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# *Melia azedarach* L. (Meliaceae): Phytochemical Analysis and Evaluation of Antibacterial Activity of Fruit Extracts.

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## ABSTRACT

### Background
Medicinal plants are still the main source of therapeutic substances for treating infectious diseases that seriously endanger human health in South Africa. The current study examined the potential therapeutic applications of the young, ripe, and mature fruits of *M. azedarach.*

### Methods
A standard protocol, which included chemical reagents and a series of reactions, was used to determine the presence of the phytochemical compound classes. The methanol and hexane extract of young, ripe, and mature fruits were applied to six bacterial strains (*Methicillin-resistant Staphylococcus aureus* (MRSA), *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Escherichia coli, Salmonella typhimurium*, and *Staphylococcus aureus*) to evaluate their antibacterial activity.

### Results
Methanol extracts of young, ripe, and mature fruits tested positive for six bioactive compounds. Hexane extracts of young, ripe, and mature fruits tested positive for four bioactive compounds. All six bacterial strains were highly susceptible to the methanol extract of fruits. *Klebsiella pneumoniae* and *P. aeruginosa* were strongly resistant to hexane extracts of the young fruits. *Klebsiella pneumoniae*, *E. coli*, MRSA, and *P. aeruginosa* were strongly resistant to hexane extracts of the ripe fruits. *Klebsiella pneumonia* and *E. coli* were strongly resistant to hexane extracts of the mature fruits.

### Conclusion
*Melia azedarach* fruits, whether young, ripe, or mature, contain bioactive therapeutic compounds (Carbohydrates, Amino acids, Alkaloids, Flavonoids, Saponins, Sterols, Steroids/Terpenoids, Phenols, Mucilage and Gums, Fixed oils, and fats) that can be used to develop medicines to treat various human ailments and display strong antibacterial potential.

### Recommendations
Future research is needed to evaluate each bioactive compound's antibacterial activity and efficacy to determine which can be used as components in producing antibacterial medicines and drugs.

***Keywords:*** *Melia azedarach*; *Bioactive compounds; Antibacterial activity; Inhibition zone; Resistance*
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## INTRODUCTION

Plants are a fundamental source of medicines for a myriad of ailments and chronic diseases (Rahman et al., 2015; Nerome et al., 2018). The extracts of roots, stems, bark, leaves, fruits, and other parts of medicinal plants have therapeutic applications as they provide antibiotics against bacterial infections [Rahman et al., 2015; Fufa et al., 2018). Other parts of medicinal plants are chewed and ingested while fresh for immediate healing effect. Over the last three to four decades, the usage of medicinal plants has grown exponentially due to the prominent antibiotic-resistant bacteria that threaten human health today (Rahman et al., 2015; Nerome et al., 2018; Abbas et al., 2017).

Historically, indigenous people have used medicinal plants to treat various infectious ailments and chronic diseases

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(Fufa et al., 2018; Wynberg, 2002). However, chronic infectious diseases and bacterial infections are, unarguably, still a dominant and leading cause of death in South Africa and worldwide (Wynberg, 2002; Nasrullah et al., 2012; Fufa et al., 2018). For example, bacterial sepsis is an emerging leading cause of many deaths (between 17.9% to 59% mortality rate or 5.3 million deaths annually) worldwide, and the mortality rate is expected to skyrocket in developing countries in Africa due to resource limitations (Ndadane et al., 2019). This shows an urgent need for an effective approach to developing new antibiotic drugs for all communicable diseases threatening public health today (Nerome et al., 2018). Therefore, scientists and health professionals have embarked on a quest to research effective antibiotics and antibacterial drugs for common infectious diseases through traditional medicinal plants (Pokhrel and Neupane, 2021; Maroyi, 2017). Indigenous Knowledge Systems (IKS) have been an important institution for many local rural communities in terms of ethnomedicines in South Africa (SA) and worldwide as reservoirs of traditional medicinal plant knowledge (Maroyi, 2017; Raj et al., 2018). In SA, almost every plant has medical applications, and *Melia azedarach* is one of these medicinal plants (Mulaudzi, 2012).

