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# EXPLORING THE ANTIBACTERIAL AND ANTIFUNGAL ACTIVITIES OF MEDICINAL PLANTS IN BIU LOCAL GOVERNMENT AREA OF BORNO STATE ON BACTERIAL AND FUNGAL PATHOGENS

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## ABSTRACT

The increasing incidence of drug-resistant pathogens raises an urgent need to identify and isolate new bioactive compounds from medicinal plants. This research investigated the phytochemical constituents and antimicrobial activities of *Azadirachta indica, Syzygium cumini* and *Moringa oleifera* collected from three locations (Nasarawa, Assorock and Dugja). The plants were analyzed using standard procedures to identify the presence of various qualitative phytochemical compounds. The experiment was completely randomized with three replications. The results were analyzed using Analysis of Variance (ANOVA) at P<0.05. Six different phytochemicals (alkaloids, flavonoids, saponins, terpenoids, phenolic and tannins), were tested in each plant extracts which showed the presence (+) of phenolics and terpenoids in *Azadirachta indica,* and *Syzygium cumini* but absent (-) in *Moringa oleifera,* alkaloids and tannins were presence (+) in *Moringa oleifera* but absent (-) in *Azadirachta indica,* and *Syzygium cumini.* There is significant reduction in the radial expansion of the bacterial and fungal pathogens using the three medicinal plants in which *Azadirachta indica,* showed the highest inhibition of Bacterial growth; *Staphylococcus aureus* (22.5 mm) and *Escherichia coli* (25.0 mm) followed by *Syzygium cumini* then *Moringa oleifera*. Furthermore, *Syzygium cumini* showed the highest inhibition on *Aspergillus niger* (42.5 mm) and *Aspergillus flavus* (12.5 mm) followed by *Azadirachta indica,* and *Moringa oleifera*. It is concluded that medicinal plants such as *Azadirachta indica, Syzygium cumini* and *Moringa oleifera* have high antimicrobial potency due to the presence of phytochemical constituents and therefore, should be used as a bio-control agents.

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## INTRODUCTION

Medicinal plant such as *Syzygium cumini*, also known as jamun or black plum, belongs to the family Myrtaceae [10][30]. The plant parts such as the leaves are widely used in folk medicine [25][29]. Studies have shown that various extracts of jamun exhibits pharmacological activities such as anti-inflammatory, antifungal, antiviral, antiulcerogenic, cardioprotective, antiallergic, anticancer, antioxidant, and hepatoprotective properties [15][26]. So, endophytes residing in *Syzygium cumini* may contain a lot of biologically active metabolites which can act as antimicrobial agents [8].

Also, plants like *Moringa oleifera* which is one of the World's most useful trees, as almost every part of the tree can be used for food, medication and industrial purposes [16]. *Moringa oleifera* leaves are highly nutritious and as such are highly recommended for infants and nursing mothers especially those from developing countries or area prone to cases of malnutrition [11]. It is also used as antiviral, anti-inflammatory, analgesic [5]. Neem (*Azadirachta indica*), a member of the Meliaceae family, has therapeutics implication in diseases cure and formulation based on the

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fact that neem is also used to treat various diseases [12]. *Azadirachta indica* has various complex constituents including nimbin, nimbidin, nimbolide, and limonoids and such types of ingredients play role in diseases management through modulation of various genetic pathways and other activities [18]. Quercetin and ß-sitosterol were first polyphenolic flavonoids purified from fresh leaves of neem were known to have antifungal and antibacterial activities. Numerous biological and pharmacological activities have been reported including antibacterial, antifungal and anti-inflammatory [19]. Earlier investigators have confirmed their role as anti-inflammatory, anti-arthritic, antipyretic, hypoglycemic, anti-gastric ulcer, antifungal, antibacterial, and antitumor activities [17].

