**Original Research Article**

# COMPARATIVE STUDY OF THE ANTIMICROBIAL PROPERTIES OF FRESH AND FREEZE-DRIED LEAF AND SEED OF WONDERFUL KOLA ( *Buccholzia coriacea)*.




## ABSTRACT

*The utilization of plant materials as alternative therapies to control pathogenic bacteria has recently attracted a lot of attention. The effect of the fresh seed, freeze-dried seed, fresh leaf and freeze-dried leaf of wonderful kola using ethanol and aqueous extracts was tested on some organisms using standard laboratory procedures. The bacteria used were Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Salmonella typhi, Klebsella pneumonia and Xanthiomonas oryzae, while the fungi used were Trichoderma harzionium, Fusconium oxysporium, Aspergillus niger, Aspergillus flavus and Penicillium notatum. The results obtained showed that the ethanolic extracts of B. coriacea fresh seed showed inhibitory zones ranging from 2–12 mm, while the aqueous extract showed inhibitory zones ranging from 2-10 mm. The ethanolic extracts of B. coriacea freeze dried seed showed inhibitory zones ranging from 5–38 mm, while the aqueous extract showed inhibitory zones ranging from 4-36 mm.The ethanolic extracts of B. coriacea fresh leaf showed inhibitory zones ranging from 2–26 mm, while the aqueous extract showed inhibitory zones ranging from 2-24 mm.The aqueous and ethanolic extracts of B. coriacea freeze dried leaf showed inhibitory zones ranging from 3-40mm respectively. The study conclude that the aqueous and ethanolic extract of freeze dried seed of B. coriacea showed better antifungal and antibacterial activity against the test organisms compared with the aqueous and ethanolic extract of fresh seed of B. coriacea. Similarly, the aqueous and ethanolic extract of freeze dried leaf of B. coriacea showed better antifungal and antibacterial activity against the test organisms compared with the aqueous and ethanolic extract of fresh leaf of B. coriacea. The ethanolic extract showed better antifungal and antibacterial activity than aqueous extract*.

**Key words:** Wonderful kola, Antimicrobial, Aqueous extract, Ethanolic extract, Freeze dried

## INTRODUCTION

Plants have been used in traditional medicine for millennia, and recent scientific research have revealed a strong link between traditional and folkloric uses of particular plants, bolstering the quest for pharmacological active components in plants (Egharevba & Kun, 2010).Medicinal plants have a high economic value in the world of herbal medicine, and they are still the primary source of primary health care for about 75-80 percent of the population, primarily in developing countries, due to their cultural acceptability, compatibility with the human body, and lack of side effects (Iroha *et al*., 2020). Phytomedicine, pharmacognosy, herbal science, and pharmaceutical chemistry are just a few of the fields where plants have proven their worth (Kigigha *et al*., 2015).The existence of bioactive and chemical compounds in essential oils found in various portions of plants (Izah *et al*., 2018) and bioactive components present in plants such as flavonoids, glycosides, saponins, and tannins (Afolabi *et al*., 2020) may have contributed to their utility. Wonderful kola is one of these therapeutic plants.

Wonderful kola also known as *Buchholziacoriacea* is a perennial plant of the Capparaceae family (Ibrahim & Fagbonun, 2013). It is a small to medium-sized evergreen plant that may grow up to 20 meters in height and is found in Nigeria, Cameroon, Gabon, Central African Republic, Congo, Angola, and Ghana, among other places (Mbata *et al.*, 2009). The leaves are big and glossy, measuring 15-25 cm long and 5-7.5 cm wide (Akinyele, 2010), with prominent creamy white blossoms and medicinally valuable edible seeds.When fresh, the seeds are blackish, covered in purple aril, and have a harsh pungent flavor with a scorching spicy flavor (Odebiyi & Sofowora, 1978). The seeds have been given a variety of local names by Nigerians. It is known as 'Ndo' in Mende (Sierra Leone), 'Doe-fiah' in Kru-basa (Liberia), 'Eson-bese' in Akan-asante (Ghana), 'Banda' in Munga (West Cameroons), 'Esson bossi' in Central Africa, 'Kola Pimente' in French, 'Owi' in Edo State, ‘Okpokolo’ in Igbo, ‘Uwuro’ and ‘Aponmu’ in Yoruba (Sofowora, 2008).

The seeds derived its popular name “wonderful kola” due to its effective potency against numerous diseases (Adelere *et al.*, 2017). Because of its capacity to improve memory, it is also known as memory nut (Ibrahim & Fagbonun, 2013). *Buchholziacoriacea* seeds have long been used to treat diabetes, rheumatism, hypertension, the common cold, catarrh, and cough (Adisa *et al*., 2011).Complications such as chest pain, wrist pain, irregular menstruation (Ezeifeka *et al*., 2004), malaria, premature ejaculation (Jaiyesimi *et al*., 2011), and diarrhea have also been alleviated by the administration of these seeds (Ibrahim & Fagbonun, 2013). *Buchholziacoriacea* is a wonderful plant that can help to boost the nervous system and purify the blood. In Africa, it has been used specifically to cure migraines (Jaiyesimi *et al.*, 2011).Wonderful kola's antibacterial qualities have been attributed to its bioactive components like as alkaloids and tannins (Doherty *et al*., 2010; Kigigha *et al*., 2015, 2016; Epidi, 2016; Kalunta, 2017; Kigigha & Kalunta, 2017).

Antimicrobial (antibacterial and antifungal) properties of Wonderful kola seed have been discovered in numerous studies (Ezekiel

& Onyeoziri, 2009; Mbata *et al*., 2009; Osadebe *et al*., 2011; Ejikeugwu *et al*., 2014; Ibrahim & Fagbohun, 2014; Umeokoli *et al*., 2016). The method of drying and the solvent used for extraction have an impact on the final result of sensitivity test of plant materials. According to Ibrahim & Fagbonun (2013), methanolic extracts of *Buchholziacoriacea* seed show a superior efficacy against a wide spectrum of bacteria when compared to ethanol extract.Fresh express extract of Wonderfulkola has a better effect than methanol and hexane extracts, according to Ezekiel and Onyeoziri (2009). Fresh express extract of Wonderful kola seed has greater efficacy compared to oven dried uncooked and cooked seed, according to Nwachukwu *et al*. (2014). Methanol has a better effect than aqueous leaf extract of Wonderful kola, according to Osadebe *et al.* (2011). In comparison to hot water extracts, Mbata *et al*. (2009) found that methanol extract has a stronger effect against various gram positive and negative bacteria.All of these of studies on the antimicrobial properties of Wonderful kola have focused only on the fresh seed, bark and leaf of the plant; and nowork has been reported on freeze dried leaf and leaf so far. Hence, this study aimed to determine the antimicrobial efficacy of fresh leaves and seeds, compared with freeze dried leaf and seed of wonderful kola.

## METHODOLOGY

### Plant Collection and Authentication

The seeds and mature leaves of *Buchholzia coriacea* were purchased from Bode market , Molete, Ibadan, Oyo-State, Nigeria and authenticated in the Department of Crop, Soil and Pest Management, The Federal University of Technology, Akure, Ondo State, Nigeria.

### Preparation of Seed and Leaf Extract

The leaves were sorted, washed, chopped and divided into two parts. The first part was blended fresh using an electric blender and refrigerated at 4ºC. The second part was freeze dried, ground into a fine powder using a dry grinder and refrigerated at 4ºC prior analysis. The seeds of Wonderful kola were also treated the same way to obtain aqueous and ethanolic extracts of fresh seed and freeze dried seed respectively. The extracts were prepared in different concentrations; 500mg, 250mg, 125mg and 50mg respectively.

