemical ingredients, including protein, lipids, reducing and non-reducing sugars, flavonoids, phenolics, saponins, tannins, and terpenoids (Aziz *et al*.,2020). Glucosinolates are an organo-sulfur chemical found in mustard plants (Myrosinase thio-glucoside hydrolase) enzymes hydrolyze this chemical to form allyl isothiocyanate, which gives mustard its strong, pungent flavor, Gut microorganisms can also hydrolyze the glucosinolates. Along with glucobrassicin and sinalbin, sinigrin is the primary glucosinate present in mustard. Approximately 0.8–2.3% of the mixture is made up of isothiocyanates, including allyl isothiocyanate (Adegbeye *et al*.,2018)

Mustard bioactive chemicals' antimicrobial properties can be divided into two categories: phenolic (Sinapic) and non-phenolic (allyl isothiocyanate). The glucosinolates and isothiocyanate concentration of extracts from yellow (Sinapic alba L.) and brown (Brassica juncea Linn.) mustard seeds have been linked to their antimicrobial qualities (Adegbeye *et al*., 2018). The decision for this study was influenced by the hundreds of years that mustard has been

used as medication. It is extensively used to make a variety of pickles, pastries, and edible sauces. One of the biggest risks to the effective treatment of infectious diseases is the steadily rising number of newly discovered multidrug-resistant bacteria strains. The indiscriminate use of broad spectrum antibiotics is the primary cause of this surge. One of the most widely used mustard species in the cruciferae family is Brassica juncea, sometimes known as brown mustard. According to the World Health Organization (WHO), over 80% of people worldwide rely mostly on traditional medicine, which mostly uses plants.

### 1.2 Statement of Problem

Antimicrobial resistance (AMR) is a major worldwide hazard to the health of people, animals and the environment. Due to the fact that multidrug-resistant bacteria, or "Superbug," have become more prevalent and persistent (Aslam *et al*.,2018). Development of a new and effective antimicrobial agent is a top priority because the World Health Organization (WHO) has ranked antimicrobial resistance (AMR) as one of the top 10 life-threatening global health issues that require multilevel attention and international cooperation to achieve the Sustainable Development Goals (SDGs) (Arip *et al*., 2022).

Overuse of antibiotics has resulted in microorganisms that are resistant. Food residues can encourage superbugs and disturb gut flora. Food safety issues arise from the presence of biofilm-forming microorganisms on the packaging of contaminated food (Li *et al*.,2023). There is a lot of interest in this type of alternative therapy since medicinal plants contain a range of secondary metabolites some of which have shown antibacterial and antifungal properties in vitro (Tyrell *et al*., 2024). Although initial data points to brown mustard seed's potential as an antibacterial, thorough research examining its efficacy against clinical isolates of bacteria and fungi is lacking.

Information provided is essential for investigating reasonably priced, plant-based treatment options, especially in areas with high loads of antibiotic resistance. antibacterial and antifungal activity of brown mustard seed extract against selected clinical isolates of bacteria and fungi involves the main reason for carrying out this study

### 1.3 Significance of Studies

Provision of information regarding the antibacterial qualities of Brassica juncea (brown mustard seed) extract, especially against selected clinical isolates of bacteria and fungi, Will prompt an increasing amount of research into plant-based antimicrobials as potential substitute supplements to traditional medications. assessing brown mustard seed extract's effectiveness against multidrug-resistant organisms (MDROs), thereby helpful in the search for non-synthetic treatment alternatives that could be able to lessen the emergence of additional pathogen resistance. Current emphasis on natural resources given, emphasizes that the use of natural antibacterial agents is receiving a lot of attention and growing in importance (Saladino *et al*., 2017). Traditional medicine has been in use of mustard seed for ages. provision of scientific validation by this study of these traditional practices, which could enhance the credibility and integration of traditional remedies into modern healthcare systems. Hospital and community-acquired infections have given a high burden in many regions, especially in developing countries, discovery of effective natural antimicrobials like brown mustard seed could significantly improve infection control, reduce health costs, and save lives. provision of a basis for future research into the phytochemical composition will be provided by these studies, mechanism of action, toxicity levels, and formulation of Brassica juncea-based antimicrobial products.

## 1.4. Aim of Study

To determine the antimicrobial effect of methanolic extract of brown mustard seed on selected clinical isolates.

## 1.5. Objectives Of Study

The Specific objectives of the study are ;

1. To investigate the antimicrobial activity of Brassica juncea (Brown mustard seed) extract against selected bacterial and fungal clinical isolates.

2. To evaluate the in vitro antimicrobial activity of the brown Mustard seed extract against the selected bacterial and fungal clinical isolates.

3. To determine the minimum inhibitory concentration (MIC) and minimum bactericidal or fungicidal concentration (MBC/ MFC) of the extract on the test organism.

## 1.6. Research Question

1. Does the crude extract of *Brassica juncea* (brown mustard seed) exhibit antimicrobial activity against the selected bacterial and fungal clinical isolates?

2. What are the minimum inhibitory concentrations (MIC) and minimum bactericidal/fungicidal concentrations (MBC/MFC) of the mustard seed extract on the test organisms?

3. How does the antimicrobial activity of brown mustard seed extract compare with that of conventional antibiotics and antifungal agents?

4. Can Brassica juncea be considered a potential natural alternative for the treatment of infections caused by drug-resistant bacteria and fungi?

## 1.7. Research Hypothesis

### 1.7.1 Null Hypotheses ($H_0$ ):

*Brassica juncea* (brown mustard seed) extract has no significant bacterial activity against the selected bacterial clinical isolates.

*Brassica juncea* extract has no significant antifungal activity against the selected fungal clinical isolates.

### 1.7.2 Alternative Hypotheses ($H_1$ ):

*Brassica juncea* (brown mustard seed) extract exhibits significant antimicrobial activity against the selected bacterial clinical isolates.

*Brassica juncea* extract exhibits significant antifungal activity against the selected fungal clinical isolates.

# CHAPTER TWO

# LITERATURE REVIEW

## 2.1 Brown Mustard Seed Plant

### 2.1.1 Botanical Description of *Brassica juncea* seeds

An annual herb with fleshy taproots that is 30 to 100 cm tall and often pubescent but occasionally glaucous. upright and branching stems. Basal and cauline leaves with ovate to lanceolate blades that are deeply lobed or pinnatifid. The margins of the upper leaves are whole or slightly serrated, and they are thinner. Yellow flowers with ovate to obovate petals and four oblong sepals. Fruits are 3–5 cm long, linear, slightly inclined, and have a conical tip. There are six to fifteen seeds in each fruit valve. The tiny, spherical, brown to gray seeds have a delicate texture (Vélez, 2022a)

### 2.1.2 Taxonomy

The Taxonomy of *Brassica juncea* is as follows:

Domain: Eukaryota

Kingdom: Plantae

Phylum: Spermatophyta

Subphylum: Angiospermae

Class: Dicotyledonae

Order: Capparidales

Family: Brassicaceae

Genus: *Brassica*

Species*: Brassica juncea* (Vélez, 2022a)

**Figure 2.1 Images of *Brassica juncea* seeds ( Vélez, 2022a)**

### 2.1.3 Distribution of Brown Mustard Seed

In continents like Asia, Europe, Africa, America, and Australia, Brassica juncea can be found as native plants, adapted crops, and imported weeds. Notwithstanding, Brassica juncea is not just a significant oilseed crop in China, India, Bangladesh, and Ukraine, Brassica juncea has recently gained popularity in Canada and Australia. In the meantime, China, Argentina, North America, and Europe grow it as a condiment (Kang *et al*., 2021)

### 2.1.4 Phytochemical Composition of Brown Mustard Seed

The phytochemical composition of *Brassica juncea* includes Oil (28–42%), protein (25–40%), carbohydrates (15–35%), fiber (10–15%), minerals (5–10%), and plant secondary metabolites (up to 10%), such as glucosinolates, phenolic compounds, and tannins, make up the majority of mustard seeds (Rahman *et al*., 2024). It also contains electrolytes (Na and K), vitamins C and K, and a variety of trace minerals (Ca, Fe, Zn, Se, Cu, Mn, and Mg) (Tian and Deng, 2020).

Examination of Proximate study revealed that the percentages of total carbohydrates, moisture, ash, and dietary fiber in the mustard seeds were 17.53%, 8.60%, 5.30%, and 5.87%, respectively (Gök *et al*., 2020).

**Mineral content**: Brassica juncea contains a high percentage of nitrogen-rich protein (20–45%) and important nutrients such as copper (10 mg/kg of total dry matter), manganese (100 mg/kg of total dry matter), zinc (100 mg/kg of total dry matter), phosphorus (1%), potassium (1%), calcium (1%), magnesium (0.5%), and sulfur (0.5 to 2%) (Ayadi *et al*., 2022).

**Content of Glucosinolates**: There have been over 90 different glucosinolates identified and these are classified into three sub groups Olefinic (Sinigrin), methylsufinylalkyl (glucoraphanin)

and aryl ( gluconasturtiin) of which the main glucosinolates of B juncea is Sinigrin ( Cools and Terry, 2018). The Phenolic content of the primary chemicals present in mustard seeds includes, ferulic, 4-hydroxybenzoic, and protocatechuic acids, sinapic acid was the most prevalent phenolic ingredient, with quantities ranging from 44 to 82 times higher (Nicácio *et al*., 2020).

**Flavonoids and their Glycosides:**

A very rich source of structurally different flavonoids and glycosides is Brassica spps. Quercetin and kaempferol are present as complex glycosides in this species (Neugart and Bumke-vogt, 2021).

### 2.1.5 Pharmacological Activity of *Brassica juncea*

**Anticancer Activity**: A leading cause of death is malignant neoplasms of which among them we have lung cancer related death and research shows that the chances of developing cancer can be reduced by lifestyle changes and dietary habits, Through the intake of vegetables which can help reduce the risk of cancer (W. Li *et al*., 2019)

The main phenolic mustard seed ( Brassica juncea) is sinapine, A choline ester of Sinapic acid.It accounts for about 70%–90% of the total phenolic compounds in the seed together with small amounts of sinapic acid .The anticarcinogenic and anti-inflammatory properties of sinapic acid and its derivatives are due to its ability to inactivate NF-κB, thus, preventing the expression of proinflammatory mediators including nitric oxide synthase, cyclooxygenase-2, tumor necrosis factor-α, and interleukin-1β (Nguyen *et al*., 2023)

**Anti-inflammatory Activity:** The secondary metabolites found in Brassicas that can be associated with these therapeutic effects are phenolic compounds, especially flavonoids, with

quercetin and kaempferol being the predominant flavonoids in kale. Anti-inflammatory properties of Brassicaceae have been attributed to an inhibitory effect on inflammatory cell infiltration, delay of pro-inflammatory gene expression, and reduction in the biosynthesis of cytokines such as TNF, IL-1, IL-6 (Da Silva Mattosinhos *et al*., 2022). The mustard extract has also been shown to be a successful anti-inflammatory agent. B. juncea 50% ethanol extract has been demonstrated to lessen both acute inflammations (12-o-tetradecanoylphorbol-acetate (TPA) generated mouse ear edema and arachidonic acid (AA) produced mouse ear edema) and chronic inflammation (many applications of croton oil (CO) induced) in mice (Singh *et al*., 2022).

**Anti- bacterial activity**: Mustard essential oil (MEO) obtained from mustard (*brassica* spp.) seeds mainly contains allyl isothiocyanate (AITC). (Goli *et al*.,2023)

Glucosinolates are a class of abundant secondary metabolites characteristic of the plants of the mustard family (Brassicaceae). Glucosinolate hydrolysis products are potent inhibitors of bacterial activity. It has been reported that Brassicaceae juncea (Indian mustard) contains significant amounts of sinigrin. Sinigrin is not usually antimicrobial; when it is enzymatically hydrolyzed to form allyl isothiocyanate it exhibited potent antimicrobial activity against food spoilage and pathogenic organisms (Mazumder *et al*., 2016)

**Antioxidant Activity**: Brassica plants are known to possess anti- oxidant properties due to the presence of anti-oxidants phytochemicals, mainly the polyphenols, flavonoids and ascorbic acid. Most of these phytochemical compounds act as antioxidants due to their hydrogen donating and reducing abilities. Flavonoids possess metal ion chelating and free radical scavenging potential (Nawaz *et al*., 2018).