*Melia azedarach*, also known as Chinaberry or Persian Lilac, is a deciduous tree from the Meliaceae family (Lusweti et al., 2011). This species, which is native to the Indian subcontinent and Southeast Asia, has gained popularity for its versatility in traditional medicine as well as its ecological adaptability to a variety of climates (Lusweti et al., 2011). Beyond its ornamental value, *M. azedarach* contains a plethora of phytochemical constituents that contribute to a variety of medicinal properties, particularly antibacterial activity.

*Melia azedarach's* phytochemical profile contains a diverse range of bioactive compounds, including limonoids, alkaloids, flavonoids, and terpenoids, many of which have shown promise in combating pathogenic bacteria (Lusweti et al., 2011; Mabona and Van Vuuren, 2013). These secondary metabolites play an important role in the tree's therapeutic applications, especially in traditional medical systems where *M. azedarach* is used to treat infections and other ailments (Marino et al., 2015). Recent research has highlighted *M. azedarach's* antibacterial properties, indicating that it could be used as an alternative or complementary antimicrobial therapy (Marino et al., 2015). Only a few studies have been conducted on the antibacterial activity of *M. azedarach* extracts from green (young), yellow (ripe), and brown (mature) fruits. According to Sanna et al 2015, the potential antiviral and antibacterial activity of limonoids from *M. azedarach* has received little attention. Tilney et al 2018, stated that *M. azedarach* contains many medicinal bioactive compounds, highlighting the need for further research into their medicinal use or pharmaceutical applications. The purpose of selecting the green, yellow, and mature stages of *M. azedarach* fruits was to identify the fruiting stage with the most bioactive compounds against bacterial subjects. It was predicted that the extract of young fruits will have higher and more effective antibacterial activity compared to ripe and mature fruits. This study investigates the phytochemistry of *M. azedarach*, fruit extracts, and evaluation of its antibacterial effects. By examining M. azedarach's phytochemical and antibacterial profile, this study aims to provide insights into its medicinal potential and possible applications in developing novel antibacterial therapies.

## MATERIALS AND METHODS

### Sample collection and preparation

The samples of young, ripe, and mature fruits of *M. azedarach* were collected in 2019 from 2 trees near the stream along Mgudulu Road (29.8123955 S, 30.9467489,680 E) and 3 trees at Papwa Sewgolum Golf Course (29.8012154 S, 30.9694767,732 E) in Reservoir Hills, Durban, KwaZulu-Natal, South Africa. Ten infructescences with a varying number of fruits were sampled from each tree. The samples of fruits were brought into The University of KwaZulu-Natal (UKZN)-Westville Campus laboratory to record morphometrics (length, width, and mass), cleaning, extraction, and phytochemical analysis.

### Drying of samples

Following the drying procedure by Rocha et al. 2011, the fruit samples were dried to allow for no enzymatic activity in each used fruit sample. In fresh, undried samples, enzymes cause phytochemical compounds to oxidize. Drying at 30 0C avoided burning the sample and evaporation of volatile chemical compounds from fruits or any used plant sample. Drying killed insects and their eggs to ensure the samples were clean. Drying also enhanced the grinding of the samples to fine powder and prolonged the shelf life of the sample material. After drying, the skins of the fruits were ground to a fine powder using a Premium Coffee grinder to allow phytochemical extraction (Figure 1).

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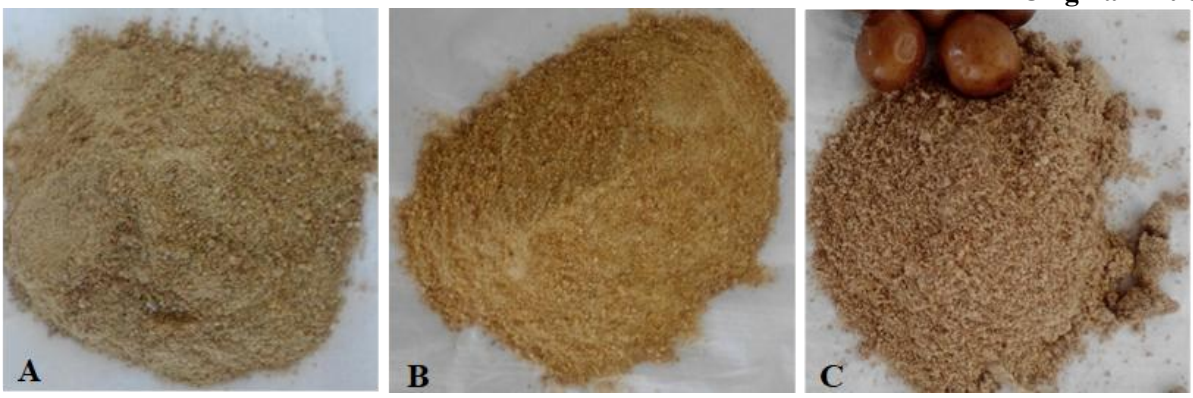