### Ethanol Extract Preparation

A Satoric AG Gottingen Electronic weighing scale was used to weigh 200 grams of pulverized kola seed. The weighed sample was soaked in 500 mL of ethanol in a conical flask, mixed and left for 24 hours with interval stirring. The mixture was filtered using Whatman No.1 filter paper (Azoro, 2002) into a clean beaker and the ethanol was recovered using a Soxhlet apparatus and was evaporated to dryness using a steam bath at $100^0$C.

**Aqueous Extract Preparation**

Two hundred grams (200 g) of the pulverized kola seed was weighed and macerated in 500ml of distilled water. The mixtures were vigorously swirled. After the elapse of 24 h with interval stirring, the mixture was filtered using Whatman No.1 filter paper (Azoro, 2002) into a clean beaker, and the filtrate was concentrated to dryness by evaporation using the steam bath at 100 ºC.

**Control Sample**

Standardized antibiotics (Gentamycin and Fluconazole) were aseptically used as the control in order to compare the diameter of zone of clearance from the extracts.

**Test Organisms**

The microorganisms used were obtained from Department Of Microbiology, Federal University Of Technology, Akure, Ondo State. The bacteria include *Escherichia coli, Bacillus subtilis*, *Staphylococcus aureus, Salmonella typhi, Klebsella pneumonia* and *Xanthiomonas oryzae*. These organisms were further streaked on nutrient agar and incubated at $37^0$C for 18 hours respectively. The isolates identities were further confirmed using standard biochemical procedures as described by Leber (2016), the isolates were stored on agar slant at $4^0$C prior to their use.The fungi used were *Trichodirma harzionum, Fusconium oxysporium, Aspergillus niger, Aspergillus flavus* and *Penicillium notatum*. These were maintained on malt extract agar.

**Screening for Antimicrobial Activities**

The process involves the use of test organisms to screen for the inhibitory properties of the extracts by measuring the diameters of slants and stored at $4^0$C. Control experiment was set up the same way but without the addition of any of the extracts. The zone of inhibition of extracts and control experiments was measured.

**Determination of antibacterial activity of the extracts:** Nutrient agar was poured into Petri dishes, allowed to set and bored with a Durham tube. Bacterial culture was used to inoculate each of the agar plates after which about 0.01 ml of the extract was added. Incubation was done at 37°C for 24 h after which the plates were inspected for zones of inhibition.

**Determination of antifungal activity of the extracts:** Nutrient agar was poured into Petri dishes, allowed to set and bored with a Durham tube. Fungal culture was used to inoculate each of the agar plates after which about 0.01 ml of the extract was added. Incubation was done at $28^0$C for 120 hours after which the plates were inspected for zones of inhibition.

The above procedure was applied for aqueous and ethanolic extracts of the fresh leaf, freeze dried leaf, fresh seed and freeze dried seeds, and concentrations of 500mg, 250mg, 125mg and 50mg of each extracts was prepared.

## RESULTS

Results of antimicrobial properties of ethanolic and aqueous extract of fresh dried seed, fresh leaf, freeze dried seed and freeze dried leaf of Wonderful kola was presented in figure 1-8.

The ethanolic extracts of *B. coriacea* fresh seed showed inhibitory zones ranging from 2–12 mm, while the aqueous extract showed inhibitory zones ranging from 2-10 mm (figure 1 & 2). From the result of antimicrobial screening it can be observed that the ethanolic and aqueous seed extract of *B. coriacea* recorded antibacterial activity against the bacterial test isolates (except *Salmonellatyphi*), with the best activity recorded against *B.subtilis*. Antifungal activity was also recorded against all fungal isolates (except *Fusconiumoxysporium*), with the best activity recorded against *Penicilliumnotatum.*The use of Gentamycin (50mg) as control only showed better antibacterial activity against *E. coli* (10mm) at high concentration (500mg) than the aqueous (5mm) and ethanolic (7mm) extract of fresh seed of wonderful kola, while the aqueous and ethanolic extract of fresh seed of wonderful kola at high concentration showed better antifungal activity than Fluconazole (50µg/ml) used as control.

Also looking at figure 3 & 4, it can be observed that the aqueous and ethanolic extract of freeze dried seed of *B. coriacea* recorded antibacterial activity against all the bacterial test isolates. The ethanolic extracts of *B. coriacea* freeze dried seed showed inhibitory zones ranging from 5–38 mm, while the aqueous extract showed inhibitory zones ranging from 4-36 mm. The highest bacterial activity of the ethanolic and aqueous extract was recorded against *Klebsellapneumonia*. Also, antifungal activity was recorded against all fungal isolates. The best fungal activity of the ethanolic and aqueous extract was recorded against *Aspergillusniger*. The ethanolic extract recorded better antifungal activity than antibacterial activity. The use of Gentamycin (50mg) as control only showed better antibacterial activity against*Bacillus subtilis* and*Staphylococcus aureus* at concentration of 50mg/mlcompared with the aqueous and ethanolic extract of freezedried seed of wonderful kola, while the aqueous and ethanolic extract of freeze dried seed of wonderful kola at all concentration showed better antifungal activity than Fluconazole (50µg/ml). The aqueous and ethanolic extract of freeze dried seed of *B. coriacea* showed better antifungal and antibacterial activity compared with the aqueous and ethanolic extract of freeze dried seed of *B. coriacea.*

The aqueous and ethanolic extract of fresh leaf of wonderful kola was presented in figure 5 & 6. The ethanolic extracts of *B. coriacea* fresh leaf showed inhibitory zones ranging from 2–26 mm, while the aqueous extract showed inhibitory zones ranging from 2-24 mm. From the result of antimicrobial screening, it can be observed that the ethanolic and aqueous fresh leaf extract of *B. coriacea* recorded antibacterial activity against the bacterial test isolates at different concentrations except for *Klebsellapneumonia* which showed antibacterial activity only at 500mg. The best antibacterial activity was recorded against *Xanthiomonasoryzae*. Antifungal activity was also recorded against all fungal isolates at different concentrations, with the best activity recorded against *Trichodermaharzonium.*The use of Gentamycin (50mg) as control only showed slightly better antibacterial activity against*Bacillus subtilis* and *Escherichia coli* at concentration of 50mg/ml compared withthe aqueous and ethanolic extract of fresh leaf of wonderful kola, while the aqueous and ethanolic extract of fresh leaf of wonderful kola at all concentration showed better antifungal activity than Fluconazole (50µg/ml).

From figure 7 & 8, the aqueous and ethanolic extracts of *B. coriacea* freeze dried leaf showed inhibitory zones ranging from 3-40mm respectively. It can be observed that the aqueous and ethanolic extract of freeze dried leaf of *B. coriacea* recorded antibacterial activity against all the bacterial test isolates at different concentrations except for *Bacillussubtilis* which did not show any antibacterial activity at 2mg. The highest bacterial activity of the ethanolic and aqueous extract was recorded against *Escherichiacoli* at a concentration of 500mg/ml. Also, antifungal activity was recorded against all fungal isolates. The highest fungal activity of the ethanolic and aqueous extract was recorded against *Penicilliumnotatum* (40mm) at a concentration of 500mg/ml. The ethanolic extract recorded better antifungal activity than antibacterial with best activity at higher concentration. The use of Gentamycin (50mg) as control only showed slightly better antibacterial activity against*Bacillus subtilis* at concentration of 50mg/ml compared withthe aqueous and ethanolic extract of freeze dried leaf of wonderful kola, while the aqueous and ethanolic extract of freeze dried leaf of wonderful kola at all concentration showed better antifungal activity than Fluconazole (50µg/ml) used as control.