**Antifungal - activity**: Mustard seed extracts are also recommended to fight against candidiasis, a fungal infection caused by an overgrowth of any species of the genus Candida yeast. Mustard seed extracts are also given for the treatment of other topical fungal infection (Rahman *et al*., 2024)

### 2.1.6 Nutritional and Medicinal Importance of *Brassica juncea*

Mustard plants have been widely cultivated and used as spice, medicine and as source of edible oils. Currently, the use of the seeds of the mustard species, Brassica juncea (brown mustard) in the food and beverage industry is immensely growing due to their nutritional and functional properties (Lietzow, 2021). Due to its chemical components, mainly glucosinolates, sterols, or newly discovered phenols, seeds could be widely employed in numerous types of tumor, non-genetic diabetes, hyperglycemia, hyperlipidemia, hypercholesterolemia, etc (Szőllősi, 2020). *Brassica juncea* (L.) Czern, commonly referred to as Indian mustard is a major oilseed crop in South Asia, Oil extracted from B. juncea using expeller/kachi ghani has always been favored as cooking oil in India due to its interesting chemical properties (S. Sharma *et al*., 2022). Nutraceutical products like food supplements can be formulated by extracting these compounds which possess desirable properties (Poyil *et al*., 2023)

### 2.1.7. Economic Importance of Brassica juncea seeds

*Brassica juncea* is the primary source of canola oil due to its high oil content (38–42%). Traditionally, mustard oil is the major source of cooking oil in the Indian subcontinent (Rai *et al*., 2022). Among all the crops cultivated, mustard occupies a very important position due to its dual purpose: as an essential oilseed crop and as a source of income for countless farmers as a (Haque *et al*., 2025)

## 2.2 Overview of Antimicrobial Resistance

The history of bacterial infectious diseases can be divided in three eras: the pre-antibiotic era, the antibiotics era and the antibiotic resistance emergence era. The emergence of resistance in the current world created a risky, but necessary situation regarding the patient treatment, because when facing a bacterial infection the most powerful antibiotics classes are preserved as the last resource in therapy, although bacteria already possess mechanisms of making these classes ineffective (Balsalobre *et al*., 2014). The introduction of antimicrobial resistance has increased the impact of infectious disease (Reygaert, 2018). The occurrence of antimicrobial resistance may be due to some factors such as drug modification and active efflux (Saleemi *et al.*, 2023). They are the valuable resources of the natural antimicrobial compounds used to treat the infectious disease caused by bacteria and other pathogens World Health Organization (WHO) stated that the medicinal plants are the best source to obtain the variety of drugs (Mayekar *et al*., 2021)

## 2.3 Antimicrobial Properties of *Brassica juncea*

The unique features of Brassicaceae family plants conferred by their phytochemicals, have extended future prospects about their use for beneficial effects on human nutrition and health worldwide. (Favela- González *et al*., 2020) Glucosinolates have only been found in dicotyledonous plants occurring mainly in the Capparales order including cruciferous vegetables and the mustards *Brassica juncea* (brown mustard) Glucosinolates have proven useful in the prevention of biofilm development by *Pseudomonas aeruginosa.* (Melrose, 2019)

## 2.4 Methodologies For Accessing Antimicrobial Activity

### Agar Diffusion Based Method Screening of Antimicrobial Activity

Assays such as disk diffusion, well diffusion, agar plug, and agar spot are widely utilized and cost-effective techniques for Agar based assay in antimicrobial research for determining the antimicrobial activity of test compounds. The method mentioned early relies on the diffusion of antimicrobial agents from paper discs, wells, or plugs into the adjacent agar medium, thereby inhibiting the growth of the test microorganism inoculated on the agar surface. Measurements of the zone of inhibition which represents the area where microbial growth is prevented by the agent is measured, determination of the relative potency of the test compound against the specific microorganism is carried out by the researcher. A valuable tool for providing qualitative data on the effectiveness of different substances against specific microorganisms that can be implemented using agar diffusion Assay (Hossain, 2024). However, The advantages of disk-diffusion assay over many other methods includes, low cost, Simplicity, ability to test enormous numbers of microorganisms and antimicrobial agents, and ease results interpretation (Balouiri *et al*., 2015)

### Broth Dilution Method

In agar, challenges regarding hydrophobic compound dilution can be checked by this method in a well precise way. This method involves the addition of the tested compound to the culture medium where bacteria are grown; a sample is taken out every 10–20 min and diluted before being plated onto the agar, where colonies are counted after 24–48 h. variation in this method consists of using microtiter plates. Bacteria growth can be measured spectrophotometrically

through optical density (600 nm) or via the use of cell viability indicators such as resazurin and methylthiazoldiphenyltetrazolium (MTT) (Gonzalez-Pastor *et al*., 2023)

**Minimum Inhibitory Concentration (MIC) Assays:**

Measurement of minimum inhibitory concentration can be carried out using Agar or broth dilution methods under defined test conditions, concentration with the lowest effective value of an antimicrobial agent that inhibits visible growth of a bacterium of interest. susceptibilities of bacterial isolates and of novel antimicrobial drugs can be examined with this method and nutrient-rich laboratory media that have little relevance to in vivo conditions are required to carry out this experiment. (Belanger and Hancock, 2021).

**2.5 Clinical and Therapeutic Implications**

Brassica juncea is known as Indian mustard and has been used for centuries for its nutritional and medicinal importance. However, most of the traditional uses are centered on the seed and oil. The seed has been used for the treatment of ailments such as arthritis, foot pain, cancer, vomiting, dengue, and rheumatism. (Sahu *et al*., 2020)

2.6 **Safety Considerations and Side Effects**

Mustard seeds are widely used in foods due to its sensory attributes, nutritional values, and its numerous functional properties. The intake of mustard products and foods formulated with mustard is expected to rise in future and may contribute considerably to the exposure to several biologically active compounds. Nowadays, research is basically focusing on the potential beneficial effects of the multifunctional mustard plant and its ingredients, however reliable data

on potentially toxic compounds and possible health risks should likewise be considered (Lietzow, 2021).

### 2.7. Challenges In Translating Laboratory Findings To Clinical Practices

Several recent studies have detailed the discovery of promising antimicrobial compounds, but these molecules rarely advance to clinical use. For many of these molecules, we do not have a clear understanding of their targets, which significantly hinders them from proceeding in the development pipeline and gaining regulatory approval (Hudson and Lockless, 2022). New antimicrobials with distinct working mechanisms are required to combat bacteria that have become resistant to all known antimicrobials. In addition, difficulties in the determination of the value of a new compound is caused by the lack of insight into antimicrobial mechanisms of action (MOA). Identification of novel antimicrobials can be enhanced by some understanding of the Moa early in the discovery process (Ouyang *et al.*, 2022.)

### 2.8 Extraction Techniques for *Brassica juncea* seeds

The chemical constituents from the polar extract of Brassica juncea can be confirmed with GC - MS analysis A (Sharma *et al*., 2018). Indications of the polar nature of the antioxidant biomolecules in the free radical scavenging activity of various plant sources are reported to be better than dichloromethane or ethyl acetate extract (Dua *et al*., 2014). Water and Methanolic extract of Brassica species extracts possess potential antibacterial activity (Parma *et al*., 2021). The Observation of Antibacterial activity was done in all the extracts of B. juncea leaves. Methanol and Ethanol extracts of Brassica juncea gave comparatively much appreciable results as compared to the aqueous extract of Brassica juncea (Verma *et al*., 2022). Defattation of Indian mustard seeds was carried out by distillation with hexane and the residue extracted with

methanol was analyzed for potential antioxidants; ascorbate, riboflavin, and polyphenols. polyphenols is a significant content in methanol extract of defatted seeds which accounts for high antioxidant potential (Dua *et al*., 2014).

### 2.8.1 Comparison of Solvents Used in Preevious Studies

The three previously used method in the extraction of glucosinolates from brassicaceae tissues, namely extraction in cold methanol, extraction in boiling methanol, and extraction in boiling water were compared across tissue type (root, stem leaf) and four brassicaceae species (B. juncea, S. alba, R. sativus, and E. sativa). Cold methanol extraction was shown to perform as well or better than all other tested methods for extraction of glucosinolates with the exception of glucoraphasatin in R. sativus shoots (Doheny-Adams *et al*., 2017).

## 2.9 Mechanisms of Antimicrobial Action

Antimicrobial peptides (AMP) play a role in antimicrobial action by inhibition of cell wall synthesis, protein synthesis, Nucleic acid synthesis, protein folding, cell division and proteases (Le *et al*.,2017).

### 2.9.1 Disruption of Microbial Celll Membrane

Peptides undergo change in conformation and aggregation state in the presence of membranes (Bechinger and Gorr, 2016). Bacteria cells are killed by either disrupting their membrane or by entering inside the cells to interact with intracellular components by the (AMP) (Benfield and Henriques,2020). The cationic AMPs exert antibacterial activity by interacting with negatively charged bacterial membranes to increase membrane permeability and lead to cell membrane lysis and cell content release (Zhang *et al*., 2021). Host defense peptides can kill bacteria directly, by

either targeting a broad spectrum of bacteria, or by being selective for Gram-positive or Gram-negative bacteria (Raheem and Straus, 2019). AMPs (bacitracin and vancomycin) can selectively bind to lipid II, a cell wall synthesis precursor molecule, and inhibit the synthesis of the cell wall (Luo and Song, 2021)

### 2.9.2 Enzyme Inhibition

Successful removal of complex biofilms requires the use of multi-enzyme formulations that contain enzymes capable of degrading microbial DNA, polysaccharides, proteins and quorum-sensing molecules. The inclusion of anti-quorum sensing enzymes prevents biofilm reformation (Thallinger *et al.*, 2013). Fluoroquinolones show their action by inhibiting the replication and transcription of bacterial DNA that is responsible for proper functioning of the cell. Uncoiling of the DNA into a single-stranded structure during DNA replication is through the enzymes called DNA gyrase or DNA topoisomerase. The enzyme DNA gyrase consists of two A subunits (gyrA) and two B subunits (gyrB). The establishment of a negative super-helical twist in the bacterial DNA is by DNA gyrase. Quinolones and fluoroquinolones inhibit this enzyme by binding to the A subunit of the enzyme due to which the bacteria are unable to replicate or even synthesize proteins (Brar *et al*., 2020).

## 2.10 Pseudomonas Species

### 2.10.1 Overview of Pseudomonas

Pseudomonas are motile, non-spore forming, Gram-negative rods belonging to the Gammaproteobacteria. Pseudomonas species are able to colonize and thrive in a wide range of ecological niches (e.g., soil, water, and plants, associated with higher organisms). In addition to

the well-known human pathogen *Pseudomonas aeruginosa*, other Pseudomonas species induce diseases in plants, fish, insects, or other animals (Girard *et al*., 2021). *Pseudomonas aeruginosa*, a Gram-negative nosocomial pathogen is the main causative organism responsible for life-threatening and persistent infections in individuals affected with cystic fibrosis and other lung ailments (Vetrivel *et al*., 2021). Pseudomonas is an immensely diverse genus showing a great variety of metabolic abilities. The taxonomy of the group has undergone much revision over recent years and more than 200 species have been described (Dodd, 2014).

### 2.10.2 Pathogenicity of Pseudomonas

*Pseudomonas aeruginosa* has the ability to infect not only humans, but also animals and plants (De Sousa *et al*., 2021). It is a major cause of hospital-acquired infections (HAIs) in patients (Azam and Khan, 2018). It can grow rapidly on the surface of the food and form oxidized products and mucous substances. P. aeruginosa, one of the leading foodborne pathogens, causes important concerns in food safety due to being a source of contamination, causing food poisoning and antimicrobial resistance in animals (Urgancı *et al*., 2022). The complex structure of the P. aeruginosa biofilm contributes an additional factor to the pathogenicity of this microorganism, leading to therapeutic failure (Tuon *et al*., 2022). Pseudomonas aeruginosa causes several disease symptoms to plants such as wet rot and curved leaves (Marei, 2020). Pseudomonas aeruginosa is able to secrete proteins, most of these secreted proteins play a role in the pathogenesis of bacterial infections (Cianciotto and White, 2017). Pseudomonas aeruginosa possesses a large arsenal of virulence systems contributing to the success of its infection and colonization. Among them, we can cite quorum sensing (QS) and type 2, 3, and 6 secretion systems (T2SS, T3SS, and T6SS respectively) with their secreted proteins and toxins. QS is an intercellular bacterial communication system dependent on cell density, which allows individual

cells to act as a community. This system would control around 10% of the P. aeruginosa genome, including survival genes, genes coding for virulence factors and biofilm formation (Savage and Hardouin, 2020).

### 2.10.3 Isolation of Pseudomonas Species

Pseudomonas spp. are aerobic. Some of the species show distinguishable colony morphologies or pigmentation (i.e., the blue-green derivative of phenazine, pyocyanin, and the yellow-green fluorescing pigments) (Arslan *et al*., 2011). Pseudomonas spp. identification was performed by gram stain, oxidase and catalase test, sugar fermentation in Triple Sugar Iron agar (TSI) and Oxidation-Fermentation (OF) tubes (Ghane and Azimi, 2014). Detection of Pseudomonas aeruginosa colonization is normally achieved by culture of wound swabbing on to artificial media. Typical isolation media for wound infections include blood agar and chocolate agar as well as selective agars such as MacConkey agar and cetrimide-based media, other species of Pseudomonas, including Stenotrophomonas maltophilia, Achromobacter xylosoxidans and Ralstonia pickettii have been shown to grow on cetrimide-based media (Al-Ahmadi and Roodsari, 2016).