**Figure 1: Young (A), ripe (B), and mature (C) fruits grounded into fine powder for phytochemical extraction.**

## Fruit extraction

A polar (90% methanol) and non-polar (75% hexane) solvent were used for extraction.

A 500 milliliter (ml) conical flask, magnetic bar, and magnetic stirrer were prepared for usage. A 65.69 g of green fruit powder was poured into a 500 ml conical flask and inserted a magnetic bar. Then, 250 ml of 90% methanol was poured into the 500 ml conical flask with 65.69 g of green fruit powder. The flask was placed onto the magnetic stirrer and stirred for 48 hours. After 2 days, the solution was filtered into a 300 ml small honey jar and sealed to airtight to avoid methanol evaporation. The methanol extract was done for green, yellow, and brown fruits. The same procedure was followed for 75% hexane extract for 3 fruit category powders.

## Phytochemical Screening

After phytochemical extraction, the crude extracts from young, ripe, and mature fruits were screened for bioactive compound classes (Figure 2).

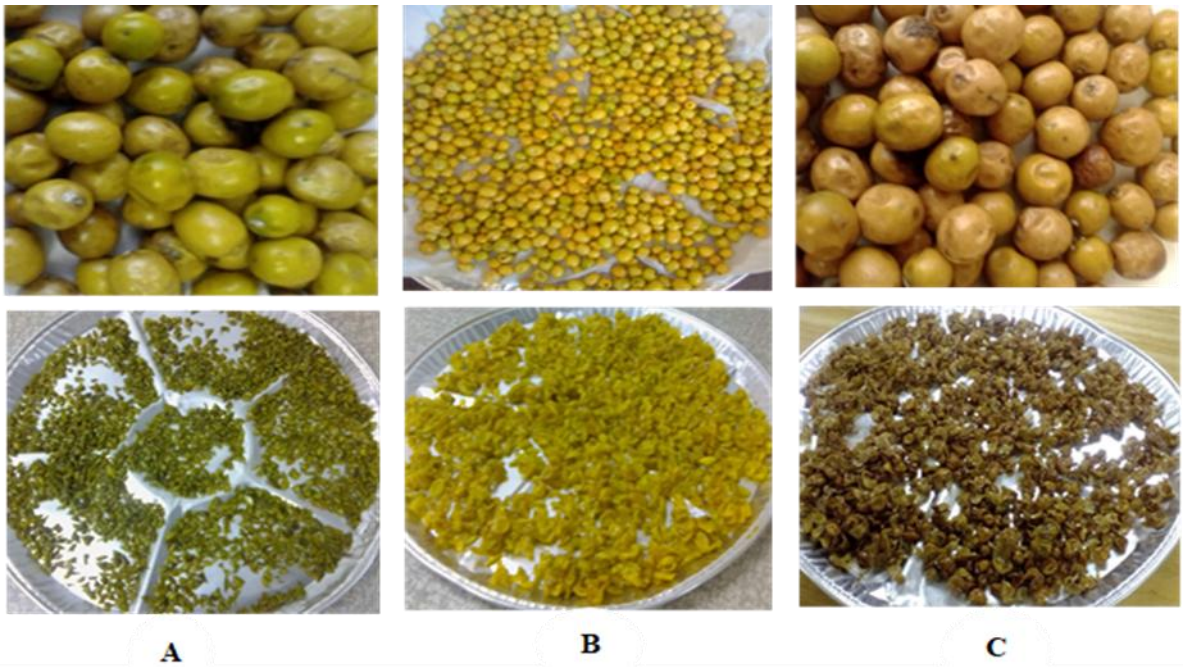


**Figure 2: Unpeeled and peeled young (A), ripe (B), and mature (C) fruits of *Melia azedarach*.**

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### Test for alkaloids

**Wagner's test** - two drops of Filtrates Wagner's reagent was added to one milliliter of extract from young, ripe, and mature fruits. The formation of a brown/reddish precipitate indicated the presence of alkaloids.