Plants with such antimicrobial activity should be tested against some microbes to inhibit their activity [2]. The application of plant extracts on bacteria and fungi has been studied by a very large number of researchers in different parts of the world [6]. Medicinal plants contain potential source of antimicrobial agents [2]. Many of the plant materials used in traditional medicine are readily available in rural areas at relatively cheaper rate than modern medicine [3][14]. Plant metabolite has been one of the better alternatives as they are known to have minimal environmental impact and danger to consumers in contrast to the synthetic pesticides [14]. Many plants have been used because of their antimicrobial traits, which are due to phytochemicals they contain and synthesized in the secondary metabolism of the plant [3]. Plants contains a wide variety of secondary metabolites such as tannins, alkaloids, phenolic compounds, and flavonoids, which have been found *in vitro* to have antimicrobial properties [5]

The demand for alternative treatment methods to meet primary health care needs and the use of these plant species in Biu Local Government Area of Borno State should be of interest among the rural and urban dwellers. The knowledge of how to use medicinal plant species is generally fast disappearing than the species themselves [21]. In order to preserve these plants and the potential in which they possess the demand for further research on this subject matter should be taken into deep consideration [21], ethnobotanical field of research which includes, the collection, uses and preservation of plants still play important role in the life of the people and therefore the need for this study. Therefore, the aim of this research is to explore the antibacterial and antifungal activities of medicinal plants on Bacteria and Fungi in Biu Local Government Area.

## MATERIALS AND METHOD

### Study Area

This research study was conducted in Biu Local Government Area, within the Nigerian Army University Biu, Borno State Nigeria. The LGA is located in the southern part of Borno State about 210 kilometres away from Maiduguri, the state capital. It is located between Latitudes 10.25$^{0}$N to 11.00$^{0}$N and Longitudes 11.42$^{0}$E to 12.30$^{0}$E with a population of about 175,760 in 2006 and a projected population of 839,460 persons for 2018 at 3.4 % growth rate. It lies on the Biu Plateau at an average elevation of 626 meters above sea level and located in the Northern Guinea savannah agro-ecological zone with a small portion in the Northeast, the Kimba area, which lies on the dryer Sudan Savannah zone [4]. The study area is bordered by Damboa, Chibok and Askira-Uba LGAs, of the State, to the north and Bayo, Kwaya-Kusar and Shani LGAs to the south. The average temperature in Biu Local Government Area is 23.7 $^{o}$C and about 888 mm of precipitation falls annually. There are two cropping season, one that starts with early onset of rain, usually in May and the dry season cropping which starts soon after harvesting of the rain-fed crops between November- December. Agriculture is the main activity in the area. The economy is mixed agriculture based on herding cattle, goats, sheep, horses and donkeys and arable farming of sorghum, millet, maize, cowpea and cotton [4].

### Plant material

Fresh leaves of *Azadirachta indica, Moringa oleifera,* and *Syzygium cumini* were collected from three different location of Biu Local Government Area (Nasarawa, Assrock and Dugja). The plants were identified taxonomically and authenticated at the Herbarium, Department of Biology, Faculty of Natural and Applied Sciences, Nigerian Army University Biu with voucher number (N-Bio#000-0505-8). These fresh leaves were washed thoroughly 2 - 3 times with running tap water and then rinsed with distilled water, it was then air-dried and pulverized into powder using sterile muter and pestle for extraction.

### Test Microorganisms

Bacteria (*Staphylococcus aureus* and *Escherichia coli)* and fungi (*Aspegillus flavus* and *Aspergillus niger*) used for the assay were collected from the laboratory, Department of Biology, faculty of natural and applied science, Nigeria Army University Biu. The entire test organisms were maintained on nutrient agar media (BHIWADI-301-019: Rajasthan, India).