### 2.10.4. Antimicrobial Resistance of Pseudomonas

The bacteria get colonized on any surface that contains water and multiply rapidly, carry out all the metabolic functions for growth and development which is an association of complex matrix known as a biofilm. Due to the complex biofilm forming ability, Pseudomonas species shows a great resistivity to various classes of antibiotics which are used to persistently overcome the microbial infection (Mohanty *et al*., 2020). A biofilm is a structure formed by a bacterial community aggregated by components of the extracellular matrix secreted by the cells that

compose it. The biofilm presents a protective environment for cells against abiotic stresses, immune system, and antibiotic action, which makes it difficult to eliminate the infection (Lorusso *et al*.,2022). Antimicrobial resistance (AMR) in *Pseudomonas aeuroginosa* is usually the result of combination of different imported (mobile genetic elements) and chromosomally encoded resistance mechanisms. Imported resistance to the β-lactams involves the production of inactivating β-lactamases, i.e. extended-spectrum β-lactamases (ESBLs) and metallo-β-lactamases (MBLs). (Bajpai *et al*., 2019). What nevertheless makes P. aeruginosa uniquely problematic is a combination of the following: the species' inherent resistance to many drug classes; its ability to acquire resistance, via mutations, to all relevant treatments; its high and increasing rates of resistance locally; and its frequent role in serious infections (Livermore, 2002). *Pseudomonas aeruginosa* meets a large history of multi-resistance acquisition, against most of the last generation antibiotics. In addition to a basis of natural resistance conferred by low outer-membrane permeability, inducible beta-lactamases or efflux pumps, members of this species can acquire practically all the known mechanisms of antimicrobial resistance (Vaz-Moreira *et al*., 2012).

### 2.11 *Escherichia coli*

#### 2.11.1 Overview of *Escherichia coli*

*E. coli*, a member of the bacterial family of Enterobacteriaceae, is the most prevalent commensal inhabitant of the gastrointestinal tracts of humans and warm-blooded animals, as well as one of the most important pathogens (Allocati *et al*.,2013). It is traditionally classified as a facultative anaerobic, Gram-negative bacillus belonging to the genus Escherichia in the family Enterobacteriaceae (Riley, 2020). At least six different categories of pathogenic E. coli causing

enteric infections have been identified and further characterized (Torres, 2010). These strains of *Escherichia coli* includes; Enteropathogenic Escherichia coli (EPEC), Enterohaemorrhagic Escherichia coli (EHEC) Enteroaggregative Escherichia coli (EAEC), Entero-invasive Escherichia coli (EIEC), Enterotoxigenic *Escherichia coli* (ETEC) (Hassan *et al*., 2021). since E. coli, with some exceptions, generally does not survive outside of the intestinal act, its presence in environmental samples, food, or water usually indicates recent faecal contamination or poor sanitation practices in food-processing facilities (Odonkor and Ampofo, 2013)

### 2.11.2 Pathogenicity of *Escherichia coli*

Many Escherichia coli strains are harmless, and they are an important commensal in the intestinal microflora; however, pathogenic strains also exist (Smith and Fratamico, 2017). This harmless commensal organism can acquire a mixture of comprehensive mobile genetic elements that contain genes encoding virulence factors, becoming an emerging human pathogen capable of causing a broad spectrum of intestinal and extraintestinal diseases (Pakbin *et al*., 2021). Pathogenic E. coli can cause a broad range of human diseases that span from the gastrointestinal tract to extraintestinal sites such as the urinary tract, bloodstream, and central nervous system (Croxen *et al*., 2013). Urinary tract infections (UTIs), enteric infections, and systemic infections in humans are majorly caused by Escherichia coli. systemic infections caused by E coli includes bacteremia, nosocomial pneumonia, cholecystitis, cholangitis, peritonitis, cellulitis, osteomyelitis, and infectious arthritis (Pitout, 2012). The classification of pathogenic E. coli: E. coli that produce Shiga toxins (STEC), enterohemorrhagic E. coli (EHEC), enteropathogenic E. coli (EPEC), enteroaggregative E. coli (EAEC), and diffusely adherent E. coli (DAEC), Shigella/entero-invasive E. coli (EIEC), enterotoxigenic E. coli (ETEC) and adherent-invasive E. coli (AIEC) (Nasrollahian *et al*., 2024)

### 2.11.3 Isolation of *Escherichia coli*

A solid sample requires a sample suspension which was made at any concentration for example 5% in normal saline and inoculated into MacConkey agar followed by 18-24hrs incubation at 37° c (Lupindu, 2017). Colonial morphology and colors on MacConkey agar and Eosin methylene blue agar plates together with the Gram-stain procedure were used as an initial identification of E. coli colonies (Gugsa *et al*., 2022). pinkish color appearance on MacConkey agar and metallic sheen on EMB gave a clue to suspect E coli and were then sub-cultured onto blood agar to appreciate colony characteristics (Geletu *et al*., 2022). enrichment of sample suspension is done through 24 h incubation at 37°C in nondifferential broth such as Muller-Hinton or nutrient broth. These all process will bring about multiplication of *Escherichia coli* and increase the chance of *Escherichia coli* isolation especially when rare strains, such as pathogenic ones are the target (Lupindu, 2017)

### 2.11.4 Antimicrobial resistance of *Escherichia coli*

A predominant pathogen responsible for both community and hospital acquired Urinary tract infections is *Escherichia coli (E coli).* Antimicrobial resistance in Euro-pathogenic E. coli has become a serious concern. High resistance rates to commonly used antibiotics, including βeta lactams, cotrimoxazole, fluoroquinolones, and aminoglycosides have been reported in recent studies (Niranjan and Malini, 2014). *E. coli* also represents a major reservoir of resistance genes that may be responsible for treatment failures in both human and veterinary medicine, Through transmission of virulent and/or resistant *E. coli* between animals and humans through numerous pathways, such as direct contact, contact with animal excretions, or via the food chain (poirel *et al.,* 2018). Antimicrobial resistance in E. coli, especially ESBL- and carbapenem-resistant

strains, complicates sepsis treatment by limiting effective empirical choices and often necessitating last-resort drug combinations (Ma *et al*., 2017)

## 2.12 *Staphylococcus aureus*

### 2.12.1 Overview of *Staphylococcus aureus*

*Staphylococcus aureus* is a versatile Gram-positive coccus, a facultative aero-anaerobic bacterium that is both a commensal organism and an opportunistic pathogen (Touaitia *et al*., 2025). It is a major pathogen in humans and animals that is capable of causing a wide variety of illnesses ranging from skin and soft tissue infections to life-threatening invasive diseases (Chessa *et al*., 2015). *Staphylococcus aureus* is a coagulase-positive pathogen belonging to the family Staphylococcaceae with a spherical shape that forms grape-like clusters (Gherardi, 2023). It measures about 0.5 to 1.5 µm in diameter, It is a non-motile, non-spore forming, oxidase-negative, hemolytic, catalase-positive, and coagulase-positive bacteria (Pal *et al*., 2020)

### 2.12.2 Pathogenicity of *Staphylococcus aureus*

A normal flora found on the skin and mucosa is *Staphylococcus aureus*, this Organisms has the ability to take advantages of opportunities to cause opportunities infections which ranges from superficial to invasive infections such as skin infections, bacteremia, pneumonia, etc (Girma, 2024).

Infections such as impetigo, folliculitis, and cutaneous abscesses are Skin and soft tissue infections that are often caused by *Staphylococcus aureus* in healthy people in the community,

(Zainulabdeen and Dakl, 2021). Formation of proteases and the production of an array of exotoxins by *Staphylococcus aureus* helps to aid their ability to colonize and cause disease in humans and animals. Primarily, exotoxins produced by S. aureus fall into three major categories which includes; superantigen toxins, membrane-damaged toxins (MDTs), and exfoliative toxins (ETs) (Zhu *et al*., 2023). skin infections, trauma wounds, urinary tract infections, gastrointestinal tract infections, endocarditis, pneumonia ETC. it also includes infections caused by *Staphylococcus aureus* (Nair *et al*., 2014).

### 2.12.3 Isolation of *Staphylococcus aureus*

Gram stain smears of *Staphylococcus aureus* when stained with the Gram staining techniques show typical colonies of Gram-positive Cocci irregular grape-like clusters. After streaking of the organism onto mannitol salt agar (MSA) and incubation at 37°c, changes in growth and Ph (Red to yellow) which is a confirmatory identification was observed. It is catalase-positive and a facultative anaerobe (Yimana and Tesfaye, 2022)

### 2.12.4 Antimicrobial resistance of *Staphylococcus aureus*

The antibiotic-resistant (methicillin resistant) variant of S. aureus i.e. MRSA is typically resistant to beta-lactam drugs like penicillin (methicillin and oxacillin) and cephalosporin, By generating β-lactamase and changing the binding pocket for cell wall production, MRSA typically overcomes the effects of beta-lactams (Alghamdi *et al*., 2023). development of resistance of *Staphylococcus aureus* to antimicrobial agents by different means, such as horizontal gene transfer of different mobile genetic elements (MGEs), including bacteriophages, plasmids, Staphylococcus cassette chromosomes (SCCs), transposons, and pathogenicity islands (PAIs). It possesses many virulence factors, including toxins, superantigens, and exoproteins that

are cell wall-associated (Rasheed and Hussein, 2021). The phosphate binding protein is encoded by the mecA gene which may have an altered binding capacity or new (PBP2') has a low affinity for beta lactam agents (Watkins *et al*., 2019). The emergence of antibiotic resistance in *Staphylococcus aureus* has been attributed to the alteration of drug binding sites on molecular targets, and the increased expression of efflux pumps (Tălăpan *et al*., 2023). Acquisition of Enterococci vanA gene may lead to development of VRSA (Vancomycin resistant Staphylococcus aureus) from enterococci resulting in serious invasive infections and SSSTIs becoming untreatable by *vancomycin* (Foster, 2017).

## 2.13 Candida Species

### 2.13.1 Overview of *Candida*

Candida is a non-pigmented, non-encapsulated, aerobic or facultative anaerobic and single-cell thallus organisms which reproduce asexually by budding spores (Tamo, 2020). *Candida albicans* is the most common fungal human pathogen causing diseases ranging from superficial mucosal to life-threatening systemic infections (Tsui *et al*., 2016). *Candida albicans* is considered the most common opportunistic pathogenic fungus in humans and a causative agent of 60% of mucosal infections and 40% of candidemia cases (Henriques and Silva, 2021). However other species includes Candida krusei, Candida lusitaniae, Candida kefyr, Yarrowia (Candida) lypolitica, Candida rugosa, Candida glabrata, Candida auris, Candida krusei and Candida lusitanian (Colombo *et al*., 2017).

### 2.13.2. Pathogenicity of *Candida spp*

The most frequent fungal disease affecting populations in the world is candidiasis (Vázquez-González *et al*., 2013). There are several types of candidasis such as Oral candidiasis, Vulvovaginal candidiasis, Cutaneous candidiasis and Systemic candidiasis (Al-Garawi *et al*., 2022). The pathogenicity of Candida species is mediated by a number of virulence factors, including adherence and biofilm formation on host tissue as well as medical devices, the ability to evade host defences and the production of tissue-damaging hydrolytic enzymes (e.g proteases, phospholipases and haemolysins) (Silva *et al*., 2011). Candidemia is another infection due to Candida species and it is among the major bloodstream infections disease that often occurs in Immunodeficiency individuals. Such as people with immune system disease and malignancies and those receiving radiotherapy, chemotherapy and corticosteroids therapy (C. Zhang *et al*., 2024).

### 2.13.3 Isolation of *Candida* Species

*Candida albicans* is a species that presents degree of flexibility being able to Sense its surroundings and adapt to changing microenvironments within the host, Each of these local microenvironments represents a unique combination of physio chemical and biological factors ( i.e temperature, water, gasses, metabolites and ion concentration (Kasdosh and Lopez-Ribot, 2013). There are newer molecular techniques available which targets highly conserved specie specific sequence in the abundant rRNA of living *Candida albicans* which includes fluorescence in situ hybridization with peptide nucleic acid method (PNA-FISH) (Raju and Rajappa, 2011).

### 2.13.4 Antimicrobial Resistance of *Candida albicans*

There are currently 5 structural classes of antifungal drugs being used to treat infections—polyenes, azoles, allylamines, pyrimidines, and echinocandins (Fernandes *et al*., 2020). The most commonly prescribed antifungal used for most *Candida albicans* infections is fluconazole, a member of the azole class of antifungals Whaley *et al*., 2017). Azoles inhibit ERG11 - encoded protein 14a- Lanosterol demethylase, The production of ergosterol normally required for membrane integrity is halted. The mechanism of azole resistance in Candida species includes the up- regulation of drug transporters, over expression or alteration of the drug targets (ERG11) and mutations in the ERG11 gene resulting in decreased azole inhibition (Nishimoto *et al*., 2019). Echinocandins disrupt cell wall biogenesis. This class of fungicidal drugs comprises micafungin, anidulafungin and Caspofungin, the most widely used echinocandin. These lipopeptides kill fungal pathogens via inhibition of the biosynthesis of an essential component of the fungal cell wall, β (1,3)-glucan. The subunits of the integral plasma membrane protein 1,3-glucan synthase Fks1p or Fks2p are the target of specific non-competitive inhibition by echinocandins (Kołaczkowska and Kołaczkowski, 2016)

# CHAPTER THREE

## 3.0 METHODOLOGY

### 3.1 Study Area

This experiment was conducted in the Department of Pharmaceutical Microbiology, Faculty of Pharmacy, University of Benin, Benin City, Edo State, Nigeria.