### Test for amino acids

**Ninhydrin test** - one drop of Ninhydrin reagent was added to one milliliter of extract. A change in color to purple indicated the presence of amino acids.

### Test for Carbohydrates

**Benedict's test** – to one milliliter of extract, one milliliter of Benedict's reagent was added and boiled in a water bath for two minutes. The formation of an orange-red precipitate indicated the presence of carbohydrates.

**Fehling's test** – to one milliliter of extract, one milliliter each of Fehling's A and B were mixed and boiled in a water bath. The formation of red precipitate confirmed the presence of carbohydrates.

**Molisch test** – in a test tube, one milliliter of the extract was treated with one drop of alcoholic α-naphthol solution. After mixing, one milliliter of concentrated hydrochloric sulphuric acid (H2SO4) was poured along the sides of the test tube. The formation of violet or purple rings at the junction of the two liquids was indicative of the presence of carbohydrates.

### Test for flavonoids

**Lead acetate test** – to five milliliters of extract, one milliliter of 5% lead acetate solution was added. A yellow precipitate formation indicated the presence of flavonoids.

### Test for fixed fats and oils

**Filter paper test** – two drops of the extract were pressed between two filter papers (Whatman No.1). Oil stains on the filter paper were a positive indication of the presence of fixed oils in the extract.

### Test for mucilage and gums

**Ruthenium red test** - two drops of 0.5% ruthenium red solution were added to one milliliter of extract. A pink-to-red color change indicated the presence of mucilage.

### Test for phenols

**Ferric chloride test** - two drops of 0.5% ferric chloride solution were added to one milliliter of extract. The formation of green or black precipitate or color change indicated a positive test for phenolics.

### Test for saponins

**Foam test** – in a test tube, one milliliter of the extract was mixed with four milliliters of water for fifteen minutes. A layer of foam that persisted for ten minutes indicated the presence of saponins.

### Test for sterols

**Salkowski's test** – two milliliters of extract were mixed with three milliliters of chloroform and two drops of concentrated sulphuric acid were poured down the side of the test tube. The formation of a red ring between the solvent layers and a green, fluorescent ring below indicated a positive test for cholesterol.

### Antibacterial Assays

Bacteria were obtained from the Discipline of Pharmaceutical Sciences, UKZN Westville Campus. Six extracts of fruits (3 methanol extracts from young, ripe, and mature fruits, and 3 extracts of hexane from young, ripe, and mature fruits) and 6 bacteria cultures were prepared.

Following the screening procedure by Singh *et al*. (2018), preliminary antibacterial screening of young, ripe, and mature fruit extracts was carried out against 2-gram-positive bacteria (*Staphylococcus aureus* ATCC 25923 and *Staphylococcus aureus* Rosenbach ATCC BAA-1683 (*Methicillin-resistant S. aureus*, MRSA)) and 4 gram-negative bacteria (*Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* ATCC 31488, *Escherichia coli* ATCC 25922 and *Salmonella typhimurium*).

The bacteria were grown overnight in Nutrient Broth (Biolab, South Africa) at 37 ℃ in a shaking incubator (100 revolutions per minute, rpm). The bacterial concentration was adjusted to 0.5 McFarland's Standard with sterile distilled water using a DEN-1B McFarland densitometer (Latvia). Mueller-Hinton agar (MHA) plates (Biolab, South Africa) were lawn inoculated with the prepared bacterial suspensions using a sterile swab, and 5 µg × ml−1 of the extracts were spotted onto the MHA plates. The plates were incubated at 37 ℃ for 24 hours.

After incubation, the plates were read to determine antibacterial activity denoted by areas or zones of inhibition (clearing zone) where the plant extracts were

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placed. The diameter of the clearing zone or zone of inhibition produced by the extract was measured in centimeters (cm) using a 30 cm ruler. The zone of inhibition was compared among the six plates with bacterial strains to determine the effectiveness of the extracts of the young, ripe, and mature fruits on each bacterium. Each antibacterial test had one replicate to make it 6 replicates for evaluating the antibacterial activity of fruits.