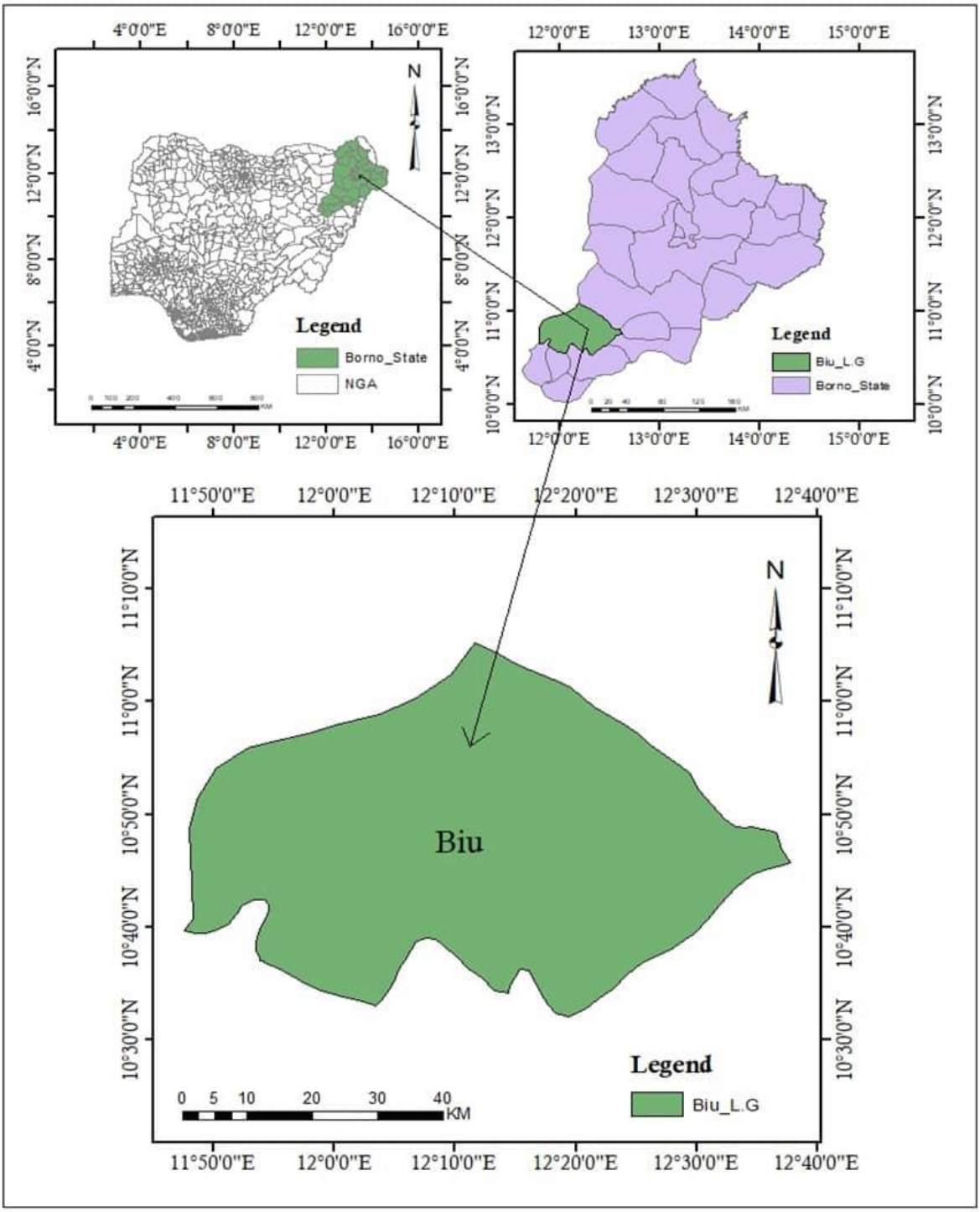



*Figure 1: Map of the Study Area, (Biu Town). Source: Modified from the Administrative Map of Borno State [1].*

**Preparation of Aqueous Plant Extracts**

In each of the pulverized plant materials, 30 grams of the powder was macerated separately with 100 ml of sterile distilled water and stored at room temperature for 24 hours. The macerates were first filtered through four layer of muslin cloth and then the filtrates were centrifuged at 8,000 rpm
for 15 min at room temperature. The Supernatant were filtered through Whatman No.1 filter paper and heat sterilized at 120ºC for 30 min. The extract was preserved aseptically in a brown bottle at 4ºC until further use [20].

**Qualitative Phytochemical Screening**

The prepared extract of all the three plants were used to test various phytoconstituents present in them. Different chemical reagents were prepared and specific test, for specific phytochemicals was done. The results revealed major phytochemical constituents such as: alkaloids, flavonoids, phenolic compounds, saponins, tannins, and terpenoids in 80 % aqueous extracts of each plant were identified. The tests were done by following standard procedures of [3] [24].

