Original Research Article

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

ABSTRACT

The utilization of plant materials as alternative therapies to control pathogenic bacteria has recently attracted. The effect of the fresh seed, freeze-dried seed, fresh leaf and freeze-dried leaf of 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 oryzae, while the fungi used were Trichoderma harzionium, Fusconium oxysporium, Aspergillus niger, Aspergillus flavus and Penicillium notatum. The results showed that the ethanol 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 ethanol 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 ethanol 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 ethanol extracts of B. coriacea freeze dried leaf showed inhibitory zones ranging from 3-40mm respectively. The study conclude that the aqueous and ethanol extract of freeze dried seed of B. coriacea showed better antifungal and antibacterial activity against the test organisms compared with the aqueous and ethanol extract of fresh seed of B. coriacea. Similarly, the aqueous and ethanol extract of freeze dried leaf of B. coriacea showed better antifungal and antibacterial activity against the test organisms compared with the aqueous and ethanol extract of fresh leaf of B. coriacea. The ethanol extract showed better antifungal and antibacterial activity than aqueous extract.

Key words: , Antimicrobial, Aqueous extract, Ethanol extract, Freeze dried

INTRODUCTION

Plants have been used in traditional medicine for millennia, and recent scientific research have revealed a link between traditional and folkloric uses of particular plants, bolstering the quest for pharmacological active components in plants (Egharevba and 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. is one of these therapeutic plants.

also known as *Buchholzia coriacea* 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 “” 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). *Buchholzia coriacea* 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). *Buchholzia coriacea* 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).'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 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), methanol extracts of *Buchholzia coriacea* seed show a superior efficacy against a wide spectrum of bacteria when compared to ethanol extract. Fresh express extract of Wonderful kola has a better effect than methanol and hexane extracts, according to Ezekiel and Onyeoziri (2009). Fresh express extract of 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 , 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 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 .

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 were also treated the same way to obtain aqueous and ethanol 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 100^0C.

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 37^0C 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 4^0C 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 4^0C. 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 28^0C for 120 hours after which the plates were inspected for zones of inhibition.

The above procedure was applied for aqueous and ethanol extracts of the fresh leaf, freeze dried leaf, fresh seed and freeze dried seeds, and

174 concentrations of 500mg, 250mg, 125mg and 50mg of each extracts was
175 prepared.
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RESULTS

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

The ethanol 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 ethanol 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 ethanol (7mm) extract of fresh seed of , while the aqueous and ethanol extract of fresh seed of 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 ethanol extract of freeze dried seed of *B. coriacea* recorded antibacterial activity against all the bacterial test isolates. The ethanol 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 ethanol and aqueous extract was recorded against *Klebsellapneumonia*. Also, antifungal activity was recorded against all fungal isolates. The best fungal activity of the ethanol and aqueous extract was recorded against *Aspergillusniger*. The ethanol 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 ethanol extract of freeze dried seed of , while the aqueous and ethanol extract of freeze dried seed of at all concentration showed better antifungal activity than Fluconazole (50µg/ml). The aqueous and ethanol extract of freeze dried seed of *B. coriacea* showed better antifungal and antibacterial activity compared with the aqueous and ethanol extract of freeze dried seed of *B. coriacea*.

The aqueous and ethanol extract of fresh leaf of was presented in figure 5 & 6. The ethanol 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 ethanol 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 with the aqueous and ethanol extract of fresh leaf of , while the aqueous and ethanol extract of fresh leaf of at all concentration showed better antifungal activity than Fluconazole (50µg/ml).

From figure 7 & 8, the aqueous and ethanol extracts of *B. coriacea* freeze dried leaf showed inhibitory zones ranging from 3-40mm respectively. It can be observed that the aqueous and ethanol 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 ethanol 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 ethanol and aqueous extract was recorded against *Penicilliumnotatum* (40mm) at a concentration of 500mg/ml. The ethanol 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 ethanol extract of freeze dried leaf of , while the aqueous and ethanol extract of freeze dried leaf of at all concentration showed better antifungal activity than Fluconazole (50µg/ml) used as control.