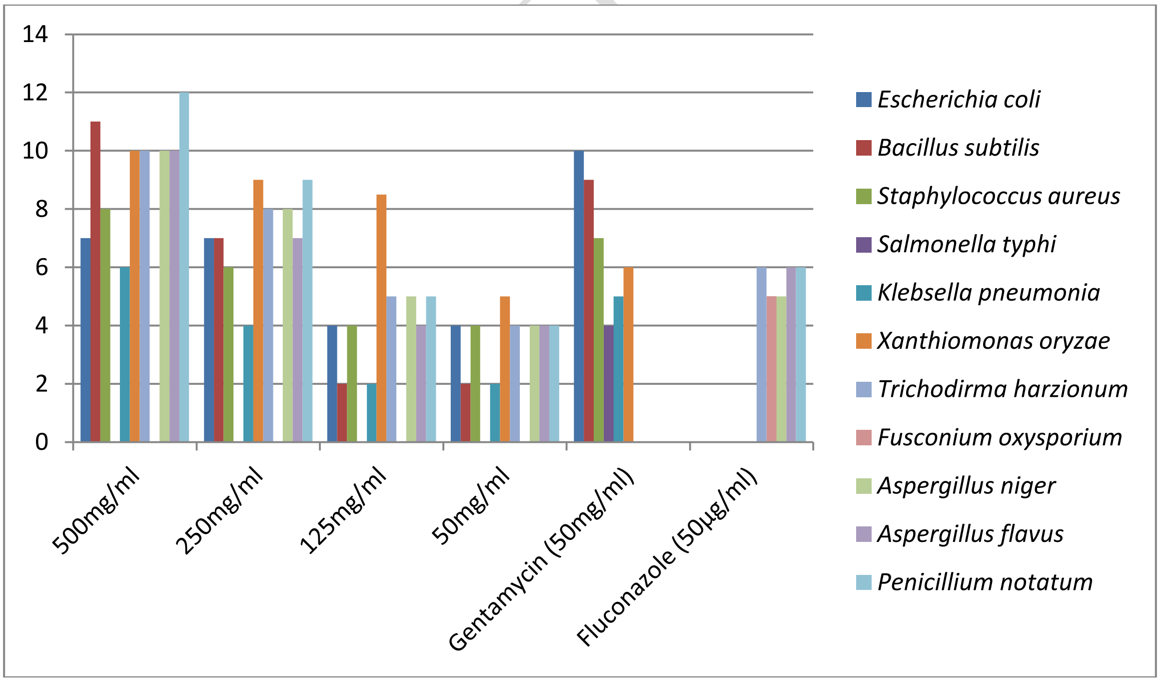


Figure 1: Result of antimicrobial screening of ethanolic extract of fresh seed of Wonderful kola with Zone of inhibition in mm

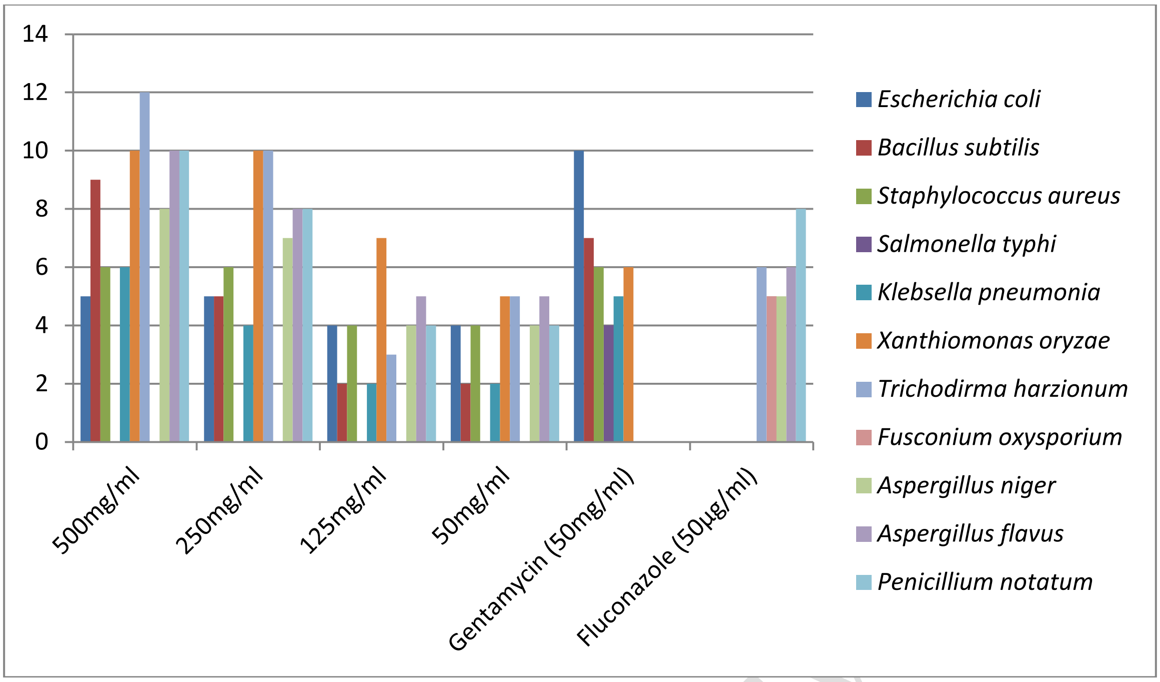


Figure 2: Result of antimicrobial screening of aqueous extract of fresh seed of Wonderful kola with Zone of inhibition in mm

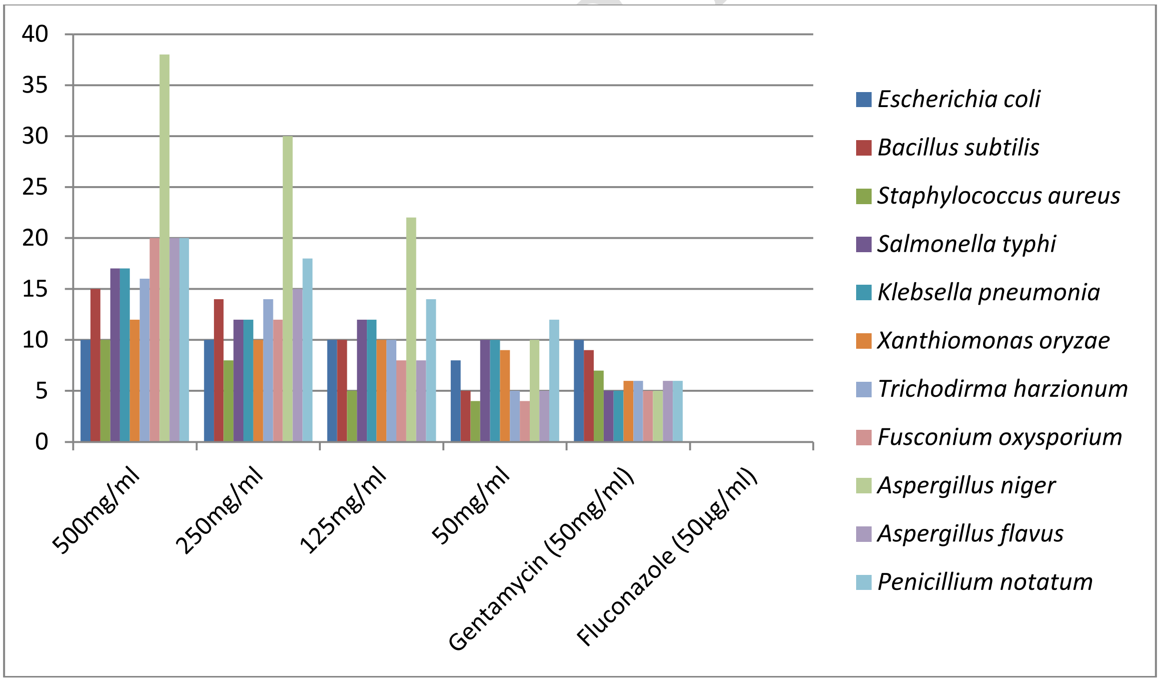


Figure 3: Result of antimicrobial screening of ethanolic extract of freeze dried seed of Wonderful kola with Zone of inhibition in mm