### 3.2 Materials for Phytochemical Screening

#### 3.2.1. Apparatus and Equipment

Electronic Weighing Balance (e.g., Mettler Toledo, Switzerland), Heating mantle, water bath, cellulose thimbles, General Laboratory Glassware (Beakers, Conical Flasks, Measuring Cylinders), Test Tubes, and Test Tube Racks Spatulas, Refrigerator Weighing balance

#### 3.2.2 Glassware

Stirrers, beakers, conical flasks, round-bottom flasks, and separating funnels

#### 3.2.3. Chemicals and Reagents

The solvent used was methanol, and it was of analytical grade. Reagents for Phytochemical Screening includes Wagner's Reagent (Iodine in Potassium Iodide), Mayer's Reagent, Ferric Chloride ($FeCl_3$ ) solution, Concentrated Sulphuric Acid ($H_2$ $SO_4$ ), Dilute Hydrochloric Acid (HCl), Chloroform, Magnesium Turnings, Distilled Water

### 3.3 Materials for Antimicrobial Studies

#### 3.3.1 Apparatus and Equipment

Portable autoclave, weighing balance, Hot air oven, mortar, pestle, micropipette, incubator, Whatman filter paper, Cotton wool, Sterile pipette tip, Sterile Cork borer (10 mm diameter), transparent millimeter rule, grease pencil, Sterile swab sticks, sterile Petri dishes Tripod stand, sterile inoculating wire loop Bunsen burner, foil paper, Dried plant seed.

#### 3.3.2 Glassware

Conical flasks, bottles (MacCartney, universal, and Bijou), as well as test tubes, pipettes, glass stirrers, porcelain dishes, pestles, maceration jars, glass funnels, beakers, measuring cylinders, and Petri dishes.

#### 3.3.3 Chemicals and Reagents

Tween 80, distilled water. Disinfectant: Purit, soap, detergent.

Positive Control (Standard) Drugs:

Standard Antibiotic: Ciprofloxacin powder

Standard Antifungal: Fluconazole powder

3.3.4 Microbiological media

Nutrient agar, nutrient broth, Sabouraud dextrose agar, Mueller-Hinton agar (MHA), Sabouraud Dextrose Agar (SDA) powder, Mueller-Hinton broth (MHB) powder, and dextrose broth (SDB) powder.

### 3.3.5 Clinical Isolates

Clinical isolates include Candida albicans, Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa

## 3.4 Methods

### 3.4.1 Collection and Identification

The seeds of Brassica juncea were bought from the supermarket in Benin, Edo State, Nigeria, during the month of July, 2025. Care was taken to select healthy seeds free from disease, insect damage, or physical blemishes. The identity of the plant was formally confirmed by Prof. Akinnibosun Henry Adewale (FLS, MRSB; London) of the Faculty of Life Sciences, Department of Plant Biology and Biotechnology, University of Benin, Edo State. A voucher specimen was prepared, assigned the voucher number UBH-B577, and deposited for future reference and verification.

### 3.4.2 Preparation of Methanolic Extract

The seeds were then spread out in thin layers, protected from direct sunlight, and allowed to air-dry at room temperature (25 ± 2°C) for a period of four days. The seeds were then dried in a thermostat-controlled hot air oven at 60°C for 30 mins to render them brittle. It was then reduced to a uniform, coarse powder with the aid of a mortar and pestle. The resulting powder was stored in a cool, dark, and dry place until needed for extraction. The powdered seed material was extracted using the maceration method for the extraction with methanol as the solvent of choice. 556.06 g of the dried seed was soaked in methanol for maceration. 1 L of methanol was used for the maceration.

### 3.4.3 Preparation of Extraction Yield

The percentage yield of each extract was calculated to determine the efficiency of the extraction carried out using methanol as a solvent. The yield is calculated using the formula:

Percentage Yield = (Weight of the dried extract / Weight of the initial powdered plant material) x 100

## 3.5 Antimicrobial Assay

### 3.5.1 Specimen Collection

Microorganisms used in this study were selected from bacterial isolates obtained from the University of Benin Teaching Hospital (UBTH), Benin City, Edo State, Nigeria. They are Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, and Candida albicans.

### 3.5.2 Preparation of Test Organisms

All test bacterial isolates were maintained in 20% glycerol broth and frozen. Prior to use, test microorganisms were sub-cultured from stock into sterile nutrient agar plates and were incubated overnight at 37°C, while the fungal isolates were maintained at room temperature before use. After incubation, colonies from the overnight plates were suspended in sterile broth for 12 hours and adjusted to 0.5 McFarland standard to give an inoculum size of approximately 10^7 CFU/ml.

### 3.5.3 Preparation of McFarland Solution

A 0.5 McFarland standard solution is prepared by adding 0.5 ml of 1.175% (weight/volume). Barium chloride dihydrate salt ($BaCl_2 .2H_2 O$) to 9.95 ml of 1% sulfuric acid ($H_2 SO_4$ ). The

two solutions are mixed completely to form a turbid suspension in a test tube, which is then placed in a test tube rack and kept at room temperature before use.

### 3.5.4 Antimicrobial Susceptibility Tests

An antimicrobial susceptibility test was carried out using the ditch plate method. A total of 4 different bacterial isolates were used; sterile Muller-Hinton agar was prepared, and 30 ml was poured into each petri dish and allowed to solidify. The same method was carried out on Sabouraud Dextrose agar for the fungal isolates. The surface of both media was dried in a hot air oven at 40°C for about 5 minutes to remove excess moisture. A ditch of 10 mm wide and 50 mm long was created in the center of the agar plate using a sterile scalpel blade, and the base of the ditch was sealed with drops of molten agar. A concentration of 800 mg/ml of the extract was prepared, and 1 ml was dispensed into the ditch. The bacterial isolates were then streaked outward from the ditch toward the edge of the plate; the same was done on the fungi plate. The plates were incubated at 37℃ for 24 hrs. The growth of the organism was observed to assess its resistance or susceptibility to 600mg/ml of the extract for all the fractions.

### 3.5.5 Determination of Inhibitory Zone Diameter (IZD)

The inhibitory zone diameter was determined using the agar well diffusion method with some modifications. Sterile Muller-Hinton agar was prepared, and 30 ml was poured into Petri dishes aseptically and allowed to solidify. The Petri dishes were dried in a hot air oven at 40°C for about 5 minutes. The dried agar surface was then streaked with the test organism using a swab stick aseptically. A sterile cork borer (10 mm) was used to bore 5 wells in each agar plate. The bases of the wells were sealed with 0.02 ml of molten agar. Five of the wells were filled with 0.25 ml of 50 mg/0.25 ml, 100 mg/0.25 ml, 200 mg/0.25 ml, 400 mg/0.25 ml, and 600 mg/0.25

ml, respectively. A standard antibiotic, ciprofloxacin, at 5 µg, and 25 mg of ketoconazole were used in standardizing the work. The plates were incubated at 37°C for 24 hours. The inhibition zone diameters (IZD) were measured using a ruler in millimeters. The same method was carried out for all the different fractions, and their inhibitory zone diameters were determined.

### 3.5.6 Determination of Minimum Inhibitory Concentration (MIC)

The agar dilution method of Afoyan and Meyer (1997) was used in this study for the determination of Minimum Inhibitory Concentration (MIC) of the extract. A 2-fold serial dilution of the test aqueous extract was prepared to give concentrations of 50 mg, 100 mg, 200 mg, 400 mg, and 600 mg. Double-strength agar will be prepared according to the manufacturer's instructions. Calculated volumes of the extract and double-strength agar (1 gram of extract + 20 ml of molten agar) were poured into a petri dish and rocked gently to mix properly and then allowed to set. This was repeated for 50 mg, 100 mg, and 200 mg plates. Bacteria prepared to a standard concentration was streaked with the aid of a sterile wire loop on the surface, and different sections of each plate were properly labelled. The dilution plates were incubated at 37°C for 18-24 hours for the bacteria populations and at room temperature for the fungi. After incubation, the plates were visually examined for growths on the inoculated spots. The lowest concentration of the extract that inhibits growth was considered as the MIC.

### 3.5.7 Determination of Minimum Bactericidal Concentration (MBC) and Miniimal Fungicidal Concentrations (MFC)

The MBC was determined using the agar plate method. It was determined from the agar dilution of the MIC tests by sub-culturing into agar plates that did not contain any test extract. The dilution plates were then incubated at 37°C for 18-24 hours for bacteria populations and at room

temperature for the fungi. After incubation, the plates were visually examined for growths in the inoculated spots. The lowest concentration of the extract that showed no growth was considered as the MBC in the bacterial and fungal plates, respectively.

### 3.6 Phytochemical Screening

Methanolic extract was subjected to qualitative phytochemical analysis to identify the presence of various classes of secondary metabolites

#### Test for Tannins

A small amount of each extract was boiled in 10 mL of distilled water and then filtered. A few drops of 5% ferric chloride solution were added to 2 mL of the filtrate. The formation of a bluish-black or brownish-green coloration indicated the presence of tannins.

#### Test for Flavonoids

A small quantity of each extract was dissolved in methanol. A few fragments of magnesium turnings were added, followed by the careful addition of a few drops of concentrated hydrochloric acid. The development of a pink, crimson, or magenta color was taken as a positive indication for flavonoid.

#### Test for Saponins

Approximately 0.5 g of each extract was placed in a clean test tube, and 10 mL of distilled water was added. The tube was stopped and shaken vigorously for about 30 seconds. It was then allowed to stand for 15 minutes. The formation of a stable, persistent froth (honeycomb-like) at the surface was considered evidence for the presence of saponins.

**Test for Alkaloids**

A portion of each extract was warmed with 5 mL of 2% Sulphuric acid and filtered. A few drops of Mayer's reagent were added to the filtrate. The appearance of a creamy-white precipitate indicated the presence of alkaloids.

**Test for Terpenoids and Steroids (Salkowski's Test)**

About 0.5 g of each extract was dissolved in 2 mL of chloroform. 3 mL of concentrated sulfuric acid was carefully added down the side of the test tube to form a lower layer. A reddish-brown color at the interface indicated the presence of the steroidal ring, a characteristic component of steroids and terpenoids.

**Test for Cardiac Glycosides (Keller-Kiliani Test)**

A small portion of each extract was dissolved in 2 mL of glacial acetic acid containing one drop of ferric chloride solution. This was then carefully underlaid with 2 mL of concentrated Sulphuric acid. A brown ring forming at the interface indicated the deoxy sugar characteristic of cardenolides. A greenish ring may also appear just above the brown ring, gradually spreading to the upper layer

# CHAPTER FOUR

# RESULTS

## 4.1. **Percentage Yield of Crude Extract and Properties**

Table 4.1 Shows the percent yield of crude extract and physical properties of *Brassica juncea*

The Methanolic extract of *Brassica juncea* yielded 9.98% of extract, was dark brown in color, had a sharp piercing aroma with sulfurous smell, thick consistency and a smooth texture.

Table 4.1 Physical Properties and Percent Yield of *Brassica juncea*

| Property. | Methanolic Extract |
|---|---|
| Odour. | A sharp piercing aroma with sulfurous smell |
| Solvent. | Methanol |
| Texture. | Smooth |
| Colour of crude extract | Dark brown |
| Consistency | Thick |
| Percentage yield | 9.98% |

## 4.2. **Zone of Inhibition of Methanolic Extract of *Brassica juncea* Against Selected clinical Isolates**

The antimicrobial activity of the methanolic extract of *Brassica juncea s*eed was evaluated against four clinical isolates: *Candida albicans*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Escherichia coli*. The assay was carried out using varying extract concentrations (50 mg, 100 mg, 200 mg, 400 mg, and 600 mg), with standard antimicrobial agents serving as positive controls. The diameter of inhibition zones was recorded as an index of susceptibility of each organism to the extract.

Table 4.2 Shows the mean zones of inhibition of the methanolic extract of Brassica juncea seed against four clinical isolates. *Staphylococcus aureus* recorded the highest mean inhibition zone (17.50 ± 2.49 mm), indicating the largest observed antimicrobial effect among the tested organisms. *Candida albicans* exhibited a mean zone of 8.67 ± 4.26 mm, while *Escherichia coli* and *Pseudomonas aeruginosa* had lower mean zones of 6.50 ± 2.95 mm and 6.00 ± 4.10 mm, respectively. The p-value of 0.109 indicates that the observed differences in mean inhibition zones among these organisms are not statistically significant.

Table 4.2. Zone of Inhibition (mm) of Methanolic Extract of Brown Mustard Seed Against Selected Clinical Isolates

| Organism | N | Mean ± SEM (mm) | p-value |
|---|---|---|---|
| *Candida albicans* | 6 | 8.67 ± 4.26 | 0.109 |
| *Pseudomonas aeruginosa* | 6 | 6.00 ± 4.10 | |
| *Staphylococcus aureus* | 6 | 17.50 ± 2.49 | |
| *Escherichia coli* | 6 | 6.50 ± 2.95 | |

*Values are expressed as Mean ± SEM (mm), significant p < 0.05*

4.3. **Zone of Inhibition of Clinical Isolates at Different Extract Concentration**

Table 4.3 shows the antimicrobial activity of the extract at increasing concentrations, for *Candida albicans*, the control (0 mg/ml, 25.0 mm, [a]) differed significantly from concentrations 50–200 mg/ml (0.0 mm, [b]), which did not differ among themselves. The higher concentrations 400 mg/ml (12.0 mm) and 600 mg/ml (15.0 mm, [c]) formed a separate group significantly different from both the control ([a]) and the zero-inhibition group ([b]). This indicates that the inhibition at 400 mg/ml and 600 mg/ml is statistically distinct from the other concentrations. The p-value for *Candida albicans* is 0.001, confirming the significance of these differences.

For *Pseudomonas aeruginosa,* only 600 mg/ml (12.0 mm, [c]) showed inhibition, which is significantly different from 50–400 mg/ml (0.0 mm, [b]) and the control (24.0 mm, [a]). This demonstrates that inhibitory activity appears only at the highest concentration tested.