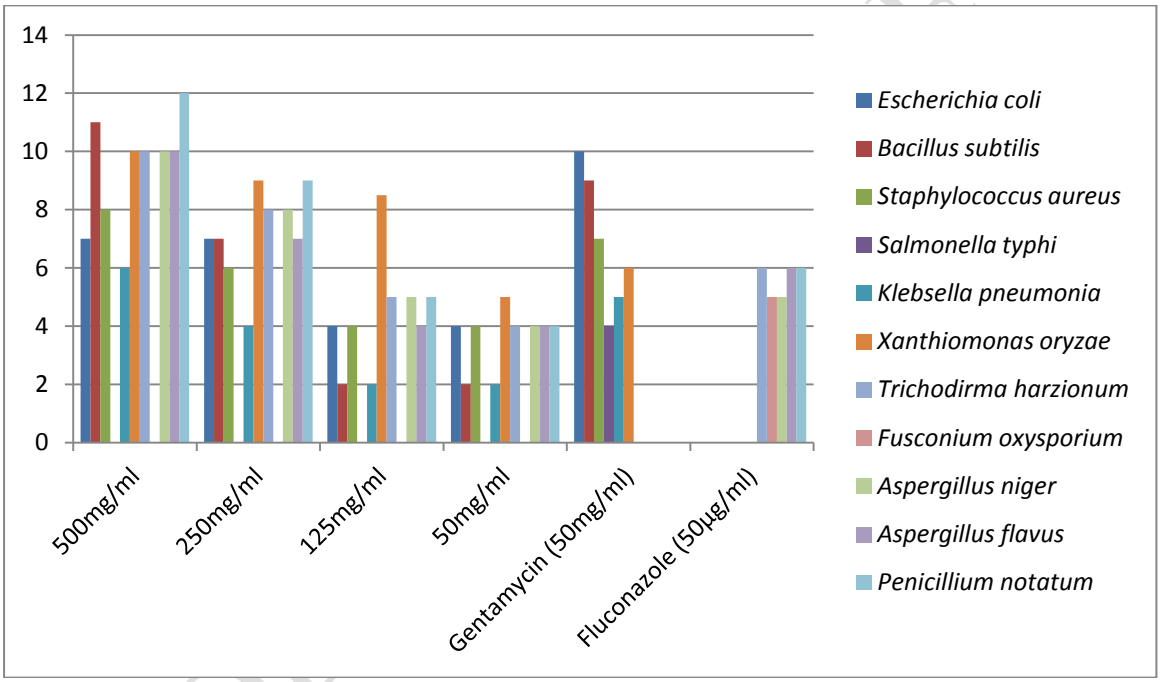

Figure 1: Result of antimicrobial screening of ethanol extract of fresh seed of with Zone of inhibition in mm