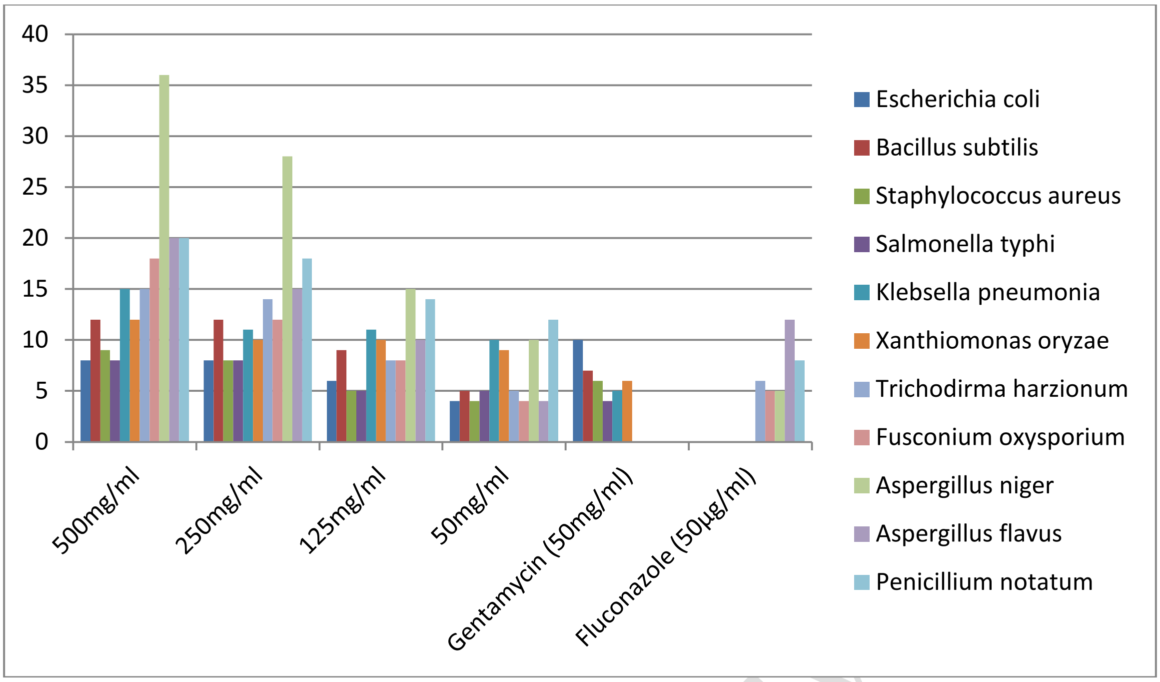


Figure 4: Result of antimicrobial screening of aqueous extract of freeze dried seed of Wonderful kola with Zone of inhibition in mm

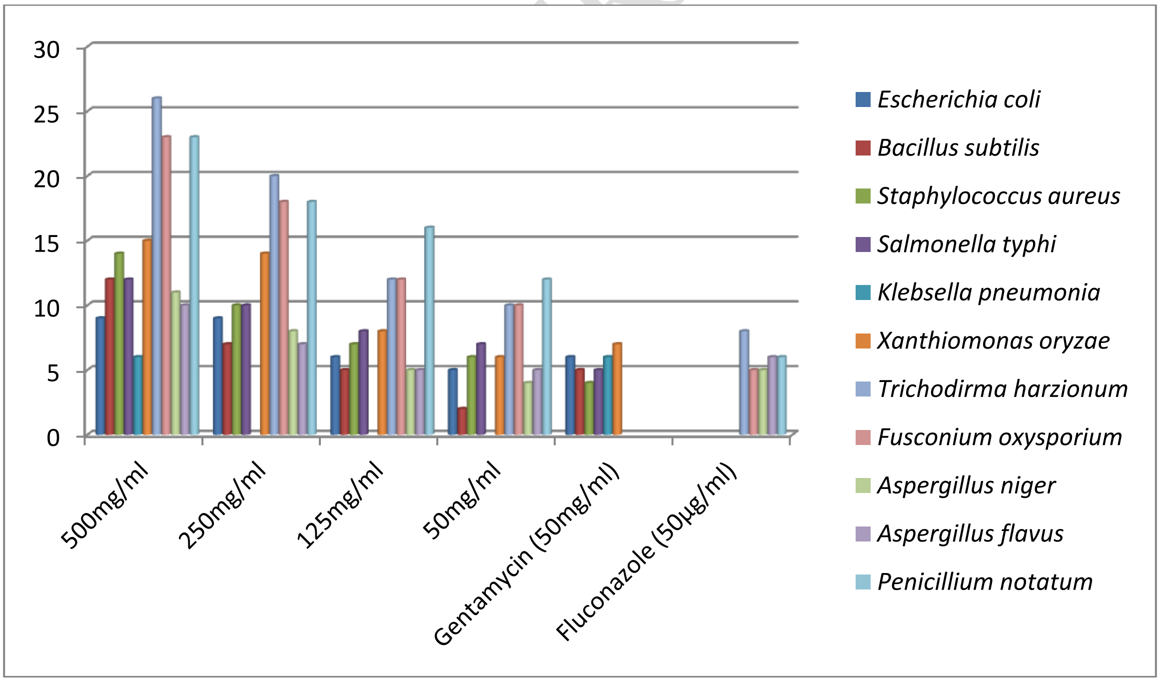


Figure 5: Result of antimicrobial screening of ethanolic extract of fresh leaf of Wonderful kola with Zone of inhibition in mm

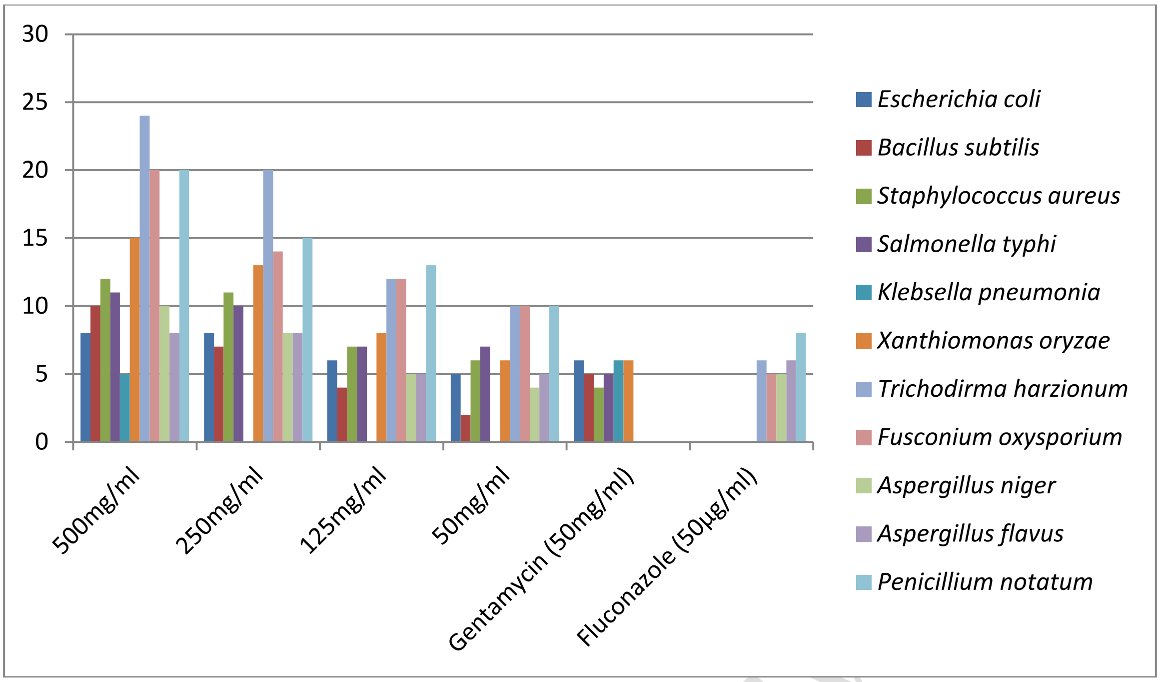


Figure 6: Result of antimicrobial screening of aqueous extract of fresh leaf of Wonderful kola with Zone of inhibition in mm