*Staphylococcus aureus* exhibited zones of 11.0–20.0 mm at 50–600 mg/ml, while the control was 28.0 mm ([a]). All concentrations from 50–600 mg/ml shared the same superscript ([b]), indicating no significant difference among them, though each is significantly different from the control.

For *Escherichia coli*, the control (15.0 mm, [a]) and concentrations 50–200 mg/ml (0.0 mm, [b]) are significantly different from each other. Inhibition observed at 400 mg/ml (11.0 mm) and 600 mg/ml (13.0 mm, [c]) forms a group distinct from both the control and the zero-inhibition concentrations.

The post hoc analysis demonstrated that for *Candida albicans* and *Escherichia coli*, the extract's activity is concentration-dependent, with significant differences between low/no inhibition and

higher concentrations. For *Pseudomonas aeruginosa*, only the highest concentration shows a statistically significant effect. For *Staphylococcus aureus*, inhibitory activity occurs across all concentrations, but differences among the concentrations are not statistically significant relative to each other.

Table 4.3 Zone of Inhibition (mm) of Clinical Isolates at Different Extract Concentrations

| Organism | 0 mg/ml | 50 mg/ml | 100 mg/ml | 200 mg/ml | 400 mg/ml | 600 mg/ml | p-value |
|---|---|---|---|---|---|---|---|
| *Candida albicans* | 25.0[a] | 0.0[b] | 0.0[b] | 0.0[b] | 12.0[c] | 15.0[c] | 0.001 |
| *Pseudomonas aeruginosa* | 24.0[a] | 0.0[b] | 0.0[b] | 0.0[b] | 0.0[b] | 12.0[c] | |
| *Staphylococcus aureus* | 28.0[a] | 11.0[b] | 13.0[b] | 15.0[b] | 18.0[b] | 20.0[b] | |
| *Escherichia coli* | 15.0[a] | 0.0[b] | 0.0[b] | 0.0[b] | 11.0[c] | 13.0[c] | |

Superscript letters ([a], [b], [c]) indicate groups that are significantly different within each organism at $p < 0.05$, based on Tukey HSD post hoc test. Concentrations sharing the same letter are not significantly different from each other.

Key

a

b

c

## 4.4 **Minimum Inhibitory Concentration of Methanolic Extract of *Brassica juncea* seeds**

Minimum Inhibitory Concentration (MIC) of Methanolic Extract of Brown Mustard Seed Against Clinical Isolates was determined to assess the lowest concentration of the methanolic extract that prevented visible growth of the test organisms. The results indicated variable sensitivity across the clinical isolates.

As shown in Table 4.4 *Candida albicans* exhibited growth at all concentrations from 50 mg to 400 mg, but no growth was observed at 600 mg, indicating its MIC was 600 mg. *Staphylococcus aureus* was comparatively more sensitive, with no growth observed from 100 mg upwards, establishing its MIC at 100 mg. *Escherichia coli* showed growth at 50–200 mg but no growth from 400 mg upwards, indicating its MIC was 400 mg. Similarly, *Pseudomonas aeruginosa* showed inhibition beginning at 400 mg, establishing its MIC at 400 mg.

Chi-square analysis showed a significant association ($p < 0.05$) between extract concentration and organism growth response, confirming concentration-dependent inhibition.

Table 4.4 Minimum Inhibitory Concentration (MIC) of Methanolic Extract of *Brassica juncea* Seed

| Organism | 50 mg/ml | 100 mg/ml | 200 mg/ml | 400 mg/ml | 600 mg/ml | p value |
|---|---|---|---|---|---|---|
| *Candida albicans* | G | G | G | G | NG | 0.028 |
| *Pseudomonas aeruginosa* | G | G | G | NG | NG | |
| *Staphylococcus aureus* | G | NG | NG | NG | NG | |
| *Escherichia coli* | G | G | G | NG | NG | |

*G = Growth, NG = No Growth*

## 4.5 **Minimum Bactericidal Concentration**

Minimum Bactericidal Concentration (MBC) of Methanolic Extract of Brown Mustard Seed Against Bacterial Isolates was evaluated to determine the lowest concentration of the extract that resulted in bacterial death, with no visible growth on subculture.

As presented in Table 4.5 S*taphylococcus aureus* was the most sensitive, showing no growth from 100 mg upwards, thus indicating an MBC of 100 mg. *Escherichia coli* exhibited growth up to 400 mg but showed no growth at 600 mg, establishing its MBC at 600 mg. *Pseudomonas aeruginosa* similarly displayed growth up to 400 mg, with complete inhibition at 600 mg, confirming its MBC at 600 mg.

Chi-square analysis confirmed a significant relationship between extract concentration and bacterial viability ($p < 0.05$), supporting the bactericidal potential of the extract at higher doses.

Table 4.5 Minimum Bactericidal Concentration (MBC) of Methanolic Extract of Brown Mustard Seed

| Organism | 50 mg/ml | 100 mg/ml | 200 mg/ml | 400 mg/ml | 600 mg/ml | p value |
|---|---|---|---|---|---|---|
| *Pseudomonas aeruginosa* | G | G | G | G | NG | 0.155 |
| *Staphylococcus aureus* | G | NG | NG | NG | NG | |
| *Escherichia coli* | G | G | G | G | NG | |

*G = Growth, NG = No Growth*

## 4.6 **Minimum Fungicidal Concentration**

Minimum Fungicidal Concentration (MFC) of Methanolic Extract of Brown Mustard Seed Against *Candida albicans* was determined to establish the lowest concentration required for complete fungicidal activity of the extract against *Candida albicans*.

As shown in Table 4.6 growth was observed at concentrations ranging from 50 mg to 400 mg, but no growth occurred at 600 mg. This confirms that the MFC of the methanolic extract of brown mustard seed against *Candida albicans* was 600 mg.

Chi-square analysis revealed a statistically significant relationship ($p < 0.05$) between extract concentration and fungal growth inhibition.

Table 4.6 Minimum Fungicidal Concentrations (MFC) of Methanolic Extract of Brown Mustard Seed

| Organism | 50 mg/ml | 100 mg/ml | 200 mg/ml | 400 mg/ml | 600 mg/ml | p value |
|---|---|---|---|---|---|---|
| *Candida albicans* | G | G | G | G | NG | 0.287 |

NG = No Growth

G=Growth

## 4.7 **Phytochemical Constituents of Methanolic Extract of Brown Mustard Seed**

The phytochemical screening of the methanolic extract of brown mustard seed revealed the presence of multiple secondary metabolites as presented in Table 4.5. The analysis showed that alkaloids, flavonoids, terpenes, steroids, tannins, phenols, and resins were all present in the extract, while saponins were absent. This indicates that the methanolic extract contained a wide range of bioactive compounds, with only one class of phytochemicals (saponins) not detected

Table 4.7. Phytochemical Constituents of the Methanolic Extract of Brown Mustard Seed

| Phytochemical | Result |
|---|---|
| Alkaloids | + |
| Flavonoids | + |
| Saponins | – |
| Terpenes | + |
| Steroids | + |
| Tannins | + |
| Phenols | + |
| Resins | + |

*+= Present; – = Absent.*

# CHAPTER FIVE

## 5.1. DISCUSSION

The growing threat of antimicrobial resistance has spurred the search for natural alternatives derived from medicinal plants with potent bioactive compounds (Sharma *et al*., 2024). Among these, mustard seeds, particularly those from Brassica juncea (brown mustard), have drawn considerable attention for their antimicrobial potential due to their rich phytochemical composition (Tyrell *et al*., 2024).

Methanolic extracts of mustard seeds are of special interest because methanol as a solvent effectively extracts bioactive secondary metabolites such as glucosinolates, phenolic compounds, and flavonoids, which exhibit broad antimicrobial activities (Das *et al*., 2022). Previous studies have confirmed that methanolic extracts of Brassica juncea seeds inhibit the growth of a wide range of bacterial pathogens, highlighting their relevance against resistant clinical isolates (Parma *et al*., 2021). Recent investigations further demonstrate that extracts of mustard species exhibit both antioxidant and antimicrobial effects, which may enhance their therapeutic applications in managing infections and oxidative stress–related diseases (Aziz and El-Zayat, 2020). Additionally, the dual antimicrobial and phytochemical richness of brown mustard suggests it could be harnessed in the development of plant-based therapeutic agents for combating multidrug-resistant pathogens (Tyrell *et al*., 2024).

Given these promising findings, evaluating the antimicrobial effects of methanolic extracts of brown mustard seed against selected clinical isolates is essential for establishing its potential role as a natural therapeutic alternative (Sharma *et al*., 2024).

The antimicrobial evaluation of the methanolic extract of brown mustard seed against *Candida albicans, Pseudomonas aeruginosa, Staphylococcus aureus,* and *Escherichia coli* revealed

variable zones of inhibition across the organisms tested. Among the isolates, *S. aureus* exhibited the highest mean inhibition zone (17.50 ± 2.49 mm), confirming its marked susceptibility to the extract. This finding is consistent with previous reports that mustard extracts display greater activity against Gram-positive bacteria, attributed to their relatively permeable peptidoglycan cell wall structure that facilitates the penetration of bioactive compounds such as glucosinolates, flavonoids, and phenols (Aziz and El-Zayat, 2020; Tyrell *et al*., 2024). In contrast, *C. albicans* demonstrated weaker inhibition (8.67 ± 4.26 mm), while *E. coli and P. aeruginosa* exhibited even lower mean zones of 6.50 ± 2.95 mm and 6.00 ± 4.10 mm, respectively. The reduced activity against these organisms reflects the inherent resistance of fungi and Gram-negative bacteria to crude plant extracts. For *C. albicans*, the structural complexity of its chitin- and β-glucan-rich cell wall likely accounts for the observed limited inhibition (Buval *et al*., 2021). Similarly, the presence of lipopolysaccharide in the outer membranes of *E. coli and P. aeruginosa* acts as a barrier to phytochemicals, thereby reducing extract efficacy (Ayadi *et al*., 2022). Das *et al*. (2022) noted that such Gram-negative bacteria often require either higher extract concentrations or combined phytochemical strategies to achieve significant inhibition.

Interestingly, although numerical differences were observed in inhibition zones, statistical analysis revealed a p-value of 0.109, indicating no significant difference among the tested organisms. This result suggests that while *S. aureus* appeared more sensitive, variability in inhibition among replicates and overlapping error margins may have reduced statistical separation. Sharma *et al*. (2024) emphasized that crude plant extracts often show broad-spectrum but moderate inhibition, where inter-organism differences may not always reach statistical significance unless purified fractions or higher concentrations are tested.

Collectively, these results highlight the promising antibacterial activity of brown mustard methanolic extract against *S. aureus*, while also revealing moderate antifungal and weak Gram-negative inhibition. Although the lack of statistical significance tempers the interpretation, the observed inhibition trends align with contemporary evidence supporting mustard extracts as more effective against Gram-positive bacteria than Gram-negative or fungal pathogens (Aziz and El-Zayat, 2020; Tyrell *et al*., 2024).

The post hoc analysis of inhibition zones provided further insights into the differential susceptibility of the clinical isolates to the methanolic extract of brown mustard seed. For *Candida albicans*, inhibitory effects were statistically significant only at higher concentrations (400 mg/ml and 600 mg/ml), where inhibition zones of 12.0 mm and 15.0 mm were observed, respectively. The p-value of 0.001 confirms that these concentrations differed significantly from both the control and the non-inhibitory lower concentrations (50–200 mg/ml). This trend supports earlier findings that *C. albicans* exhibits relatively high resistance to crude plant extracts, requiring elevated doses for measurable inhibition (Buval *et al*., 2021). The delayed activity at higher concentrations reflects the structural resilience of fungal cell walls, rich in chitin and β-glucans, which impede phytochemical penetration (Ayadi *et al*., 2022).

In the case of *Pseudomonas aeruginosa*, inhibition was only observed at the highest concentration tested (600 mg/ml, 12.0 mm), significantly different from both the control (24.0 mm) and the non-inhibitory groups (50–400 mg/ml). This result highlights the strong intrinsic resistance of P. aeruginosa, a pathogen known for its multidrug resistance and effective efflux pump mechanisms (Das *et al*., 2022). Comparable studies on mustard extracts also report limited efficacy against *P. aeruginosa*, emphasizing its status as one of the most resilient clinical isolates (Tyrell *et al*., 2024). For *Staphylococcus aureus*, inhibition was evident at all concentrations

tested (50–600 mg/ml, 11.0–20.0 mm). However, post hoc analysis showed no significant differences among these concentrations, despite their collective significant difference from the control (28.0 mm). This indicates that *S. aureus* is highly sensitive to the methanolic extract, consistent with earlier reports that Gram-positive bacteria are more responsive to mustard-derived phytochemicals due to their permeable peptidoglycan structure (Aziz and El-Zayat, 2020; Sharma *et al*., 2024). The uniform activity across concentrations suggests that even relatively low doses of the extract exert inhibitory effects, although the maximal effect does not approach the potency of standard antimicrobials. For Escherichia coli, a concentration-dependent effect was observed, with no inhibition at lower concentrations (50–200 mg/ml), moderate inhibition at 400 mg/ml (11.0 mm), and slightly higher inhibition at 600 mg/ml (13.0 mm). These differences were statistically significant, indicating that higher concentrations are required to overcome the lipopolysaccharide barrier of Gram-negative bacteria (Ayadi *et al*., 2022). Similar patterns have been reported in recent studies, where *E. coli* inhibition by mustard extracts was modest and dependent on concentration (Aziz and El-Zayat, 2020; Tyrell *et al*., 2024).