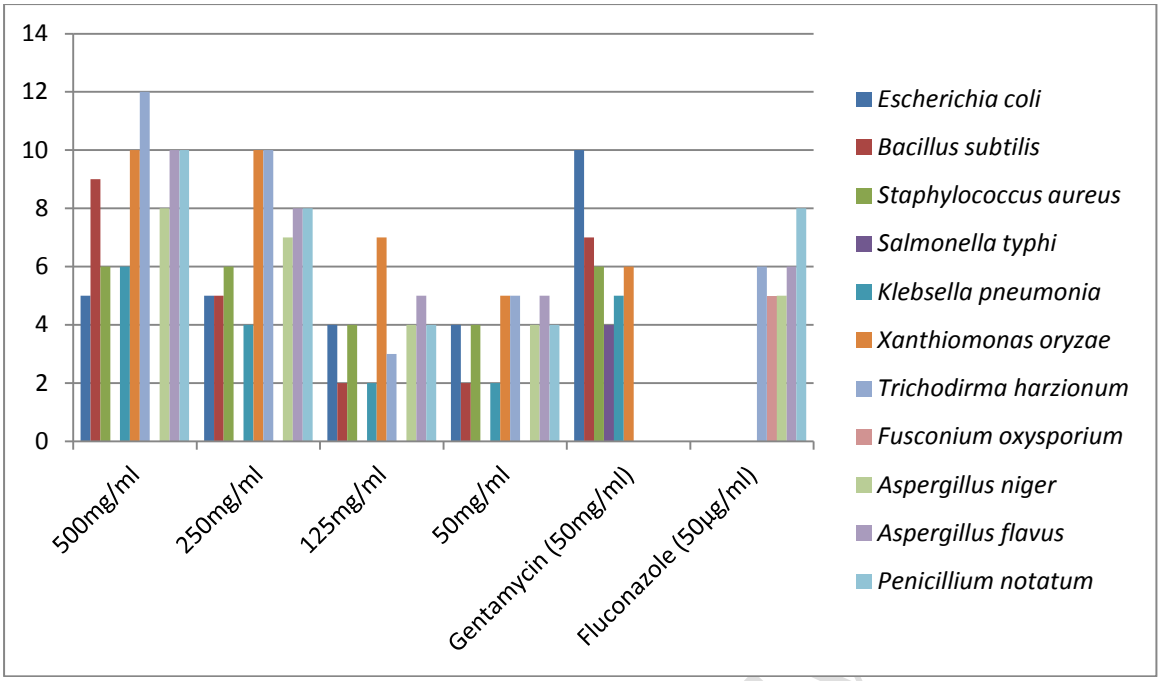

Figure 2: Result of antimicrobial screening of aqueous extract of fresh seed of with Zone of inhibition in mm

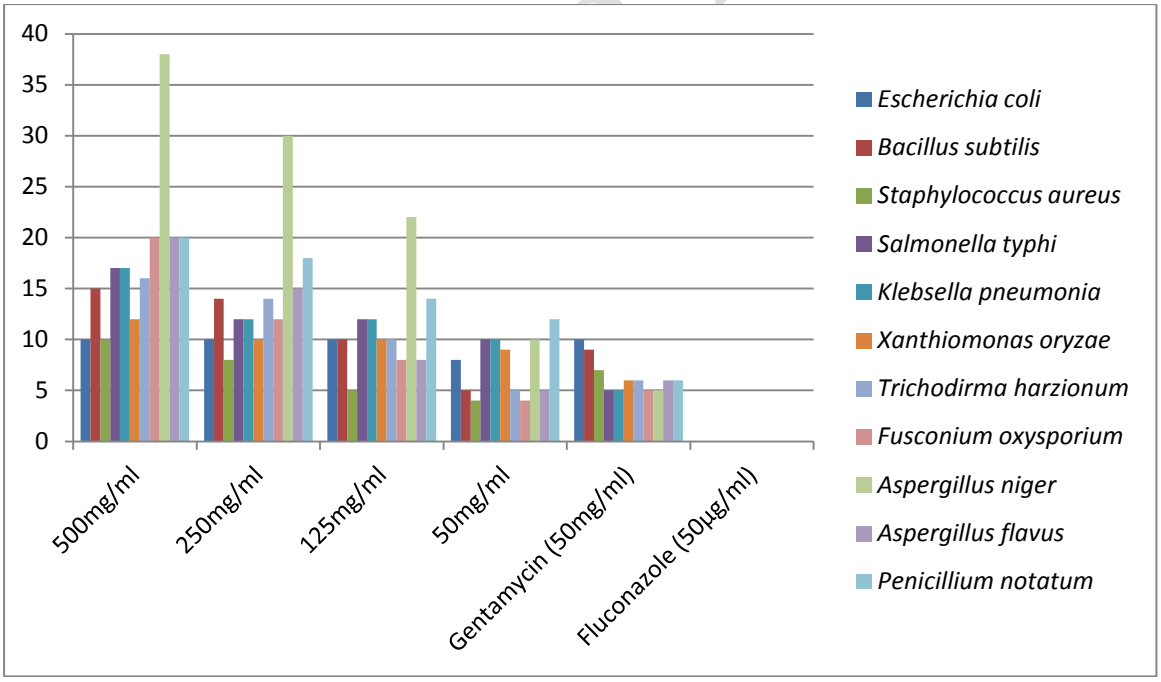

Figure 3: Result of antimicrobial screening of ethanol extract of freeze dried seed of with Zone of inhibition in mm