i. **Test for Alkaloids:** To 2 mL of extract, 1 mL of Meyer's reagent was added. The presence of pale-yellow precipitate indicated the presence of alkaloids.
ii. **Test for Flavonoids:** 2 ml of the Extract aqueous solution + few drops 10 % ferric chloride solution was added. The presence of green precipitate indicated the presence of flavonoids
iii. **Test for Phenolics:** 1 mL of the aqueous extract, few drops of diluted Iodine solution was added the presence of a transient red precipitate indicated the presence of phenol.
iv. **Test for Saponin:** 2 g of powdered sample was boiled in 20 mL of distilled water. 10 mL of filtrate, 5 mL of distilled water were quivered vigorously. The appearance of frothing indicated the presence of saponins.
v. **Test for Tannins:** 1 mL of the plant extract, 3 mL distilled water was added + 3 drops 10 % Ferric chloride solution was added the presence of a blue green precipitate indicated the presence of tanins.
vi. **Test for Terpenoids:** 2 ml of aqueous extract was mixed with 2 mL chloroform, 3 mL conc. $H_2SO_4$. The appearance of Reddish-brown coloration indicated the presence of terpenoids.

**Anti-bacterial Assay**

Antibacterial activities of aqueous extracts were determined by well diffusion method on nutrient agar medium (BHIWADI-301-019:Rajasthan, India). Wells were made by using a sterilized cork-borer on the solid nutrient agar in a Petri dish to which the test organism was inoculated, 1 ml of 80 % aqueous plant extract was added into the well. The treatments also included control (without any extracts). The plates were incubated for 3 days at 37ºC for fungi and 24hrs for bacteria. Minimal Zone of inhibition around the wells were measured in mm (millimeter). Each treatment consists of three replicates and repeated at least thrice [9].

**Statistical Analysis**

The experiment was completely randomized with three replications. Data obtained were analyzed using statistical package for social sciences (SPSS) version 20. The results were analyzed using Analysis of Variance (ANOVA) at $P<0.05$. The significant means were separated using the least significant difference (LSD).

**RESULTS**

**Qualitative Determination of Phytochemical Components.**

Table 1 showed the presence (+) of phenolics and terpenoids in *Azadirachta indica,* and *Syzygium cumini* which were absent (-) in *Moringa oleifera,* alkaloids and tannins were presence (+) in *Moringa oleifera* but absent (-) in *Azadirachta indica,* and *Syzygium cumini.* Flavanoids and Saponins are present in all the three extracts. However, the concentration of the phytochemicals varies among the plant materials.

**Minimal Zone of Inhibition using 80 % Aqueous Extracts of *Azadirachta indica. Moringa oleifera,* and *Syzygiumn cumini* Leaves Extracts on Bacterial and Fungal Pathogens**

The Result in Table 2 below shows the significant difference between the inhibition of bacterial pathogens using aqueous extract of *Azadirachta indica* at $P<0.05$. It also shows the variation between the control (without extract) and the pathogens which the extract was applied to and also significant variation between *Staphylococcus aureus* (22.5 mm) and *Escherichia coli* (25.0 mm).

There is significant difference in inhibition between the Fungal pathogens using aqueous extract of *Azadirachta indica* at P<0.05 in Table 3. It also shows the variation between the control (without extract) and the pathogens which the extract was applied to and also significant variation between *Aspergillus niger* (15.0 mm) and *Aspergillus flavus* (12.5 mm).

Table 4 shows the significant difference between the inhibition of bacterial pathogens using aqueous extract of *Moringa oleifera* at P<0.05. It also shows the variation between the control (without extract) and the pathogens which the extract was applied to and also significant variation between *Staphylococcus aureus* (7.5 mm) and *Escherichia coli* (10.0 mm).

The Result in Table 5 shows the significant difference between the inhibition of Fungal pathogens using aqueous extract of *Moringa oleifera* at P<0.05. It also shows the variation between the control (without extract) and the pathogens which the extract was applied to and also significant variation between *Aspergillus niger* (5.0 mm) and *Aspergillus flavus* (7.5 mm)

**Table 1: Qualitative Phytochemicals Composition of *Azadirachta indica, Moringa oleifera,* and *Syzygiumn cumini* Leaves Extracts**

| Phytochemicals | *Azadirachta indica* | *Moringa oleifera* | *Syzygiumn cumini* |
|---|---|---|---|
| Phenolic | + + + | − | + + + |
| Terpenoids | + + | − | + + + |
| Saponins | + + + | + + | + + + |
| Tannins | − | + + | − |
| Flavanoids | + + + | + + + | + + + |
| Alkaloids | − | + + | − |