## RESULTS

### Phytochemical Screening

Carbohydrates tested positive (+) in methanol and hexane extracts for all fruit categories (young, ripe, and mature). Amino acids and saponins tested negative (–) in hexane extract for all fruit categories. For amino acids, a brown meniscus was observed instead of a deep purple color. However, for saponins, a pale-yellow thick layer or separation formed on top of the solution in the test tube and a colorless solution below it in the hexane extracts, while methanol extracts showed a white foam for a positive test. All other methanol and hexane extracts tested positive for all fruit categories (Table 1).

**Table 1: Bioactive phytochemical compounds detected in the methanol and hexane extracts of young, ripe, and mature fruits of *Melia azedarach*.**

| | | Methanol extracts | | | Hexane extracts | | |
|---|---|---|---|---|---|---|---|
| Compound | Test | Young | Ripe | Mature | Young | Ripe | Mature |
| Carbohydrates | Fehlings | + | + | + | + | + | + |
| Amino acids | Ninhydrin | + | + | + | – | – | – |
| Alkaloids | Wagner's | + | + | + | + | + | + |
| Flavonoids | Lead acetate | + | + | + | + | + | + |
| Saponins | Foam | + | + | + | – | – | – |
| Sterols | Sterols | + | + | + | + | + | + |
| Steroids/Terpenoids | Chloroform | + | + | + | + | + | + |
| Phenols | Phenols | + | + | + | + | + | + |
| Mucilage and Gums | Ruthenium Red | + | + | + | + | + | + |
| Fixed oils and fats | Filter paper | + | + | + | + | + | + |

Note: + indicates presence, – indicates absence of compound.

### Antimicrobial activity

After incubation of gram-positive and gram-negative bacteria (Table 2), plates were read to evaluate the antibacterial activity of fruits. Antibacterial activity was denoted by the inhibition zone where fruit extracts were applied (Figure 3). *Pseudomonas aeruginosa, Methicillin-resistant Staphylococcus aureus (MRSA), Klebsiella pneumoniae, Staphylococcus aureus, Salmonella typhimurium,* and *Escherichia coli* were highly susceptible to the methanol extracts of young, ripe, and mature fruits.

**Table 2: Gram-positive and gram-negative bacterial stains used to test for antibacterial activity of the extracts of young, ripe, and mature fruits of *Melia azedarach*.**

| Gram-positive | Gram-negative |
|---|---|
| *Methicillin-resistant staphylococcus aureus* (MRSA). | *Pseudomonas aeruginosa* (Pa) |
| *Staphylococcus aureus* (Sa). | *Escherichia coli* (*E. coli*) |
| | *Klebsiella pneumoniae* (Kp) |
| | *Salmonella typhimurium* (St) |

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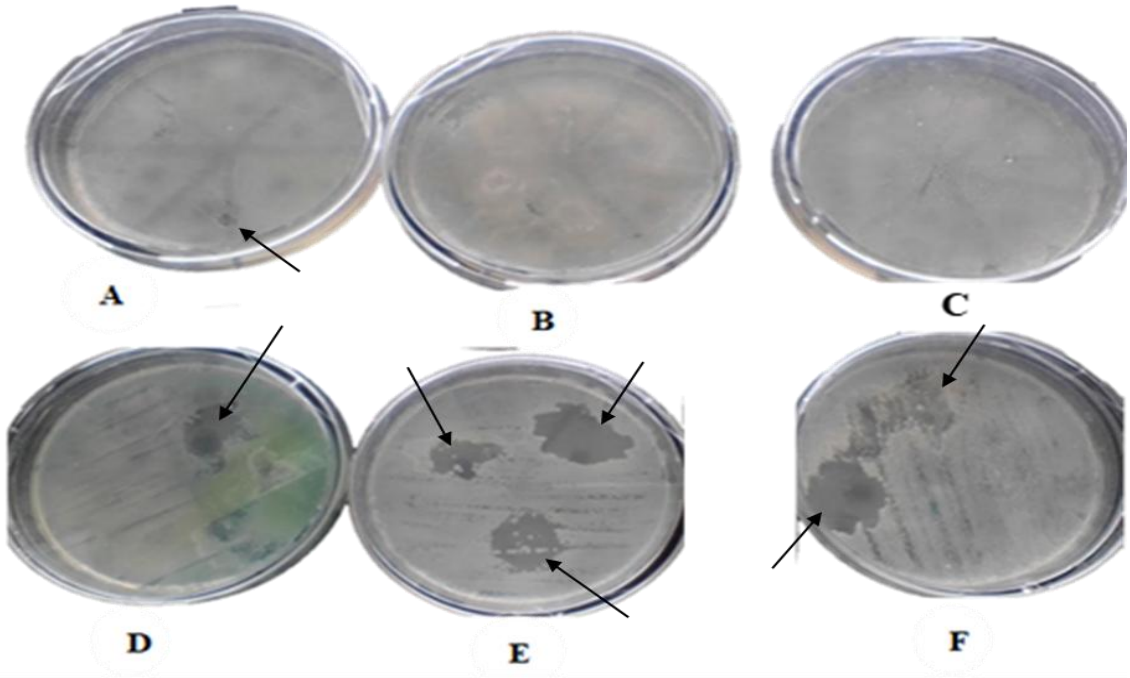