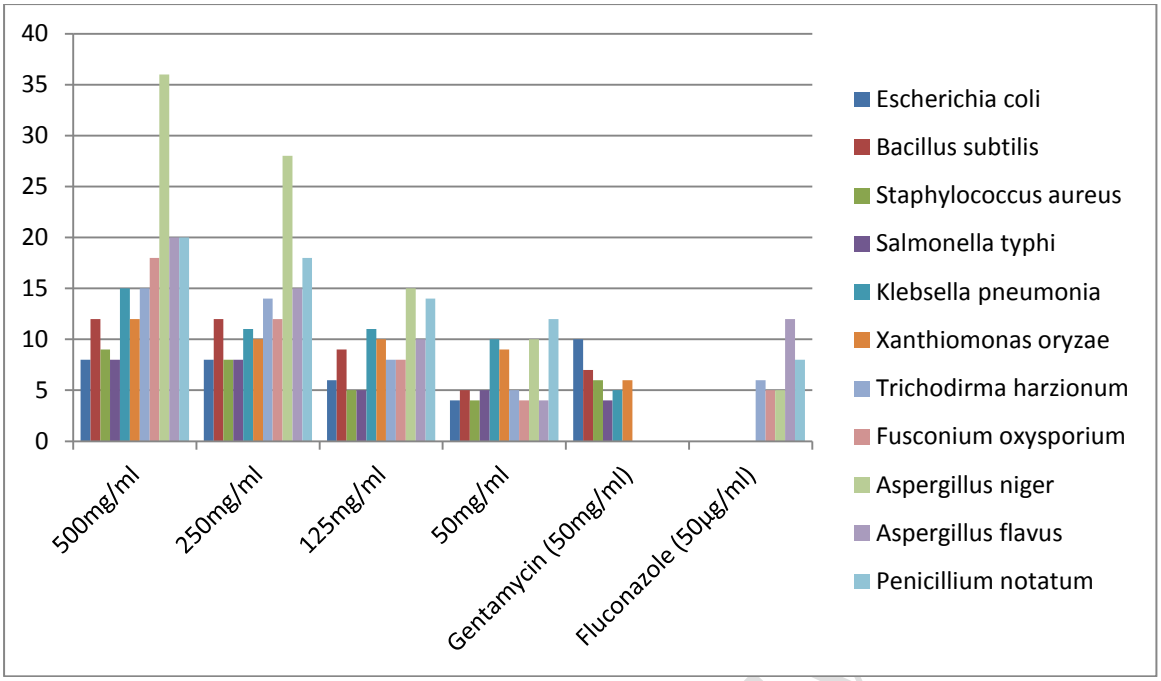

Figure 4: Result of antimicrobial screening of aqueous extract of freeze dried seed of with Zone of inhibition in mm