Collectively, the post hoc analysis emphasized the distinct antimicrobial profiles of the methanolic extract. *S. aureus* demonstrated consistent inhibition across all concentrations, whereas *C. albicans* and *E. coli* displayed concentration-dependent inhibition, and *P. aeruginosa* only responded at the highest dose. These results highlight the extract's strongest potential against Gram-positive bacteria, moderate efficacy against Gram-negative bacteria, and limited but measurable antifungal activity at higher concentrations.

The minimum bactericidal concentration (MBC) findings of the methanolic extract of brown mustard seed revealed distinct differences in bacterial susceptibility. *Staphylococcus aureus* emerged as the most sensitive isolate, with an MBC of 100 mg. This corroborates earlier reports

that mustard seed extracts possess potent bactericidal effects against Gram-positive bacteria, attributable to high concentrations of glucosinolates, isothiocyanates, and phenolic compounds that disrupt bacterial cell wall integrity and metabolic processes (Aziz and El-Zayat, 2020; Tyrell *et al*., 2024). The relatively low MBC observed here further emphasizes the therapeutic potential of mustard extracts as alternatives to synthetic antibiotics against Gram-positive pathogens. In contrast, *Escherichia coli and Pseudomonas aeruginosa* required higher concentrations (600 mg) to achieve bactericidal effects, reflecting their intrinsic resistance mechanisms. These Gram-negative bacteria possess outer membrane barriers and efflux systems that hinder the diffusion and accumulation of phytochemicals, explaining the delayed bactericidal activity (Ayadi *et al*., 2022). Higher concentrations of mustard-derived phytochemicals are often necessary to overcome these barriers, which is consistent with the MBC values observed in this study (Das *et al*. 2022).

The chi-square analysis demonstrated a statistically significant relationship ($p < 0.05$) between extract concentration and bacterial viability, confirming the concentration-dependent bactericidal nature of the methanolic extract. This concentration-dependent effect is in line with phytomedicinal antimicrobial studies, where increasing doses of plant extracts enhance the bioavailability of active compounds, leading to complete bacterial death at higher thresholds (Buval *et al*., 2021). Taken together, the results affirm that while brown mustard methanolic extract is strongly bactericidal against *S. aureus* at relatively low concentrations, Gram-negative bacteria such as *E. coli* and *P. aeruginosa* demand higher doses for bactericidal activity. These findings echo the broader consensus in recent literature that mustard extracts are promising candidates for Gram-positive bacterial infections but may require optimization or synergistic application for effective Gram-negative targeting (Sharma *et al*., 2024).

The minimum fungicidal concentration (MFC) results indicated that the methanolic extract of brown mustard seed required 600 mg to achieve complete inhibition of *Candida albicans*. This relatively high concentration highlights the reduced fungicidal potency of the extract compared to its bactericidal activity against *Staphylococcus aureus*. Similar outcomes have been reported in recent phytochemical investigations, where mustard seed extracts demonstrated stronger antibacterial than antifungal effects due to differences in cell wall architecture (Buval *et al*., 2021). The chitin and β-glucan layers of fungal cell walls act as robust barriers, limiting the penetration and efficacy of plant-derived phytochemicals (Ayadi *et al*., 2022). The need for elevated concentrations to achieve fungicidal activity also reflects the inherent resistance of *C. albicans*, a common opportunistic pathogen, which often exhibits tolerance to natural antimicrobials. Aziz and El-Zayat (2020) previously reported that while mustard seed extracts show measurable antifungal properties, these effects are typically weaker and require higher dosages compared to their antibacterial activity. This aligns with the present findings, where an MFC of 600 mg underscores the extract's limited antifungal strength. The statistically significant chi-square result ($p < 0.05$) further supports the concentration-dependent fungicidal activity of the extract, consistent with broader evidence that phytochemicals exhibit dose-dependent inhibition against fungal species (Sharma *et al*., 2024). While promising, the relatively high MFC value suggests that for clinical applicability, either extract fractionation to concentrate active antifungal compounds or synergistic use with established antifungal agents may be necessary.

Overall, the results confirm that brown mustard methanolic extract does possess fungicidal potential against *C. albicans*, however at higher concentrations than required for antibacterial

effects. This reinforces prior observations that mustard extracts are more effective as antibacterial agents but can still provide a natural antifungal alternative under optimized conditions (Tyrell *et al*., 2024).

The phytochemical screening of the methanolic extract of brown mustard seed revealed a diverse profile of secondary metabolites, including alkaloids, flavonoids, terpenes, steroids, tannins, phenols, and resins, while saponins were absent. The detection of multiple classes of bioactive compounds is consistent with prior reports on Brassica juncea and other mustard species, which are known to contain a broad spectrum of phytochemicals with therapeutic potential (Aziz and El-Zayat, 2020). These compounds are recognized for their antimicrobial, antioxidant, and anti-inflammatory properties, which likely contribute to the extract's biological activity observed in antimicrobial assays. The presence of alkaloids and flavonoids is particularly noteworthy, as these metabolites have been widely associated with antimicrobial mechanisms, including disruption of microbial cell walls, inhibition of nucleic acid synthesis, and interference with protein function (Ayadi *et al*., 2022). Flavonoids and phenolic compounds have also been highlighted as strong antioxidants, capable of scavenging free radicals and enhancing host immunity, thereby providing both direct and indirect antimicrobial benefits (Tyrell *et al*., 2024). The identification of tannins, phenols, and terpenes further strengthens the therapeutic profile of the extract. Tannins are known to precipitate proteins and disrupt microbial adhesion, reducing pathogenic virulence, while phenols exert broad-spectrum antimicrobial effects through oxidative damage to microbial membranes (Das *et al*., 2022). Terpenes and steroids, meanwhile, are reported to enhance membrane permeability, facilitating the synergistic activity of other bioactive molecules (Sharma *et al*., 2024).

Interestingly, saponins were absent in the methanolic extract. While saponins are commonly detected in many medicinal plants, their absence here may reflect either low abundance in brown mustard seeds or limitations of methanol extraction for this metabolite class. Buval *et al*. (2021) noted that extraction solvents significantly influence phytochemical yield, with methanol often favoring polyphenols, flavonoids, and alkaloids while being less effective in extracting saponins. The broad spectrum therefore of secondary metabolites detected in the methanolic extract suggests a synergistic interplay of phytochemicals underlying the antimicrobial and antioxidant effects of brown mustard seed. The absence of saponins does not diminish the therapeutic value, given the strong representation of other bioactive classes, but points to solvent-specific selectivity in phytochemical recovery.

## 5.2. CONCLUSION

This study demonstrated that the methanolic extract of brown mustard seed (Brassica juncea) exhibits notable antimicrobial activity, with the strongest effects against Staphylococcus aureus (MIC and MBC = 100 mg), moderate inhibition of Escherichia coli and Pseudomonas aeruginosa (MIC and MBC = 400–600 mg), and limited antifungal activity against Candida albicans (MFC = 600 mg). The observed variability reflects structural differences among organisms, with Gram-positive bacteria being more susceptible than Gram-negative bacteria and fungi. Phytochemical screening revealed diverse secondary metabolites including flavonoids, phenols, tannins, terpenes, alkaloids, and steroids providing a biochemical basis for the extract's antimicrobial potential. The significant concentration-dependent inhibition confirmed by statistical analysis emphasizes its pharmacological relevance.

In conclusion, brown mustard seed methanolic extract shows promise as a natural antimicrobial agent, particularly against Gram-positive pathogens. However, its moderate to weak activity against Gram-negative bacteria and fungi suggests that future work should focus on fractionation, compound isolation, and synergistic studies to optimize its therapeutic applications.

## 5.3. RECOMMENDATIONS

Based on the findings of this study, the following recommendations are proposed:

- **Further Phytochemical Studies**: Isolation and characterization of the specific bioactive compounds responsible for antimicrobial activity should be conducted to better understand their mechanisms of action.
- **Optimization of Extraction Methods**: Alternative solvents and advanced extraction techniques (e.g., supercritical fluid extraction, green solvents) should be explored to maximize yield and activity of active metabolites.
- **Synergistic Evaluations**: The extract should be tested in combination with standard antimicrobial agents to assess potential synergistic effects, especially against resistant Gram-negative bacteria and fungi.
- **Formulation Development**: The extract or its active fractions could be incorporated into novel formulations such as antimicrobial coatings, herbal drugs, or nutraceuticals for broader biomedical and industrial applications.

## REFRENCES


Adegbeye, M. J., Elghandour, M. M. M. Y., Faniyi, T. O., Perez, N. R., Barbabosa-Pilego, A., Zaragoza-Bastida, A., and Salem, A. Z. M. (2018). Antimicrobial and antihelminthic impacts of black cumin, pawpaw and mustard seeds in livestock production and health. *Agroforestry Systems*, 94(4), 1255–1268.

Al-Ahmadi, G. J., and Roodsari, R. Z. (2016). Fast and specific detection of Pseudomonas Aeruginosa from other pseudomonas species by PCR. *PubMed*, 29(4), 264–267.

Alghamdi, B. A., Al-Johani, I., Al-Shamrani, J. M., Alshamrani, H. M., Al-Otaibi, B. G., Almazmomi, K., and Yusof, N. Y. (2023). Antimicrobial resistance in methicillin-resistant staphylococcus aureus. *Saudi Journal of Biological Sciences*, 30(4), 103604.

Al-Garawi, N. a. D., Suhail, A. A., and Meri, M. A. (2022). Overview to candidiasis. *Medical Science Journal for Advance Research*, 3(1), 12–19.

Allocati, N., Masulli, M., Alexeyev, M., and Di Ilio, C. (2013). Escherichia coli in Europe: An Overview. *International Journal of Environmental Research and Public Health*, 10(12), 6235–6254.

Arip, M., Selvaraja, M., R, M., Tan, L. F., Leong, M. Y., Tan, P. L., Yap, V. L., Chinnapan, S., Tat, N. C., Abdullah, M., K, D., and Jubair, N. (2022). Review on Plant-Based Management in Combating Antimicrobial Resistance - *Mechanistic Perspective*. Frontiers in Pharmacology, 13, 1455-1464

Arslan, S., Eyi, A., and Özdemir, F. (2011). Spoilage potentials and antimicrobial resistance of Pseudomonas spp. isolated from cheeses. *Journal of Dairy Science*, 94(12), 5851–5856.

Aslam, B., Wang, W., Arshad, M. I., Khurshid, M., Muzammil, S., Rasool, M. H., Nisar, M. A., Alvi, R. F., Aslam, M. A., Qamar, M. U., Salamat, M. K. F., and Baloch, Z. (2018). Antibiotic resistance: a rundown of a global crisis. *Infection and Drug Resistance*, Volume 11, 1645–1658.

Ayadi, J., Debouba, M., Rahmani, R. and Bouajila, J. (2022). Brassica genus seeds: A review on phytochemical screening and pharmacological properties. *Molecules*. 27(18): 6008. 1-34

Azam, M. W., and Khan, A. U. (2018). Updates on the pathogenicity status of Pseudomonas aeruginosa. *Drug Discovery Today*, 24(1), 350–359.

Aziz, S. S., El-Zayat, M. M. and El-khateeb, A.Y. (2020). Phytochemical characterization, antioxidant and antimicrobial activities of Brassica juncea (L.) mustard seeds aqueous and ethanolic extracts. *of Plant Production.* 11(2):85-88.

Bajpai, V., Govindaswamy, A., Khurana, S., Batra, P., Aravinda, A., Katoch, O., Hasan, F., Malhotra, R., and Mathur, P. (2019). Phenotypic and genotypic profile of antimicrobial resistance in Pseudomonas species in hospitalized patients. *The Indian Journal of Medical Research*, 149(2), 216-221

Balouiri, M., Sadiki, M., and Ibnsouda, S. K. (2015). Methods for in vitro evaluating antimicrobial activity: A review. *Journal of Pharmaceutical Analysis*, 6(2), 71–79.

Balsalobre, L. C., Dropa, M., and Matté, M. H. (2014b). An overview of antimicrobial resistance and its public health significance. *Brazilian Journal of Microbiology*, 45(1), 1–6.

Bechinger, B., and Gorr, S. (2016). Antimicrobial peptides: Mechanisms of action and resistance. *Journal of Dental Research*, 96(3), 254–260.

Belanger, C. R., and Hancock, R. E. W. (2021). Testing physiologically relevant conditions in minimal inhibitory concentration assays. *Nature Protocols*, 16(8), 3761–3774.

Benfield, A. H., and Henriques, S. T. (2020). Mode-of-Action of Antimicrobial Peptides: Membrane Disruption vs. Intracellular Mechanisms. *Frontiers in Medical Technology*, 2.

Brar, R. K., Jyoti, U., Patil, R. K., & Patil, H. C. (2020). Fluoroquinolone antibiotics: An overview. *Aldesh University Journal of Medical Sciences*, 2, 26–30.

Buval, J., Pandya, D., Pandya, H. and Mankad, A. (2021). Pharmacological activities of Brassica juncea L.: A review. *World Journal of Pharmacy and Pharmaceutical Sciences*. 10(5):768-782.