**Figure 3: Hexane and methanol extracts from young (A and D), ripe (B and E), and mature (C and F) fruits of *Melia azedarach*, respectively**

Arrows indicate the zone of inhibition, where bacteria did not grow due to the application of fruit extracts. The absence of an inhibition zone indicates the resistance of bacteria to fruit extract. Hexane extracts: A, B, C; Methanol extracts: D, E, F.

The zone of inhibition or inhibitory zone (Table 3) is the area of media where bacteria are unable to grow, due to the presence of a drug (fruit extracts, in this case) that impedes their growth (Balouiri *et al*., 2016). Zeros (0) denoted no zone of inhibition; the bacteria were resistant to fruit extract.

Methanol and hexane extracts of the young and mature fruits had a larger inhibition zone compared to the methanol and hexane extracts of the ripe fruits. Both methanol and hexane extract of the young and mature fruits were highly effective and toxic against bacterial strains. However, in other bacterial strains, the diameter of the inhibition zone was very small to no inhibition zone when methanol or hexane extract of the ripe fruits was applied (Figure 3).

*Escherichia coli* was partially resistant to the methanol extract of the ripe fruits and the hexane extract of the young fruits. *Klebsiella pneumoniae* and *P. aeruginosa* had no inhibition zone when the extract of hexane from the young fruits was applied. *Klebsiella pneumoniae*, *E. coli, Methicillin-resistant Staphylococcus aureus* (MRSA), and *P. aeruginosa* had no inhibition zone when the extract of hexane from the ripe fruits was applied. Moreover, *K. pneumoniae* and *E. coli* had no inhibition zone when the extract of hexane from the mature fruits was applied (Table 3).

**Table 3: Diameter (cm) of inhibition zone of methanol and hexane extracts from young, ripe, and mature fruits of *Melia azedarach***

| | Methanol extracts | | | Hexane extracts | | |
|---|---|---|---|---|---|---|
| Bacterial strain | Young | Ripe | Mature | Young | Ripe | Mature |
| *Pseudomonas aeruginosa* (Pa) | 2.3 | 1.5 | 2 | 0 | 0 | 2.5 |
| *Methicillin-resistant Staphylococcus aureus* (MRSA) | 2.8 | 1.3 | 1.8 | 2.5 | 0 | 1.5 |
| *Escherichia coli (E. coli)* | 1.7 | 0.5 | 1 | 0.5 | 0 | 0 |
| *Salmonella typhimurium* (St) | 1.3 | 1.5 | 1.5 | 1 | 1 | 1.5 |
| *Klebsiella pneumoniae* (Kp) | 2.5 | 2 | 2.5 | 0 | 0 | 0 |
| *Staphylococcus aureus* (Sa) | 1 | 2 | 1.6 | 1.5 | 1 | 2.6 |

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## Controls

Methanol was effective against the rest of the bacteria, except *P. aeruginosa*. Moreover, *P. aeruginosa*, *Methicillin-resistant Staphylococcus aureus Staphylococcus* (MRSA), *K. pneumoniae*, and *S. aureus* were resistant to hexane. Nevertheless, hexane was effective against *E. coli* and *S. typhimurium* bacteria only.