Keys: + Reactive; ++ Weakly Reactive; +++ Strongly Reactive; Non-Reactive

**Table 2. Minimal Zone of Inhibition of Bacterial Pathogens using Aqueous Extracts of *Azadirachta indica.***

| Pathogens | Zone of Inhibition (mm) |
|---|---|
| *Staphylococcus aureus* | 22.5 |
| *Escherichia coli* | 25.0 |
| Control | 90.0 |
| LSD (0.05) | 0.25 |

**Keys**: Least Significant Difference (LSD)

**Table 3: Minimal Zone of Inhibition of Fungal Pathogens using Aqueous Extracts of *Azadirachta indica***

| Pathogens | Zone of inhibition (mm) |
|---|---|
| *Aspergillus niger* | 15.0 |
| *Aspergillus flavus* | 12.5 |
| Control | 90.0 |
| LSD (0.05) | 0.75 |

**Keys**: Least Significant Difference (LSD)

**Table 4: Minimal Zone of Inhibition of Bacterial Pathogens using Aqueous Extracts of *Moringa oleifera***

| Pathogens | Zone of inhibition (mm) |
|---|---|
| *Staphylococcus aureus* | 7.5 |
| *Escherichia coli* | 10.0 |
| Control | 90.0 |
| LSD (0.05) | 0.25 |

Keys: Least Significant Difference (LSD)

**Table 5: Minimal Zone of Inhibition of Fungal Pathogens using Aqueous Extracts of *Moringa oleifera***

| Pathogens | Zone of inhibition (mm) |
|---|---|
| *Aspergillus niger* | 5.0 |
| *Aspergillus flavus* | 7.5 |
| Control | 90.0 |
| LSD (0.05) | 0.30 |

**Keys**: Least Significant Difference (LSD)

The Result in Table 6 shows the significant difference between the inhibition of bacterial pathogens using aqueous extract of *Syzygium cumini* at P<0.05. It also shows the variation between the control (without extract) and the pathogens which the extract was applied to and also significant variation between *Staphylococcus aureus* (17.5 mm) and *Escherichia coli.* (22.5 mm)

Table 7 shows the significant difference between the inhibition of Fungal pathogens using aqueous extract of *Syzygium cumini* at P<0.05. It also shows the variation between the control (without extract) and the pathogens which the extract was applied to and also significant variation between *Aspergillus niger* (42.5 mm) and *Aspergillus flavus* (12.5 mm).

**Table 6: Minimal Zone of Inhibition of Bacterial Pathogens using Aqueous Extracts of *Syzygium cumini***

| Pathogens | Zone of inhibition (mm) |
|---|---|
| *Staphylococcus aureus* | 17.5 |
| *Escherichia coli* | 22.5 |
| Control | 90.0 |
| LSD (0.05) | 0.33 |

**Keys**: Least Significant Difference (LSD)

**Table 7: Minimal Zone of Inhibition of Fungal Pathogens using Aqueous Extracts of *Syzygium cumini***

| Pathogens | Zone of inhibition (mm) |
|---|---|
| *Aspergillus niger* | 42.5 |
| *Aspergillus flavus* | 12.5 |
| Control | 90.0 |
| LSD (0.05) | 0.67 |

**Keys**: Least Significant Difference (LSD)

## DISCUSSIONS

Plants are a source of large number of secondary metabolites which are claimed to possess the bioactive compounds which are responsible for their antibacterial, antifungal, and, repellent properties [22]. The results in Table 1 revealed the presence of medicinally important chemicals in the leaves of three different medicinal plants (*Azadirachta indica, Moringa oleifera,* and *Syzygiumn cumini*). Among the various qualitative tests done for the presence of secondary metabolites (alkaloids, flavonoids, saponins, terpenoids, phenolic and tannins), in the plant extracts of *Azadirachta indica,* phenolic, tannins, flavanoids and terpenoids were present (in different phytochemical concentrations), while tannins and alkaloids were absent, whereas saponins, tannins, flavanoids and alkaloids were present (in different phytochemical concentrations) in the plant extract of *Moringa oleifera* while phenolic and terpenoids were absent, and phenolic, terpenoids, saponins and flavanoids were present in the plant extract of *Syzygium cumini* (in different phytochemical concentrations) while tannins and alkaloids were absent. [6] conducted a research on the qualitative phytochemicals analysis on the barks of the ethanol extracts of *Moringa oleifera* in Osun State, Nigeria and his results revealed the presence of Cardiac Glycosides, saponins, tannins, flavanoids and alkaloids. [31], also conducted a photochemical screening analysis on *Psidium guajava L* in Mubi Local Government Area of Adamawa State, Nigeria and the tests results shows the presence of alkaloids, flavanoids, tannins, terpenoids, steroids, saponins