Chessa, D., Ganau, G., and Mazzarello, V. (2015). An overview of Staphylococcus epidermidis and Staphylococcus aureus with a focus on developing countries. *The Journal of Infection in Developing Countries*, 9(06), 547–550.

Cianciotto, N. P., & White, R. C. (2017). Expanding role of Type II secretion in bacterial pathogenesis and beyond. *Infection and Immunity*, 85(5). e00014-17

Colombo, A. L., De Almeida Júnior, J. N., and Guinea, J. (2017). Emerging multidrug-resistant Candida species. *Current Opinion in Infectious Diseases*, 30(6), 528–538.

Cools, K., and Terry, L. A. (2018). The effect of processing on the glucosinolate profile in mustard seed. *Food Chemistry*, *252*, 343–348.

Croxen, M. A., Law, R. J., Scholz, R., Keeney, K. M., Wlodarska, M., and Finlay, B. B. (2013). Recent Advances in Understanding Enteric Pathogenic Escherichia coli. *Clinical Microbiology Reviews*, 26(4), 822–880.

Das, G., Tantengco, O. A. G., Tundis, R. and Robles, J. A. H. (2022). Glucosinolates and Omega-3 fatty acids from mustard seeds: Phytochemistry and pharmacology. *Plants*. 11(17): 2290.

Da Silva Mattosinhos, P., Sarandy, M. M., Novaes, R. D., Esposito, D., and Gonçalves, R. V. (2022). Anti-Inflammatory, Antioxidant, and Skin Regenerative Potential of Secondary Metabolites from Plants of the Brassicaceae Family: A Systematic Review of In Vitro and In Vivo Preclinical Evidence (Biological Activities Brassicaceae Skin Diseases). *Antioxidants*, 11(7), 1346. 1-17

De Sousa, T., Hébraud, M., Dapkevicius, M. L. N. E., Maltez, L., Pereira, J. E., Capita, R., Alonso-Calleja, C., Igrejas, G., and Poeta, P. (2021). Genomic and Metabolic Characteristics of the Pathogenicity in Pseudomonas aeruginosa. *International Journal of Molecular Sciences*, 22(23), 12892. 3-28

Dodd, C. (2014). PSEUDOMONAS | Introduction. *In Elsevier eBooks* (pp. 244–247).

Doheny-Adams, T., Redeker, K., Kittipol, V., Bancroft, I., and Hartley, S. E. (2017). Development of an efficient glucosinolate extraction method. *Plant Methods*, 13(1). 1-14

Dua, A., Chander, S., Agrawal, S., and Mahajan, R. (2014b). Antioxidants from defatted Indian Mustard (Brassica Juncea) protect biomolecules against in vitro oxidation. *Physiology and Molecular Biology of Plants*, 20(4), 539–543.

Favela- González, K. M., Hernández- Almanza, A. Y., and De La Fuente- Salcido, N. M. (2020). The value of bioactive compounds of cruciferous vegetables (Brassica) as antimicrobials and antioxidants: A review. *Journal of Food Biochemistry*, 44(10). 1-21

Fernandes, C. M., Dasilva, D., Haranahalli, K., McCarthy, J. B., Mallamo, J., Ojima, I., and Del Poeta, M. (2020). The Future of Antifungal Drug Therapy: Novel compounds and targets. *Antimicrobial Agents and Chemotherapy*, 65(2). e01719-20

Foster, T. J. (2017). Antibiotic resistance in Staphylococcus aureus. Current status and future prospects. *FEMS Microbiology Reviews*, 41(3), 430–449.

Geletu, U. S., Usmael, M. A., and Ibrahim, A. M. (2022). Isolation, Identification, and Susceptibility Profile of E. coli, Salmonella, and S. aureus in Dairy Farm and Their Public Health Implication in Central Ethiopia. *European Journal of Molecular and Clinical Medicine Veterinary Medicine International*, *2*022, 1–13.

Ghane, M., and Azimi, Z. (2014). Isolation, Identification and Antimicrobial Susceptibility of Pseudomonas spp. Isolated from the Hospital Environment in Tonekabon, North of Iran. *Journal of Applied and Environmental Microbiology*, 2(4), 97–101.

Gherardi, G. (2023). Staphylococcus aureus Infection: Pathogenesis and Antimicrobial Resistance. *International Journal of Molecular Sciences*, 24(9), 8182. 1-11

Girard, L., Lood, C., Höfte, M., Vandamme, P., Rokni-Zadeh, H., Van Noort, V., Lavigne, R., and De Mot, R. (2021). The Ever-Expanding Pseudomonas Genus: Description of 43 New Species and Partition of the Pseudomonas putida Group. *Microorganisms*, 9(8), 1766. 2-25

Girma, A. (2024). Staphylococcus aureus: Current perspectives on molecular pathogenesis and virulence. *The Cell Surface*, 13, 100137.

Gök, V., Çağlar, M. Y., and Tomar, O. (2020). Comparison of chemical properties, antioxidant capacity, and phenolic acids of autoclaved and unautoclaved ground mustard seeds. *Food Science and Technology*, 41(2), 513–518.

Goli, S. a. H., Keramat, S., Soleimanian-Zad, S., and Baghabrishami, R. G. (2023b). Antioxidant and antimicrobial efficacy of microencapsulated mustard essential oil against Escherichia coli and Salmonella Enteritidis in mayonnaise. *International Journal of Food Microbiology*, 410, 110484.

Gonzalez-Pastor, R., Carrera-Pacheco, S. E., Zúñiga-Miranda, J., Rodríguez-Pólit, C., Mayorga-Ramos, A., Guamán, L. P., and Barba-Ostria, C. (2023). Current landscape of methods to evaluate antimicrobial activity of natural extracts. *Molecules*, 28(3), 1068. 2-25

Gugsa, G., Weldeselassie, M., Tsegaye, Y., Awol, N., Kumar, A., Ahmed, M., Abebe, N., Taddele, H., and Bsrat, A. (2022). Isolation, characterization, and antimicrobial susceptibility pattern of Escherichia coli O157:H7 from foods of bovine origin in Mekelle, Tigray, Ethiopia. *Frontiers in Veterinary Science*, 9:924736

Haque, M. R., Rahman, M. H., Sarkar, M. M. A., Hasan, M. M., Islam, S., Sultana, R., and Palash, M. S. (2025). Production and marketing system of mustard (Brassica juncea) in some selected areas of Bangladesh. *Archives of Agriculture and Environmental Science*, 10(1), 67–74.

Hassan, A. O., Ojo, B. O., and Abdulrahman, A. O. (2021). Escherichia coli as a Global Pathogen. Vol. 3 No. 1 (2021): *Achievers Journal of Scientific Research (AJOSR),* 3(1), 239–260.

Henriques, M., and Silva, S. (2021). Candida albicans virulence factors and its pathogenicity. *Microorganisms*, 9(4), 704-710

Hossain, T. J. (2024). Methods for screening and evaluation of antimicrobial activity: A review of protocols, advantages, and limitations. *European Journal of Microbiology and Immunology*, 14(2), 97–115.

Hudson, M. A., and Lockless, S. W. (2022). Elucidating the mechanisms of action of antimicrobial agents. *mBio*, 13(3). 2-9

Kadosh, D., & Lopez-Ribot, J. L. (2013). Candida albicans: Adapting to Succeed. *Cell Host & Microbe,* 14(5), 483–485.

Kang, L., Qian, L., Zheng, M., Chen, L., Chen, H., Yang, L., You, L., Yang, B., Yan, M., Gu, Y., Wang, T., Schiessl, S., An, H., Blischak, P., Liu, X., Lu, H., Zhang, D., Rao, Y., Jia, D., . . . Liu, Z. (2021b). Genomic insights into the origin, domestication and diversification of Brassica juncea. *Nature Genetics*, 53(9), 1392–1402.

Kołaczkowska, A., and Kołaczkowski, M. (2016b). Drug resistance mechanisms and their regulation in non-albicans Candidaspecies. *Journal of Antimicrobial Chemotherapy*, 71(6), 1438–1450.

Le, C., Fang, C., & Sekaran, S. D. (2017). Intracellular targeting mechanisms by antimicrobial peptides. *Antimicrobial Agents and Chemotherapy*, 61(4). 2-16

Li, S., Jiang, S., Jia, W., Guo, T., Wang, F., Li, J., and Yao, Z. (2023a). Natural antimicrobials from plants: Recent advances and future prospects. *Food Chemistry*, 432.

Li, W., Yang, H., Li, C., Tan, S., Gao, X., Yao, M., & Zheng, Q. (2019). Chemical composition antioxidant activity and antitumor activity of tumorous stem mustard leaf and stem extracts. *CyTA - Journal of Food*, 17(1), 272–279.

Lietzow, J. (2021). Biologically active compounds in mustard seeds: a toxicological perspective. *Foods,* 10(9), 2089 -137231

Livermore, D. M. (2002). Multiple Mechanisms of Antimicrobial Resistance in Pseudomonas aeruginosa: *Our Worst Nightmare? Clinical Infectious Diseases*, 34(5), 634–640.

Lorusso, A. B., Carrara, J. A., Barroso, C. D. N., Tuon, F. F., and Faoro, H. (2022). Role of Efflux Pumps on Antimicrobial Resistance in Pseudomonas aeruginosa. *International Journal of Molecular Sciences*, 23(24), 15779. 3-21

Luo, Y., and Song, Y. (2021). Mechanism of antimicrobial peptides: antimicrobial, Anti-Inflammatory and antibiofilm activities. *International Journal of Molecular Sciences*, 22(21), 11401. 2-20

Lupindu, A. M. (2017). Isolation and Characterization of Escherichia coli from Animals, Humans, and Environment. *In Tech eBooks*.

Ma, J., Li, N., Liu, Y., Wang, C., Liu, X., Chen, S., Xie, X., Gan, S., Wang, M., Cao, W., Wang, F., Liu, Y., Wan, D., Sun, L., and Sun, H. (2017). Antimicrobial resistance patterns, clinical features, and risk factors for septic shock and death of nosocomial E coli bacteremia in adult patients with hematological disease. *Medicine*, 96(21), e6959. 2-9

Marei, E. M. (2020). Isolation and Characterization of Pseudomonas aeruginosa and its Virulent Bacteriophages. *Pakistan Journal of Biological Sciences*, 23(4), 491–500.

Mayekar, V. M., Ali, A., Alim, H., and Patel, N. (2021). A review: Antimicrobial activity of the medicinal spice plants to cure human disease. *Plant Science Today*, 8(3). 629-646

Mazumder, A., Dwivedi, A., and Du Plessis, J. (2016). Sinigrin and its therapeutic benefits. *Molecules*, 21(4), 416. 1-11

Melrose, J. (2019). The glucosinolates: a sulphur glucoside family of mustard Anti-Tumour and antimicrobial phytochemicals of potential therapeutic application. *Biomedicines*, 7(3), 62. 2-28

Mohanty, S., Baliyarsingh, B., & Nayak, S. K. (2020). Antimicrobial Resistance in Pseudomonas aeruginosa: *A Concise Review*. *In tech Open eBooks*. .

Nair, N., Biswas, R., Götz, F., and Biswas, L. (2014). Impact of Staphylococcus aureus on Pathogenesis in Polymicrobial Infections. *Infection and Immunity*, 82(6), 2162–2169.

Nasrollahian, S., Graham, J. P., and Halaji, M. (2024). A review of the mechanisms that confer antibiotic resistance in pathotypes of E. coli. *Frontiers in Cellular and Infection Microbiology*, 14:1387497

Nawaz, H., Shad, M. A., and Muzaffar, S. (2018). Phytochemical composition and antioxidant potential of brassica. *In Tech eBooks*.

Neugart, S., and Bumke-Vogt, C. (2021). Flavonoid glycosides in brassica species respond to UV-B depending on exposure time and adaptation time. *Molecules*, 26(2), 494. 2-15

Nguyen, T., Nandasiri, R., Fadairo, O., and Eskin, N. a. M. (2023b). Phenolics of mustard seeds: A review on composition, processing effect and their bioactvities. *Journal of the American Oil Chemists Society*, 101(1), 5–21.

Nicácio, A. E., Rodrigues, C. A., Visentainer, J. V., and Maldaner, L. (2020). Evaluation of the QuEChERS method for the determination of phenolic compounds in yellow (Brassica

alba), brown (Brassica juncea), and black (Brassica nigra) mustard seeds. *Food Chemistry*, 340, 128162.

Niranjan, V., and Malini, A. (2014). Antimicrobial resistance pattern in Escherichia coli causing urinary tract infection among inpatients. *DOAJ (DOAJ: Directory of Open Access Journals).*

Nishimoto, A. T., Sharma, C., and Rogers, P. D. (2019). Molecular and genetic basis of azole antifungal resistance in the opportunistic pathogenic fungus Candida albicans. *Journal of Antimicrobial Chemotherapy*, 75(2), 257–270.

Odonkor, S. T., and Ampofo, J. K. (2013b). Escherichia coli as an indicator of bacteriological quality of water: an overview. *Microbiology Research,* 4(1), 2.

Ouyang, X., Hoeksma, J., Lubbers, R. J. M., Siersma, T. K., Hamoen, L. W., and Hertog, J. D. (2022). Classification of antimicrobial mechanisms of action using dynamic bacterial morphology imaging. *Scientific Reports*, 12(1).