## Methanol extracts

The methanol extract of young fruits was highly effective against *Methicillin-resistant Staphylococcus aureus Staphylococcus* (MRSA), *K. pneumoniae*, *P. aeruginosa*, *E. coli, S. typhimurium*, and *S. aureus* bacteria, respectively. The methanol extract of ripe fruits was effective against *K. pneumoniae* and *S. aureus* bacteria. However, the methanol extract of ripe fruits was partially active against *E. coli*, *MRSA*, *P. aeruginosa*, and *S. typhimurium*. The methanol extract of mature fruits was highly effective against *K. pneumoniae* bacteria. The methanol extract of mature fruits was also effective against *P. aeruginosa*, *MRSA*, *S. aureus*, *S. typhimurium*, and *E. coli* bacteria, respectively (Table 3).

## Hexane extracts

The hexane extract of young fruits was highly effective against *Methicillin-resistant Staphylococcus aureus Staphylococcus* (MRSA), *S. aureus*, *S. typhimurium,* and *E. coli* bacteria, respectively. *Pseudomonas aeruginosa* and *K. pneumoniae* were resistant to the hexane extract of young fruits. Moreover, *P. aeruginosa*, *Methicillin-resistant Staphylococcus aureus Staphylococcus* (MRSA), *E. coli,* and *K. pneumoniae* bacteria were resistant to the hexane extract of ripe fruits. However, the hexane extract of the ripe fruits was effective against *S. typhimurium* and *S. aureus* bacteria. The hexane extract of the mature fruits was highly effective against *S. aureus* and *P. aeruginosa* bacteria, respectively. In addition, the hexane extract of mature fruits was effective against *Methicillin-resistant Staphylococcus aureus Staphylococcus* (MRSA) and *S. typhimurium* bacteria. However, *E. coli* and *K. pneumoniae* bacteria were resistant to the hexane extract of mature fruits.

# DISCUSSION

The present study revealed that the fruit extracts of Melia azedarach L. (Meliaceae) possess significant phytochemical constituents and exhibit antibacterial activity against various bacterial strains. These findings align with previous studies demonstrating the pharmacological potential of this plant.

## Phytochemical screening

The phytochemical analysis of *M. azedarach* fruits confirmed the presence of alkaloids, flavonoids, tannins, saponins, and terpenoids. These secondary metabolites are well-known for their therapeutic properties. For instance, flavonoids and tannins are recognized for their antioxidant and antimicrobial activities (Kumar et al., 2021). Similarly, saponins have been implicated in disrupting bacterial membranes, thereby enhancing antibacterial efficacy (Raut et al., 2019).

Comparable findings were reported by Singh et al. (2018), who identified similar phytoconstituents in M. azedarach leaves and bark. However, the higher concentration of flavonoids and terpenoids in fruit extracts, as observed in this study, suggests a unique metabolic profile that may contribute to their distinct antibacterial activity. The variability in phytochemical composition may be attributed to factors such as geographical location, environmental conditions, and extraction methods, as noted by Ghareeb et al. (2020).

## Antibacterial activity

The antibacterial activity of *M. azedarach* fruit extracts was tested against both Gram-positive and Gram-negative bacterial strains, with encouraging results. The ethanolic extract had the highest antibacterial activity, especially against *Escherichia coli* and *Staphylococcus aureus*. These findings are consistent with those of Munir et al. (2017), who found that *M. azedarach* extracts have potent antibacterial activity against similar pathogens.

*Klebsiella pneumoniae* (KP) and *P. aeruginosa* (PA) showed strong resistance to the extract of hexane from the young fruits. *Klebsiella pneumoniae*, *E. coli*, MRSA, and *P. aeruginosa* showed strong resistance to the extract of hexane from the ripe fruits. *Klebsiella pneumoniae* and *E. coli* showed strong resistance to the hexane extract of mature fruits. *Klebsiella pneumoniae* was resistant to the extract of hexane from the young, ripe, and mature fruits (Figures 3 and 4).