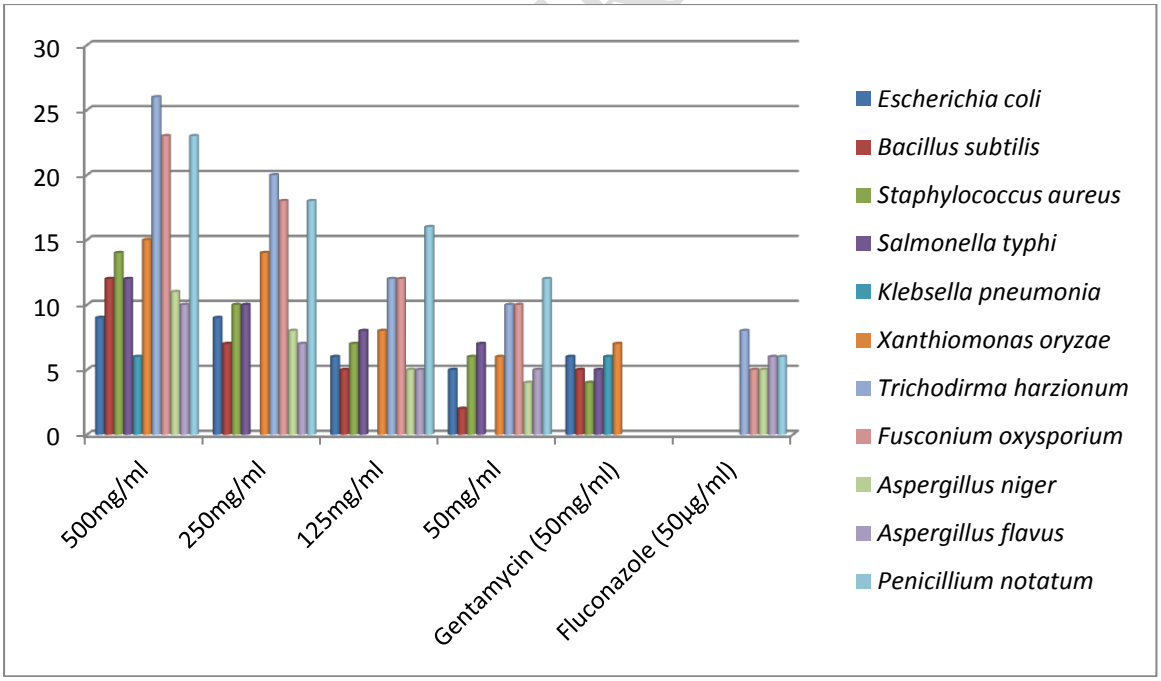

Figure 5: Result of antimicrobial screening of ethanol extract of fresh leaf of with Zone of inhibition in mm

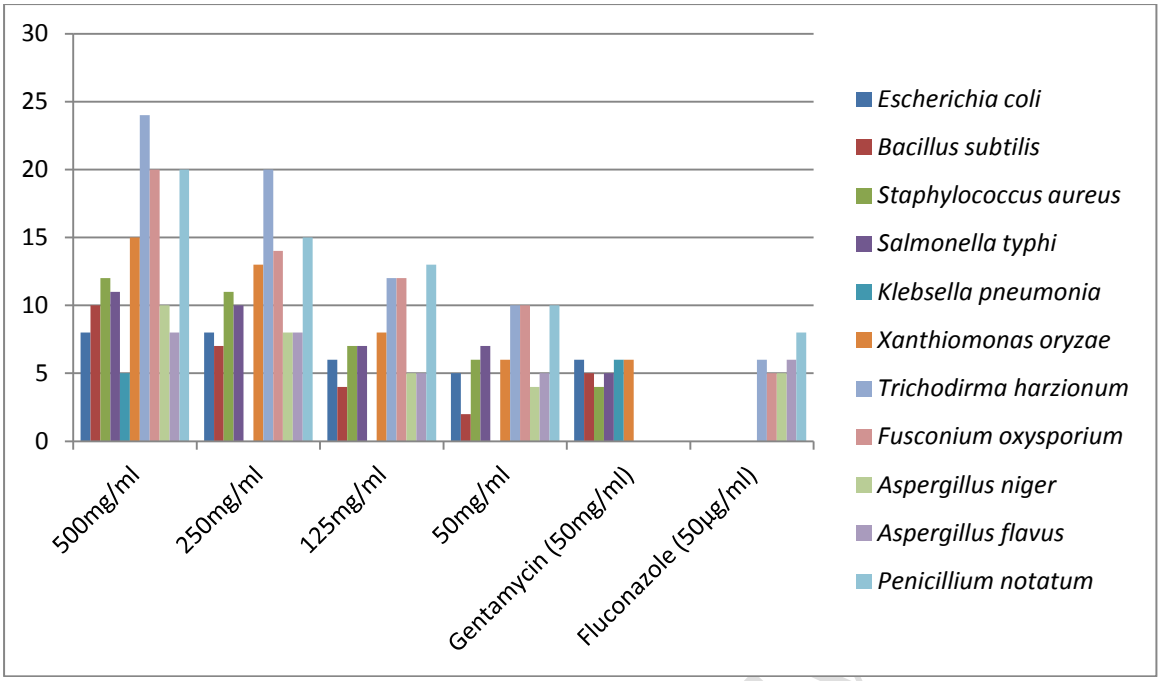

Figure 6: Result of antimicrobial screening of aqueous extract of fresh leaf of with Zone of inhibition in mm