and glyceroides his results shows similarities with that of [7] they conducted a screening on the leaves of *Manginefera indica* in Lafia Local Government Area, Nasarawa State Nigeria, there result show the present of Mangiferins, Tannins, Flavonoid , Alkaloids, Terpenoids, Saponins , Glycoside, Phenol and Steroids.

These differences in phytochemical concentrations and constituent were as a result of environmental conditions, aseptic conditions, geographical location, type of plant used, parts of plant used and type of extract used [23].

Also in the study, antimicrobial activities of three medicinal plants *(Azadirachta indica*, *Moringa oleifera, and Syzygiumn cumini),* from Biu Local Government Area were assessed by well diffusion method (using the aqueous leaves extract). In Table 2 The result showed potential antibacterial effects of *Azadirachta indica* extracts against bacterial strains tested, which there was a statistical significant differences between the two bacterial strains and control (without extract) which the pathogens are allowed to grow over all the plate, so as to ascertain the effectiveness of the plates with extract. Also, the extract was tested on *Aspergillus flavus* which has more effect than *Aspergillus niger,* thus showing the antifungal effect of the aqueous leaf extract of *Azadirachta indica* as shown above in Table 3. Similarly, [13] studies has revealed the efficacy of medicinal plants as an alternative therapy in tackling the development and spread of multidrug-resistant pathogens coupled with the toxic effects of some antibiotics. However, the study shows the lack of adequate characterization of the chemical composition of the antimicrobial medicinal plants in Nigeria.

Whereas, in Table 4 *Moringa oleifera* was effective against *Escherichia coli* and there was a minimum inhibition on *Staphylococcus aureus* which also shows a significant difference with the control. In Table 5 The extract also exhibit a minimum inhibition on the fungi tested (*Aspegillus flavus* and *Aspergillus niger*) as compared with the extract of *Azadiratcha indica*, there was also significant differences between both fungi tested and the control. In contrast with the work of [28] which was conducted in Kaduna State Nigeria, this research made used of the ethanol leaf extracts of *Morinda lucida, Tithonia diversifolia* and *Carica papaya* which contained important bioactive components with efficient antibacterial activity against the tested organisms. The outcomes showed the activeness of the ethanol decoction of these plants as bacteria strain dependent. While ethanol extract of *Carica papaya* actively inhibited *Staphylococcus aureus*, extract of *Tithonia diversifolia* showed moderate effects and *Morinda lucida* was not effective.

Lastly, Table 6 *Syzygium cumini* was highly effective on *Escherichia coli* as compared to the inhibition on *Starphylococcus aureus* which there was a statistically significant differences between the pathogens and the control. Table 7 also shows the effectiveness of *Syzygium cumini* which was very effective on *Aspergillus niger* with a low minimum inhibition rate on *Aspergillus flavus* with a significant difference with the control. In contrast [27] also conducted a research in Wukari Local Government Area of Taraba State, Nigeria, on *Senna occidentalis,* this study isolates and characterizes various bacteria, including *Staphylococcus spp*., *Klebsiella pneumoniae*, *Escherichia coli*, and *Pseudomonas aeruginosa*. Antimicrobial susceptibility testing revealed that methanol extract is more effective than aqueous extract of the plant *Senna occidentalis.*

## CONCLUSION

Medicinal plants such as *Azadirachta indica, Moringa oleifera* and *Syzigium cumini* are alternative bio-control agents due to the presence of secondary metabolites that includes alkaloids, flavonoids, saponins, terpenoids, phenolic and tannins which attributed to their effectiveness in inhibiting the growth of Fungal and bacterial pathogens.

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## AUTHORS' CONTRIBUTION

SMK designed the research work, PDB carried out the research project under the supervision of SMK. SMK is responsible for drafting the original manuscript.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest. All data are available with the authors on request.

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