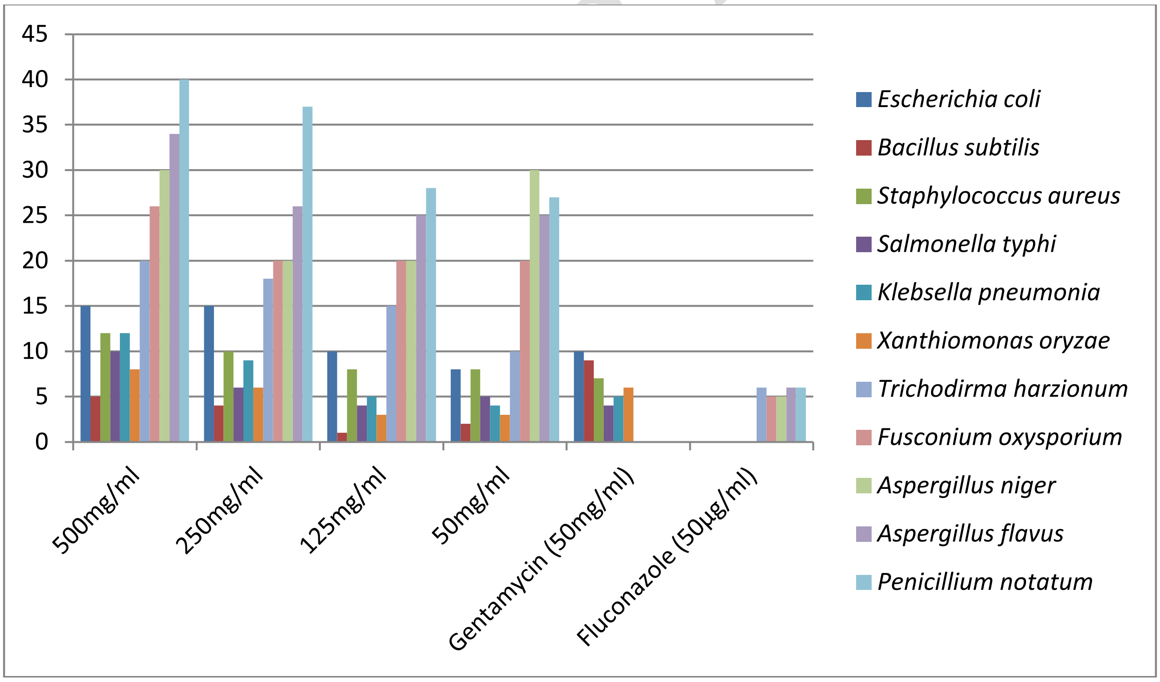


Figure 7: Result of antimicrobial screening of ethanolic extract of freeze dried leaf of Wonderful kola with Zone of inhibition in mm

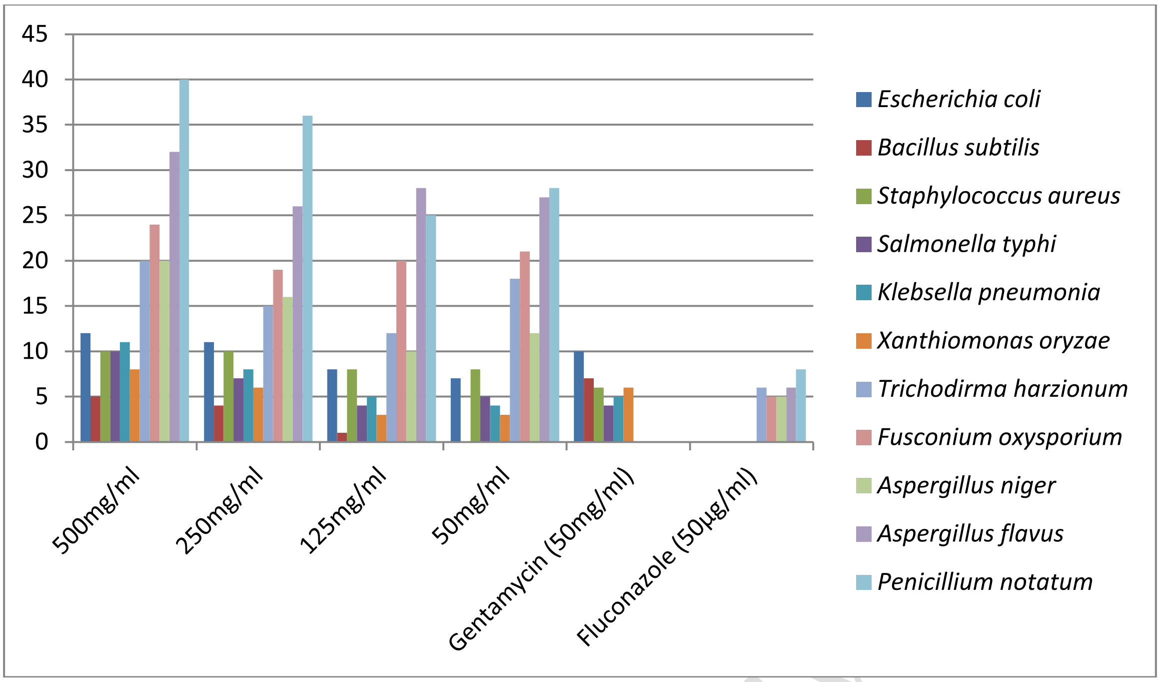


Figure 8: Result of antimicrobial screening of aqueous of freeze dried leaf of Wonderful kola with Zone of inhibition in mm

**Figure 9:** The pictures of *Buchholzia coriacea* tree, leaves and seeds.

Table 1: Fresh seed of Wonderful cola with Zone of inhibition in mm at different concentration in mg/ml.

| S/N | Microorganism | | 500mg | 250mg | 125mg | 50mg | CONTROL |
|---|---|---|---|---|---|---|---|
| | Bacteria | | | | | | Gentamycin |
| 1 | Escherichia coli | Eth | 7 mm | 7 mm | 4 mm | 4 mm | 10 mm |
| | | Aq | 5 mm | 5 m | 4 mm | 4 mm | 10 mm |
| 2 | Bacillus subtilis | Eth | 11mm | 7 mm | 2 mm | 2 mm | 9 mm |
| | | Aq | 9 mm | 5 mm | 2 mm | 2 mm | 7 mm |
| 3 | Staphylococcus aureus | Eth | 8 mm | 6 mm | 4 mm | 4 mm | 7 mm |
| | | Aq | 6 mm | 6 mm | 4 mm | 4 mm | 6 mm |
| 4 | Salmonella typhi | Eth | - | - | - | - | 4 mm |
| | | Aq | - | - | - | - | 4 mm |
| 5 | Klebsella pneumonia | Et | 6 mm | 4 mm | 2 mm | 2 mm | 5 mm |
| | | Aq | 6 mm | 4 mm | 2 mm | 2 mm | 5 mm |
| 6 | Xanthiomonas oryzae | Eth | 10mm | -9mm | 8.5mm | 5 mm | 6 mm |
| | | Aq | 10mm | 10mm | -7mm | 5 mm | 6 mm |
| | FUNGI | | | | | | Fluconazole |
| 1 | Trichoderma harzionium | Eth | 10mm | 8 mm | 4 mm | 5 mm | 6 mm |
| | | Aq | 12mm | 10mm | 3 mm | 5 mm | 6 mm |
| 2 | Fusconium oxysporium | Eth | - | - | - | - | 5 mm |
| | | Aq | - | - | - | - | 5 mm |
| 3 | Aspergillus niger | Eth | 10mm | 8 mm | 5 mm | 4 mm | 5 mm |
| | | Aq | 7 mm | 8 mm | 4 mm | 4 mm | 5 mm |
| 4 | Aspergillus flavus | Eth | 10mm | 7 mm | 5 mm | 4 mm | 6 mm |
| | | Aq | 10mm | 8 mm | 5 mm | 5 mm | 6 mm |
| 5 | Penicillium notatum | Eth | 12mm | 9 mm | 4 mm | 5 mm | 6 mm |
| | | Aq | 10mm | 8 mm | 4 mm | 5 mm | 8 mm |