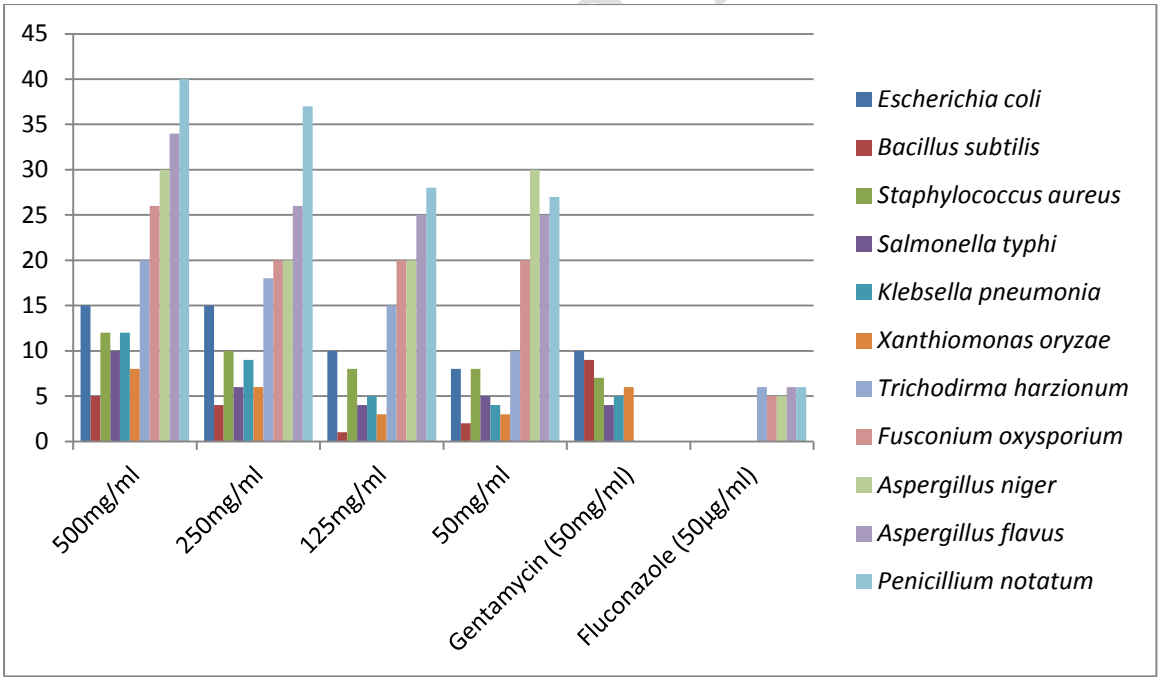

Figure 7: Result of antimicrobial screening of ethanol extract of freeze dried leaf of with Zone of inhibition in mm

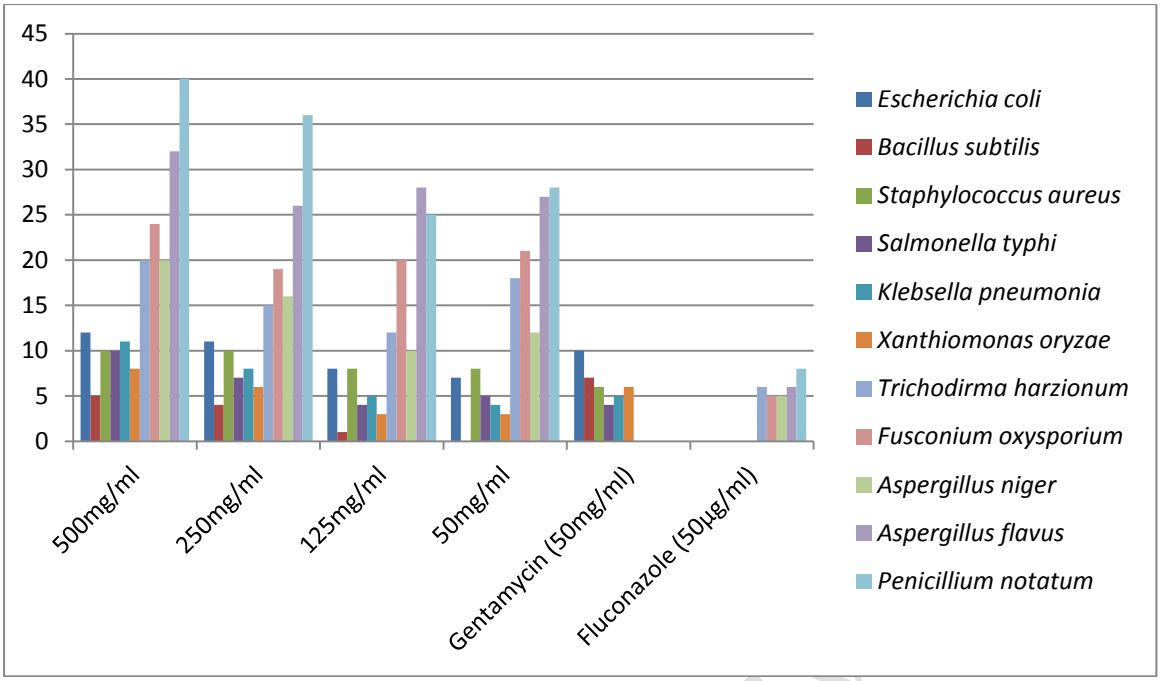

Figure 8: Result of antimicrobial screening of aqueous of freeze dried leaf of with Zone of inhibition in mm