The methanol extract of young and mature fruits showed the highest antibacterial activity against MRSA, *K. pneumoniae*, *P. aeruginosa*, *E. coli*, *S. typhimurium*, and *S. aureus*, indicating the potential of young and mature fruits of *M. azedarach* against pathogenic microorganisms that cause infectious diseases to humans. The methanol extract of ripe fruits also showed effectiveness against *K. pneumoniae* and *S. aureus*, *P. aeruginosa*, *S. typhimurium*, and MRSA, and partially active against *E. coli*, respectively.

The hexane extract of the young fruits was effective against MRSA, *S. aureus*, *S. typhimurium*, and partially active against *E. coli*. *Pseudomonas aeruginosa* and *K.*

*pneumoniae* bacteria showed resistance to the extract of the young fruits. The hexane extract of the ripe fruits was highly effective against *S. typhimurium* and *S. aureus*. However, *P. aeruginosa*, MRSA, *E. coli*, and *K. pneumoniae* showed resistance to the hexane extract of the ripe fruits. The hexane extract of the mature fruits was highly active against *S. aureus*, *P. aeruginosa*, MRSA, and *S. typhimurium*. *Escherichia coli* and *K. pneumoniae* showed resistance to the hexane extract of the mature fruits.

The efficacy of the extract of the young and mature fruits indicated a high concentration of toxins in mature green and brown fruits. This shows the potential antibacterial and therapeutic applications of the young and mature fruits of *M. azedarach*. The resistance of bacteria to the ripe fruits indicated the decreased toxicity and efficacy due to the high sugar content (Marino et al., 2015). This was evidenced by the frequent visits of frugivores to the trees to eat the ripe fruits. When the fruit ages from ripe to mature there is a breakdown of cells and anatomical changes, releasing toxins that have antibacterial properties (Borges et al., 2016).

The resistance to the hexane extract of the young, ripe, and mature fruits of *M. azedarach* could be a result of changes in the membrane permeability of bacteria, making it difficult to penetrate or kill with toxins of fruits or antibiotics (Li et al., 2015). Enzymatic degradation also results in the inactiveness of the antibiotics from the ripe and mature fruits of *M. azedarach* (Bistrović et al., 2018). Moreover, bacteria may alter their proteins that are antibacterial targets, developing more resistance mechanisms and strategies (Domalaon et al., 2018).

The antibacterial activity results for the current investigation correlate with Abbas et al. 2017, where ethanolic extracts of the flowers of *M. azedarach* showed effective antioxidant and antifungal activity against *Micrisporum canis, Aspergillus fumigatus, Aspergillus niger*, and *Aspergillus flavus*. Akihisa et al. 2013, found that limonoids from the fruits of *M. azedarach* have high cytotoxic activities against human cancer cells. In addition, Balouiri et al. 2016, and Fufa et al. 2018, also found excellent antimicrobial and antibacterial activity in the leaves and stem bark of *M. azedarach.*

The observed antibacterial activity is due to the synergistic effects of the identified phytochemicals. Alkaloids and flavonoids, for example, have been demonstrated to inhibit bacterial enzymes and disrupt cell membranes, resulting in cell death (Ali et al., 2022). The pronounced efficacy of ethanolic extracts over aqueous ones is consistent with previous research indicating that ethanol is more effective in extracting bioactive compounds with antimicrobial properties (Ghasemzadeh and Jaafar, 2014).

## CONCLUSION

The results highlight *M. azedarach* as a promising candidate for developing plant-based antibacterial agents. Future research should focus on isolating and characterizing individual bioactive compounds to understand their specific mechanisms of action. Additionally, investigating the synergistic effects of combining *M. azedarach* extracts with conventional antibiotics could help address antibiotic resistance issues.

Future studies are required to assess the antibacterial activity and efficacy of each (carbohydrates, amino acids, alkaloids, flavonoids, saponins, sterols, steroids and terpenoids, phenols, mucilage and gums, and oils and fats) bioactive compound to determine which can be constituted as components in the manufacturing of antibacterial medicines and drugs. In addition, it is recommended to test or evaluate the most effective antibacterial bioactive compound on human health and toxicity (toxicity testing) to humans for pharmaceutical purposes.

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South African Medical Research Council

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## AUTHOR CONTRIBUTIONS

Mr. Masuku was responsible for conceptualizing the project, data collection and analysis, and writing the manuscript. Dr Singh and Prof Baijnath assisted with data analysis and editing of the manuscript**.**

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