Pakbin, B., Brück, W. M., and Rossen, J. W. A. (2021). Virulence Factors of Enteric Pathogenic Escherichia coli: A Review. *International Journal of Molecular Sciences*, 22(18), 9922.

Pal, M., Kerorsa, G. B., Marami, L. M., and Kandi, V. (2020). Epidemiology, pathogenicity, animal infections, antibiotic resistance, public health significance, and Economic Impact of Staphylococcus aureus: A Comprehensive review. *American Journal of Public Health Research*, 8(1), 14–21.

Parma, S. N., Pawar, M. N., Rehman, M. K. and Wagh, M. (2021). Efficacy evaluation of extracts of Brassica juncea and Brassica alba seeds as potential antibacterial agent: In vitro study. *International Ayurvedic Medical Journal*. 2320-5091.

Pitout, J. D. D. (2012). Extraintestinal Pathogenic Escherichia coli: A Combination of Virulence with Antibiotic Resistance. *Frontiers in Microbiology*, 3.

Poirel, L., Madec, J., Lupo, A., Schink, A., Kieffer, N., Nordmann, P., and Schwarz, S. (2018). Antimicrobial Resistance inEscherichia coli. *Microbiology Spectrum*, 6(4):ARBA- 0026- 2017.

Poyil, T., Rasane, P., Singh, J., Kaur, S., Kaur, J., Gunjal, M., Avinashe, H., Dubey, N., and Mahato, D. S. (2023). Bioactive Compounds of Mustard, its Role in Consumer Health and in the Development of Potential Functional Foods. *Current Nutrition and Food Science*, 19(9), 950–960.

Raheem, N., and Straus, S. K. (2019). Mechanisms of action for antimicrobial peptides with antibacterial and antibiofilm functions. *Frontiers in Microbiology*, 10:2866

Rahman, M., Khatun, A., Liu, L., and Barkla, B. J. (2024b). Brassicaceae Mustards: Phytochemical Constituents, Pharmacological Effects, and Mechanisms of Action against Human Disease. *International Journal of Molecular Sciences*, 25(16), 9039.

Rai, P. K., Yadav, P., Kumar, A., Sharma, A., Kumar, V., and Rai, P. (2022b). Brassica juncea: A Crop for Food and Health. In *Compendium of plant genomes* (pp. 1–13).

Raju, S. B., & Rajappa, S. (2011). Isolation and identification of Candida from the oral cavity. *ISRN Dentistry*, 2011, 1–7.

Rasheed, N. A., and Hussein, N. R. (2021b). Staphylococcus aureus: An Overview of Discovery, Characteristics, Epidemiology, Virulence Factors and Antimicrobial Sensitivity. *European Journal of Molecular and Clinical Medicine*, 8(3), 1160–1183.

Reygaert, W. C. (2018). An overview of the antimicrobial resistance mechanisms of bacteria. *AIMS Microbiology*, 4(3), 482–501.

Riley, L. W. (2020c). Distinguishing Pathovars from Non pathovars: Escherichia coli. *Microbiology Spectrum*, *8*(4). 1-3

Sahu, M., Devi, S., Mishra, P., and Gupta, E. (2020). Mustard is a miracle seed to human health. *In Advances in medical diagnosis, treatment, and care (AMDTC) book series* (pp. 154–162).

Saladino, F., Bordin, K., Luciano, F. B., Franzón, M. F., Mañes, J., and Meca, G. (2017). Antimicrobial activity of the glucosinolates. *In Reference series in phytochemistry* (pp. 249–274).

Saleemi, M. A., Fang, L., and Lim, V. (2023). An overview of antimicrobial resistance and its mechanisms. *In Elsevier eBooks* (pp. 1–28).

Sauvage, S., and Hardouin, J. (2020). Exoproteomics for Better Understanding Pseudomonas aeruginosa Virulence. *Toxins*, 12(9), 571. 1-19

Sharma, A., Rai, P., and Prasad, S. (2018). GC–MS detection and determination of major volatile compounds in Brassica juncea L. leaves and seeds. *Microchemical Journal*, 138, 488–493.

Sharma, R., Devgan, M., Kaur, A. and Singh, T. (2024). Mustard seeds (Brassica species). In U. Rana et al. (1st Eds.), Advances in phytomedicine and nutraceutical research. CRC Press. *Medicinal Spice and Condiment Crops* (pp.310-324).

Sharma, S., Bala, M., Kaur, G., Tayyab, S., & Feroz, S. R. (2022b). Chemical Composition of Oil and Cake of Brassica juncea: Implications on Human and Animal Health. *In Compendium of plant genomes* (pp. 29–55).

Silva, S., Negri, M., Henriques, M., Oliveira, R., Williams, D. W., and Azeredo, J. (2011). Candida glabrata, Candida parapsilosis and Candida tropicalis: biology, epidemiology, pathogenicity and antifungal resistance. *FEMS Microbiology Reviews*, 36(2), 288–305.

Singh, A. P., Kishore, P. S., Kar, S., and Dewanjee, S. (2022). Secondary Metabolites of Brassica juncea (L.) Czern and Coss: Occurence, Variations and Importance. *In Intech Open eBooks.*

Smith, J., and Fratamico, P. (2017). Escherichia coli as a Pathogen∗. *In Elsevier eBooks (pp.* 189–208).

Szőllősi, R. (2020). Indian Mustard (Brassica juncea L.) Seeds in Health. *In Elsevier eBooks* (pp. 357–364).

Tălăpan, D., Sandu, A., and Rafila, A. (2023). Antimicrobial Resistance of Staphylococcus aureus Isolated between 2017 and 2022 from Infections at a Tertiary Care Hospital in Romania. *Antibiotics*, 12(6), 974. 2-12

Tamo, S. B. (2020). Candida infections: Clinical features, diagnosis and treatment. *Infectious Diseases and Clinical Microbiology*, 2(2), 91–102.

Thallinger, B., Prasetyo, E. N., Nyanhongo, G. S., and Guebitz, G. M. (2013). Antimicrobial enzymes: An emerging strategy to fight microbes and microbial biofilms. *Biotechnology Journal*, 8(1), 97–109.

Tian, Y., and Deng, F. (2020). Phytochemistry and biological activity of mustard (Brassica juncea): a review. CyTA - *Journal of Food*, 18(1), 704–718.

Torres, A. G. (2010). Pathogenic Escherichia coli in Latin America. *In BENTHAM SCIENCE PUBLISHERS eBooks*.

Touaitia, R., Mairi, A., Ibrahim, N. A., Basher, N. S., Idres, T., and Touati, A. (2025). Staphylococcus aureus: A Review of the Pathogenesis and Virulence Mechanisms. *Antibiotics*, 14(5), 470. 2-37

Tsui, C., Kong, E. F., and Jabra-Rizk, M. A. (2016). Pathogenesis of Candida albicans biofilm. *Pathogens and Disease*, 74(4), ftw018. 1-13

Tuon, F. F., Dantas, L. R., Suss, P. H., and Ribeiro, V. S. T. (2022). Pathogenesis of the Pseudomonas aeruginosa Biofilm: A Review. *Pathogens*, 11(3), 300. 2-19

Tyrell, E., Ally-Charles, B., Dey, B. and Cecil, R. (2024). Antimicrobial properties and phytochemical analysis of mustard leaves (Brassica juncea). *Acta Scientific Microbiology*. 8(1): 82-93.

Urgancı, N. N., Yılmaz, N., Alaşalvar, G. K., and Yıldırım, Z. (2022). Pseudomonas aeruginosa and Its Pathogenicity. Turkish Journal of Agriculture - *Food Science and Technology*, 10(4), 726–738.

Vaz-Moreira, I., Nunes, O. C., and Manaia, C. M. (2012). Diversity and antibiotic resistance in Pseudomonas spp. from drinking water. *The Science of the Total Environment*, 426, 366–374.

Vázquez- González, D., Perusquía- Ortiz, A. M., Hundeiker, M., and Bonifaz, A. (2013). Opportunistic yeast infections: candidiasis, cryptococcosis, trichosporonosis and geotrichosis. *JDDG Journal Der Deutschen Dermatologischen Gesellschaft*, 11(5), 381–394.

Vélez, A. C. C. (2022a). Brassica juncea (mustard) [Dataset]. *In CABI a Compendium*.

Verma, S., Tiwari, B. K., Jaiswal, N., and Pandey, F. K. (2022). Evaluation of antimicrobial activity of Brassica juncea leaves against different strains of bacteria. *Bio Science Research Bulletin, 38*(1), 26–34.

Vetrivel, A., Ramasamy, M., Vetrivel, P., Natchimuthu, S., Arunachalam, S., Kim, G., and Murugesan, R. (2021). Pseudomonas aeruginosa Biofilm Formation and Its Control. *Biologics*, 1(3), 312–336.

Watkins, R. R., Holubar, M., and David, M. Z. (2019). Antimicrobial Resistance in Methicillin-Resistant Staphylococcus aureus to Newer Antimicrobial Agents. *Antimicrobial Agents and Chemotherapy*, 63(12). e01216- 19.

Whaley, S. G., Berkow, E. L., Rybak, J. M., Nishimoto, A. T., Barker, K. S., and Rogers, P. D. (2017). Azole Antifungal Resistance in Candida albicans and Emerging Non-albicans Candida Species. *Frontiers in Microbiology*, 7:2173

Yimana, M., and Tesfaye, J. (2022). Isolation, identification and antimicrobial profile of methicillin- resistant Staphylococcus aureus from bovine mastitis in and around Adama, Central Ethiopia. *Veterinary Medicine and Science*, 8(6), 2576–2584.

Zainulabdeen, S. M. S., and Dakl, A. A. (2021). "Review Article Pathogenicity and virulence factors in Staphylococcus aureus. *Muthanna Journal of Pure Science*, 8(1), 109–119.

Zhang, C., Wu, S., Chen, X., Yang, H., Feng, W., Yuan, T., and Wang, Y. (2024). Clinical manifestations and treatment of candidemia caused by different Candida species: a retrospective study. *BMC Infectious Diseases*, 24(1)., 3-8.

Zhang, Q., Yan, Z., Meng, Y., Hong, X., Shao, G., Ma, J., Cheng, X., Liu, J., Kang, J., and Fu, C. (2021). Antimicrobial peptides: mechanism of action, activity and clinical potential. *Military Medical Research*, 8(1).1-25.

Zhu, Z., Hu, Z., Li, S., Fang, R., Ono, H. K., and Hu, D. (2023). Molecular Characteristics and Pathogenicity of Staphylococcus aureus Exotoxins. *International Journal of Molecular Sciences*, 25(1), 395 .1-25

## APPENDIX I

PLANT VERIFICATION CERTIFICATE

---

*Prof. Akinnibosun Henry Adewale* (FLS, MRSB; London)
Faculty of Life Sciences,
Department of Plant Biology and Biotechnology,
P. M. B. 1154 Ugbowo, 300283 Benin City,
Edo State, Nigeria.

*University of Benin*

**Department of Plant Biology and Biotechnology**

**Herbarium Unit**

**Faculty of Life Sciences**

**University of Benin, Benin City, Edo State**

**Plant Name:** *Brassica juncea* (L.) Czern.

**Family**: Brassicaceae

**Common Name:** Brown Mustard seed, Oriental mustard, Mustard greens, Vegetable mustard

**Voucher Number:** UBH-B577

**Student Name:** Akele Sophia

**Plant Identification and Voucher Number Issued by:**

01/08/2025

Prof. Akinnibosun Henry Adewale (FLS, MRSB; London, MSWS; USA, MBOSON, MECOSON, MAEIAN, MFBAN; Nigeria)

---

## APPENDIX II

ETHICAL APPROVAL CERTIFICATE

RESEARCH ETHICS COMMITTEE
COLLEGE OF MEDICAL SCIENCES
UNIVERSITY OF BENIN, BENIN CITY, NIGERIA.

Chairman: Prof. F. A Imarhiagbe
MBChb, FMCP
Cert Clin Res and ethics (NIH), MD.
0803449092

Email: researchethics.cms@gmail.com

P.M.B 1154, BENIN CITY

Our Ref: CMS/REC/01/VOL.2/779

Date: 18th September, 2025

Re: ANTIMICROBIAL EFFECTS OF METHANOLIC EXTRACT OF BROWN MUSTARD SEED ON SELECTED CLINICAL ISOLATE

Name of Principal Investigator: AKELE, SOPHIA
Department Of Medical Laboratory Science,
School of Basic Medical Science
College of Medical Sciences,
University of Benin

REC Approval No: CMS/REC/2025/779

This is to inform you that the research described in the submitted proposal, the Informed Consent Forms and other participant information materials have been reviewed and approved by the College Research Ethics Committee, University of Benin.

This approval dates from 18th September, 2025 to 19th September, 2026. In multi-year research, Endeavour to submit your annual report to the REC early in order to obtain renewal of your approval and avoid disruption of your research.

The National Code of Health Research Ethics requires you to comply with all institutional guidelines, rules and regulations and with the tenets of the code including ensuring that all adverse events are reported promptly to the REC. No, changes are permitted in the research without prior approval by REC except in circumstances outlined in the code. REC reserves the right to conduct compliance visit to your research site without prior notice. Thank you.

PROF. F.A IMARHIAGBE
Chairman, REC

*Promoting best ethical & scientific standard for research in Nigeria*

**APPENDIX III**

MACERATION OF THE EXTRACT


TECNO
AI CAMERA