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 mm	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

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

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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	9 mm	6 mm	5 mm	6 mm
	Aq	8mm	8 mm	6 mm	5 mm	6 mm
2	Bacillus subtilis	12mm	7 mm	5 mm	2 mm	5 mm
		10mm	7 mm	4 mm	2 mm	5 mm
3	Staphylococcus aureus	14mm	10 mm	7 mm	6 mm	4 mm
		12mm	11 mm	7 mm	6 mm	4 mm
4	Salmonella typhi	12mm	10 mm	8 mm	7 mm	5 mm
		11mm	10 mm	7 mm	7 mm	5 mm
5	Klebsella pneumonia	6mm	-	-	-	6 mm
		5mm	-	-	-	6 mm
6	Xanthiomonas oryzae	15mm	14 mm	8 mm	6 mm	7 mm
		15mm	13 mm	8 mm	6 mm	6 mm
	Fungi					**Fluconazole**
1	Trichoderma harzonium	26mm	20 mm	12mm	10mm	8 mm
		24mm	20 mm	12mm	10mm	6 mm
2	Fusconium oxysporium	23mm	18 mm	12mm	10mm	5 mm
		20mm	14 mm	12mm	10mm	5 mm
3	Aspergillus niger	11mm	8 mm	5 mm	4 mm	5 mm
		10mm	8 mm	5 mm	4 mm	5 mm
4	Aspergillus flavus	10mm	7 mm	5 mm	5 mm	6 mm
		8mm	8 mm	5 mm	5 mm	6 mm
5	Penicillium notatum	23mm	18 mm	17mm	12mm	6 mm
		20mm	15 mm	13mm	10mm	8 mm

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	10 mm
		12mm	11mm	8 mm	7mm	10 mm
2	Bacillus subtilis	5mm	4 mm	1 mm	-	9 mm
		5mm	4 mm	1 mm	-	7 mm
3	Staphylococcus aureus	12mm	10mm	8 mm	8	7 mm
		10mm	10mm	8 mm	8	6 mm
4	Salmonella typhi	10mm	6 mm	4 mm	5mm	4 mm
		10mm	7 mm	4 mm	5mm	4 mm
5	Klebsella pneumonia	12mm	9 mm	5 mm	4mm	5 mm
		11mm	8 mm	5 mm	4mm	5 mm
6	Xanthiomonas oryzae	8mm	6 mm	3 mm	3mm	6 mm
		8mm	6 mm	3 mm	3mm	6 mm
	Fungi					Fluconazole
1	Trichoderma harzionum	20mm	18mm	15mm	10mm	6 mm
		20mm	15mm	12mm	1.9mm	6 mm
2	Fusconium oxysporium	26mm	20mm	20mm	30mm	5 mm
		24mm	19mm	20mm	21mm	5 mm
3	Aspergillus niger	30mm	20mm	20mm	20mm	5 mm
		20mm	16mm	10mm	12mm	5 mm
4	Aspergillus flavus	34mm	26mm	25mm	26mm	6 mm
		32mm	26mm	28mm	27mm	6 mm
5	Penicillium notatum	40mm	37mm	27mm	28mm	6 mm
		40mm	36mm	25mm	28mm	8 mm

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 compared with its freeze dried leaf and seed.

The result of this study showed that the ethanol 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 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 ethanol 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(*Escherichia 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 ethanol extract of freeze dried seed of *B. coriacea* showed better antifungal and antibacterial activity against the test organisms compared with the aqueous and ethanol extract of fresh seed of *B. coriacea.*Similarly, the aqueous and ethanol extract of freeze dried leaf of *B. coriacea* showed better antifungal and antibacterial activity against the test organisms compared

with the aqueous and ethanol extract of fresh leaf of *B. coriacea*.The ethanol 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.

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