Control ; 50mg/ml

Table 2 : Freeze dried seed of Wonderful cola with Zone of inhibition at different concentration in mm

| s/n | Microorganism | 500mg | 250mg | 125mg | 50mg | Control |
|---|---|---|---|---|---|---|
| | Bacteria | | | | | Gentamycin |
| 1 | Escherichia coli | 10mm | 10mm | 10mm | 8mm | 10mm |
| | | 8mm | 8mm | 6mm | 4mm | 10mm |
| 2 | Bacillus subtilis | 15mm | 14mm | 10mm | 5mm | 9mm |
| | | 12mm | 12mm | 9mm | 5mm | 7mm |
| 3 | Staphylococcus aureus | 10mm | 8mm | 5mm | 4mm | 7mm |
| | | 9mm | 8mm | 5mm | 4mm | 6mm |
| 4 | Salmonella typhi | 10mm | 10mm | 7mm | 6mm | 4mm |
| | | 8mm | 8mm | 5mm | 5mm | 4mm |
| 5 | Klebsella pneumonia | 17mm | 12mm | 12mm | 10mm | 5mm |
| | | 15mm | 11mm | 11mm | 10mm | 5mm |
| 6 | Xanthiomonas oryzae | 12mm | 10mm | 10mm | 9mm | 6mm |
| | | 12mm | 10mm | 10mm | 9mm | 6mm |
| | Fungi | | | | | Flucomazole |
| 1 | Trichoderma harzionium | 16mm | 14mm | 10mm | 5mm | 6mm |
| | | 15mm | 14mm | 8mm | 5mm | 6mm |
| 2 | Fusconium oxysporium | 20mm | 12mm | 8mm | 4mm | 5mm |
| | | 18mm | 12mm | 8mm | 4mm | 5mm |
| 3 | Aspergillus niger | 38mm | 30mm | 22mm | 10mm | 5mm |
| | | 36mm | 28mm | 15mm | 10mm | 5mm |
| 4 | Aspergillus flavus | 20mm | 15mm | 8mm | 5mm | 6mm |
| | | 20mm | 5mm | 10mm | 4mm | 6mm |
| 5 | Penicillium notatum | 20mm | 19mm | 14mm | 12mm | 6mm |
| | | 20mm | 19mm | 14mm | 12mm | 8mm |

Table 3 : Fresh leaf of Wonderful cola with Zone of inhibition at different concentration in mm

| s/n | Microorganism | 500mg | 250mg | 12mg | 50mg | Control |
|---|---|---|---|---|---|---|
| | Bacteria | | | | | Gentamycin |
| 1 | Escherichia coli Eth | 9mm | 9mm | 6mm | 5mm | 6mm |
| | Aq | 8mm | 8mm | 6mm | 5mm | 6mm |
| 2 | Bacillus subtilis | 12mm | 7mm | 5mm | 2mm | 5mm |
| | | 10mm | 7mm | 4mm | 2mm | 5mm |
| 3 | Staphylococcus aureus | 14mm | 10mm | 7mm | 6mm | 4mm |
| | | 12mm | 11mm | 7mm | 6mm | 4mm |
| 4 | Salmonella typhi | 12mm | 10mm | 8mm | 7mm | 5mm |
| | | 11mm | 10mm | 7mm | 7mm | 5mm |
| 5 | Klebsella pneumonia | 6mm | - | - | - | 6mm |
| | | 5mm | - | - | - | 6mm |
| 6 | Xanthiomonas oryzae | 15mm | 14mm | 8mm | 6mm | 7mm |
| | | 15mm | 13mm | 8mm | 6mm | 6mm |
| | **Fungi** | | | | | **Fluconazole** |
| 1 | Trichoderma harzonium | 26mm | 20mm | 12mm | 10mm | 8mm |
| | | 24mm | 20mm | 12mm | 10mm | 6mm |
| 2 | Fusconium oxysporium | 23mm | 18mm | 12mm | 10mm | 5mm |
| | | 20mm | 14mm | 12mm | 10mm | 5mm |
| 3 | Aspergillus niger | 11mm | 8mm | 5mm | 4mm | 5mm |
| | | 10mm | 8mm | 5mm | 4mm | 5mm |
| 4 | Aspergillus flavus | 10mm | 7mm | 5mm | 5mm | 6mm |
| | | 8mm | 8mm | 5mm | 5mm | 6mm |
| 5 | Penicillium notatum | 23mm | 18mm | 17mm | 12mm | 6mm |
| | | 20mm | 15mm | 13mm | 10mm | 8mm |

Table 4: Freeze dried leaf of Wonderful cola with Zone of inhibition at different concentration in mm

| s/n | Microorganism | 500mg | 250mg | 125mg | 50mg | Control |
|---|---|---|---|---|---|---|
| | Bacteria | | | | | Gentamyan |
| 1 | Escherichia coli | 15mm | 15mm | 10mm | 8mm | 10mm |
| | | 12mm | 11mm | 8mm | 7mm | 10mm |
| 2 | Bacillus subtilis | 5mm | 4mm | 1mm | - | 9mm |
| | | 5mm | 4mm | 1mm | - | 7mm |
| 3 | Staphylococcus aureus | 12mm | 10mm | 8mm | 8 | 7mm |
| | | 10mm | 10mm | 8mm | 8 | 6mm |
| 4 | Salmonella typhi | 10mm | 6mm | 4mm | 5mm | 4mm |
| | | 10mm | 7mm | 4mm | 5mm | 4mm |
| 5 | Klebsella pneumonia | 12mm | 9mm | 5mm | 4mm | 5mm |
| | | 11mm | 8mm | 5mm | 4mm | 5mm |
| 6 | Xanthiomonas oryzae | 8mm | 6mm | 3mm | 3mm | 6mm |
| | | 8mm | 6mm | 3mm | 3mm | 6mm |
| | Fungi | | | | | Fluconazole |
| 1 | Trichoderma harzionum | 20mm | 18mm | 15mm | 10mm | 6mm |
| | | 20mm | 15mm | 12mm | 1.9mm | 6mm |
| 2 | Fusconium oxysporium | 26mm | 20mm | 20mm | 30mm | 5mm |
| | | 24mm | 19mm | 20mm | 21mm | 5mm |
| 3 | Aspergillus niger | 30mm | 20mm | 20mm | 20mm | 5mm |
| | | 20mm | 16mm | 10mm | 12mm | 5mm |
| 4 | Aspergillus flavus | 34mm | 26mm | 25mm | 26mm | 6mm |
| | | 32mm | 26mm | 28mm | 27mm | 6mm |
| 5 | Penicillium notatum | 40mm | 37mm | 27mm | 28mm | 6mm |
| | | 40mm | 36mm | 25mm | 28mm | 8mm |

**DISCUSSION**

The utilization of plant materials as alternative therapies to control pathogenic bacteria has recently sparked a lot of attention (Nostro *et al*., 2006). Because of the increasing failure of chemotherapeutics and infections' antibiotic resistance, various medicinal plants have been investigated for their antibacterial efficacy (Iroha *et al*., 2020).This study was carried out to determine the antimicrobial efficacy of fresh leaves and seeds of wonderful kola compared with its freeze dried leaf and seed.

The result of this study showed that the ethanolic and aqueous seed extract of *B. coriacea* recorded antibacterial activity against bacterial test isolates (*B.subtilis, E. coli, S. aureus, K. pneumonia* and *X. oryzae*). Antifungal activity was also recorded against *A. niger,A. flavus, T. harzionum* and *P.notatum*. This observation is in agreement with previous studies which have variously shown that wonderful kola seed and leaf contain antimicrobial (antibacterial and antifungal) activities (Ezekiel and Onyeoziri, 2009; Mbata *et al*., 2009; Osadebe *et al*., 2011; Ejikeugwu *et al*., 2014; Ibrahim and Fagbohun, 2014; Umeokoli *et al*., 2016).

The impact of fresh kola, hexane, and methanol extracts of *B. coricea* on various food borne pathogens (*Escherichia coli, Enterococcus faecalis, Staphylococcus aureus, Trichoderma viride*, and *Aspergillus niger*) was studied by Ezekiel and Onyeoziri (2009). The fresh kola showed inhibitoryzones with the test bacteria: *E. coli* (62 mm), *E.faecalis* (40 mm) and *S. aureus* (50 mm). The growthof the two test fungi *T. viride* and *A. niger* was completely inhibited.According to Umeokoli *et al*. (2016), the aqueous seed extract of *B. coriacea* has antibacterial activity against all of the bacterial test isolates (excluding *E. coli* and *K. pneumoniae*), with *B. subtilis* having the best activity. Only *C.*

*albicans*was found to have antifungal action. Antibacterial activity was also seen in the methanol seed extract of *B. coriacea* against all of the bacterial test isolates, as well as antifungal activity against *Candida albicans* and *Aspergillus niger*.The methanol extract had superior antifungal activity than antibacterial activity, with the highest action against the mold *A. niger*, which is consistent with our findings.

In this study, the ethanolic extracts of *B. coriacea* fresh seed showed inhibitory zones ranging from 2–12 mm with all test organisms (*B. subtilis*, *E. coli*, S. *typhi* , *K. pneumonia*, *X. oryzae* and *S. aureus*). The aqueous extract of *B. coriacea* fresh seed showed inhibitory zones of 2-10 mm with the test bacteria. Obidegwe & Okazi (2016) reported that the ethanol extracts of *B. coriacea* showed inhibitory zones ranging from 14–27 mm with all test organisms (*Pseudomonas spp., E. coli, S. aureus, Klesiella sp., Streptococcus sp.,* and *Candida albicans*), while the aqueous extract of *B. coriacea* showed inhibitory zones of 2-14mm (Obidegwe & Okazi, 2016).The isolates were treated with n-hexane, methanol, and chloroform extracts of *B. coriacea* leaf in a related study by Chika *et al*. (2012), and it elicited modest antibacterial activities against the test isolates with *E. coli*, *Staphylococcus aureus, Shigella species, Klebsiella pneumoniae,* and *Bacillus subtilis* susceptible. According to Okoli *et al*. (2010), extracting solvents can cause variations in spice extractive components, which can affect antibacterial activity. *S.*

*aureus, E. coli, S. typhii, P. aeruginosa, Candida albicans,* and *A. flavus* have all been found to be inhibited by stem bark portions of *B. coriacea*(Ajayeoba *et al*., 2003).

The freeze dried leaf and seed exhibited greater inhibitory effect on the test organisms than the fresh seed and leaf, showing inhibitory zones ranging from 3-40 mm with the test bacteria (*B. subtilis, E. coli,* S. *typhi, K. pneumonia, X. oryzae*and *S. aureus*) it was exposed to and it completely inhibited the growth of *T. harzionum, F. oxysporium, A. niger, A. flavus* and *P. notatum.* When Ezekiel and Onyeoziri (2009) investigated the effect of fresh kola, hexane, and methanol extracts of *B. coricea* on several food-borne pathogens(*Esherichia coli, Enterococcus faecalis, Staphylococcus aureus, Trichoderma viride* and *Aspergillusniger*), they found a similar result. The heat applied during drying may account for the dried leaf extracts of *B. coriacea* having a lower inhibitory activity than the frozen seed and freeze dry leaf of *B. coriacea* (Savitri *et al*., 1986).Freeze drying (Ratti, 2008) is a low-temperature dehydration method that involves freezing the product, reducing the pressure, and then sublimating the ice (Fellows, 2017). This is in contrast to most traditional methods of dehydration, which use heat to evaporate water (Prosapio *et al*., 2017). Because of the low temperature employed in processing, the rehydrated product has good quality as most of the bioactive compounds has been preserved which could explain why freeze seed and freeze dry leaf had a better inhibitory impact on the test organisms than other drying processes employed in other studies reported.

Changes in the inhibitory impact of freeze dried seed and freeze dried leaf on the test organisms could potentially be attributable to differences in the solvents' polarity, specificity, and affinity level(Ezekiel and Onyeoziri, 2009). Furthermore, the differences in zone of inhibition could be attributable to the concentration of plant extract employed in the study (Izah *et al.*, 2018). The physiology, metabolism, nutrition, and biochemistry of the microbial isolates may also have an impact on the sensitivity of an extract to and organisms (Kigigha *et al.*, 2016; Epidi *et al.*, 2016). Variations in sensitivity could be caused by the age and type of plants employed, as well as environmental factors (Kigigha *et al.*, 2016; Epidi *et al.*, 2016).



**CONCLUSION AND RECOMMENDATIONS**

The study conclude that the aqueous and ethanolic extract of freeze dried seed of *B. coriacea* showed better antifungal and antibacterial activity against the test organisms compared with the aqueous and ethanolic extract of fresh seed of *B. coriacea*.Similarly, the aqueous and ethanolic extract of freeze dried leaf of *B. coriacea* showed better antifungal and antibacterial activity against the test organisms compared with the aqueous and ethanolic extract of fresh leaf of *B. coriacea*.The ethanolic extract showed better antifungal and antibacterial activity than aqueous extract.The extracts' reduced inhibitory activities in traditional drying procedures demonstrate that excessive exposure to air, sunlight, too much artificial heat, and quick drying can result in loss of bioactive compounds. Plant products should be developed into standardized, quality-controlled phytopharmaceuticals, and the characterization of *B. coriacea* bioactive components should be promoted and researched.



COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

# REFERENCES

Adelere, I.A. and Lateef, A. (2016). Novel Approach to the Green Synthesis of Metallic Nanoparticles: The use of agro-wastes, enzymes and pigments. *Nanotechnology Reviews,* 15(4), 196-200.

Adelere, I.A., Agbaje, L., Aboyeji, D.O., Abdulsalam, R., Adabara, N.U. and Bala, J.D. (2017). Biosynthesis of silver nanoparticles using aqueous extract of *Buchholzia coriacea* (Wonderful kola) seeds and their antimicrobial activities. *Annals of Food Science and Technology,* 18 (4), 671-679

Adisa, R.A., Choudhary, M.I. and Olorunsogo, O.O. (2011). Hypoglycemic activity of *Buchholzia coriacea* seeds in streptozotocin-induced diabetic rats and mice. *Experimental and Toxicological Pathology*, 63(5),619-625.

Afolabi, B.T., Agu, G.C. and Onajobi, I.B. (2020). Phytochemical screening and Antibacterial activity of *Garciniakola* (Hackel) and Kola Nitida (vent) Extracts. *Nigerian Journal of Technology,* 39 (2), 379 - 385

Ajaiyeoba, E.O., Onocha, P.A., Nwozo, S.O. and Sama, W. (2003). Antimicrobial and cytotoxicity evaluation of *Buchholzia coriacea* stem bark. *Fitoterapia,* 7(1), 706-709.

Akinyele, A.O. (2010). Effects of growth hormones, rooting media and leaf size on juvenile stem cuttings of *Buchholzia coriacea* Engler. *Annual Forrest Reserve,* 53(2), 127-133.

Chika, E., Ikegbunam, M., Ugwu, C., Araka, O., Iroha, I., Adikwu, M. l. and Esimone, C.

(2012). Evaluation of antibacterial activity of the leave extracts of *Buchholzia coriacea*. *Asian Journal of Pharmaceutical and Biological Research*,*2*(4), 204-208.

Doherty, V.F., Olaniran, O.O., and Kanife, U.C. (2010). Antimicrobial activities of
*Aframomummelegueta* (Alligator pepper).*International Journal of Biology*,2(2), 126-131

Egharevba, H.O. and Kun, O.F. (2010). Preliminary Phytochemical and proximate
Analysis of the Leaves of *Piliostigmathioniningii* (Schumach) Mile-Redhead. *EthanobotanicalLeaflets*, 14(4), 570-577.

Ejikeugwu, C., Umeokoli, B., Iroha, I., Ugwu, M. and Esimone, C. (2014).
Phytochemical and antibacterial screening of crude extracts from leaves and wonderful kola. *American Journal of Life Sciences*, 2(6), 9-12

Epidi, J.O., Izah, S.C. and Ohimain, E.I. (2016). Antibacterial and synergistic efficacy of
extracts of *Alstoniaboonei* tissues. *British Journal of Applied Science*, 1(1), 21-26
Ezeifeka, G.O., Orji, M.U., Mbata, T.I. and Patrick, A.O. (2004). Antimicrobial activities
of *Cajanus cajan*, *Garcinia kola* and *Xylopia aethiopica* on pathogenic microorganisms. *Biotechnology*,**3**(1), 41-43.

Ezekiel, O.O. and Onyeoziri, N.F. (2009). Preliminary studies on the antimicrobial
properties of *Buchholziacoriacea* (wonderful kola). *African Journal of Biotechnology*, 8 (1), 472-478.

Fellows, P. (2017). Freeze drying and freeze concentration. Food processing technology :
principles and practice (4th ed.). Kent: Woodhead Publishing/Elsevier Science, Pp. 929–940.

Ibrahim, T.A. and Fagbohun, E.D. (2014). Antibacterial and antifungi activities of ethanolic and methanolic extract of dried seeds of *Buchhloziacoriacea*, Research and Reviews: *Journal of Microbiology and Biotechnology*, 3(2), 5-9

Ibrahim, T.A. and Fagbonun, E.D. (2013). Antibacterial and Antifungal Activity of
Ethanolic and Methanolic Extract of Dried Seeds of *Buchhloziacoriacea*. *Greener Journal of Agricultural Sciences,* 3(6), 458-463.

Iroha, I.R., Ozor, H.O., Moses, I.B., Onuora, A.L. and Kalu, A.C. (2020). Antimicrobial
Activity of Ethyl Acetate and Ethanol Seed Extracts of *Buchholziacoriacea* (Wonderful kola) against Multidrug-resistant Clinical Isolates of *Salmonella*species and *Escherichiacoli*. *Arch Clin Microbiology*, 11(3), 109-113.

Izah, S.C., Uhunmwangho, E.J. and Eledo, B.O. (2018). Medicinal potentials of
*Buchholzia coriacea* (wonderful kola).*Medicinal Plant Research*, 8(5), 27-42

Jaiyesimi, A.F., Ogbole, O., Anthony, O.and Egbebunmi, O. (2011). Larvicidal effect of
pet, ether, chloroform fractions and methanol extract of *Buccholzia coriacea* Engle seed. *International Journal of Pharmaceutical Sciences and Research*, **2**(7), 1736-1738.

Kalunta, C.G. (2017). Antimicrobial effect of different seed extracts of Piper nigrum
against *Escherichiacoli*, *Staphylococcusaureus* and *Candidaalbican*.*Biotechnology Research*, 3(3), 71-76

Kigigha, L.T. and Kalunta, C.G. (2017). Antimicrobial efficacy of leaf extracts of *Piper*
*nigrum* against *Escherichiacoli*, *Staphylococcusaureus* and *Candidaalbicans. Journal of Basic Pharmacology and Toxicology*, 1(2), 32-36

Kigigha, L.T., Biye, S.E.and Izah, S.C. (2016). Phytochemical and antibacterial activities
of *Musanga cecropioides* tissues against*Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Proteus* and

*Bacillus* species. *International Journal of Applied Research and Technology*, 5(1), 100-107.

Leber, A. (2016). Biochemical procedures. In:Clinical Microbiology Procedures,
Handbook, Fourth Edition, Pp. 11-16.

Mbata, T.I., Duru, C.M. and Onwumelu, H.A. (2009). Antibacterial activity of crude seed
extracts of *Buchholzia coriacea* on some pathogenic bacteria. *Journal of Developmental Biology and Tissue Engineering*, **1** (1): 1 -5.

Nostro, A., Cellini, L. and Bartolomeo, S. (2006). Effects of Combining extracts (From
Propolis or *Zingiberofficinale*) with Clarithromycin on *Helicobacterpylori*. *Phytotherapy Research*,20 (3), 187-190.

Nwachukwu, M. I., Duru, M. K. C., Amadi, B. A. and Nwachukwu, I. O. (2014).
Comparative Evaluation of Phytoconstituents, Antibacterial Activities and Proximate Contents of Fresh, Oven Dried Uncooked and Cooked Samples of *Buchholzia coriacea* Seed and Their Effects on Hepatocellular Integrity. *International Journal of Pharmaceutical Science Invention*, 3 (6), 41-49.

Obidegwe, A.O. and Okazi, P.I. (2016). Antimicrobial and Antihelmintic Effect of
Wonderful kola (*Buchholzia coriacea*). *Journal of Developmental Biology and Tissue Engineering*, 11(1), 11-15.

Odebiyi, A. and A. E. Sofowara, A. E. (1978). Phytochemical screening of Nigerian
medicinal plants. *Lloydia*,41 (2), 234-246.

Okoli, B. J., Okere, O. S. and Adeyemo, S. O. (2010). Antiplasmodial activity of
*Buchholzia coriancea. Journal of Medical and Applied Biosciences*,2(1), 21-29.

Osadebe, P.O., Awemu, G.A., Ezealisiji, K.M. and Agbo, M.O. (2011). Phytochemical and antimicrobial studies of the leaf extracts of *Bucholziacoriaceae*, *International Journal of Current Research and Review*, 13(2), 17-22

Prosapio, V., Norton, I. and De-Marco, I. (2017). Optimization of freeze-drying using a Life Cycle Assessment approach: Strawberries' case study. *Journal of Cleaner Production*, 16 (8), 1171–1179.

Ratti, C. (2008). Advances in Food Dehydration. CRC Press, Pp. 209–235.

Sofowora, E. A. (2008). Medicinal Plants and Traditional medicine in Africa: Nigeria`s useful plants. *Nigeria Field*, 24 (3), 13-34.

Umeokoli, B.O., Abba, C., Ezeh, P. and Ajaghaku, D. (2016). Antimicrobial, anti-inflammatory, and chemical evaluation of *Buchholziacoriacea* seed (Wonderful kola).*American Journal of Life Sciences*, 4(5), 106-112.