Original Research Article

COMPARATIVE STUDY OF THE ANTIMICROBIAL PROPERTIES OF FRESH AND FREEZE-DRIED LEAF AND SEED OF WONDERFUL KOLA (*Bucholzia 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 (*Buchholzia coriacea*) using ethanol and aqueous extracts was tested on some organisms/isolates? using standard laboratory procedures. The bacteria used were *Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Salmonella typhi, Klebsiella pneumoniae* and Xanthomonas*Xanthiomonas oryzae*, while the fungi used were *Trichoderma harzionium, Fusarium Fusconium oxysporium, Aspergillus niger, Aspergillus flavus* and *Penicillium notatum*. The results obtained showed that the ethanolic extracts of fresh seed 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 freeze dried seed 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 fresh leaf 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 freeze dried leaf of *B. coriacea* ~~freeze dried leaf~~ showed inhibitory zones ranging from 3-40 mm 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/isolates?, 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/isolates?, 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: *B. coriacea*~~Wonderful kola~~, Antimicrobial, Aqueous extract, Ethanolic extract, Freeze dried

1. INTRODUCTION

Plants have been used as ~~in~~ traditional medicine for millennia, and recent scientific research have revealed a ~~strong~~ link between traditional and folkloric uses of particular plants, strengthening ~~bolstering~~ the search

~~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 main~~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 compounds ~~components~~ present in plants such as flavonoids, glycosides, saponins, and tannins (Afolabi *et al*., 2020) may have contributed to their utility. *Buchholzia coriacea* or Wonderful kola is one of these ~~therapeutic~~ plants.

~~Wonderful kola 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 can ~~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. F~~When f~~resh~~, the~~ seeds are blackish in

Comment [PM1]: Glycosides is a broad term. Be specific.

Comment [PM2]: How big is big? Delete.

color, covered in purple arils, and have a harsh pungent spicy flavor ~~with a scorching spicy flavor~~ (Odebiyi & Sofowora, 1978). The seeds have been given a number ~~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, and 'Kola Pimente' in French. In Nigeria it is commonly called , '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). *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 (Reference needed). In Africa, it has been used specifically to cure migraines (Jaiyesimi *et al.*, 2011).The ~~Wonderful kola's~~ antibacterial qualities of *B. coriacea* have been attributed to its bioactive compounds~~components~~ such as ~~like as~~

Comment [PM3]: which country?

Comment [PM4]: Alleviate/ease migraines? Nobody has yet cured migraines?

alkaloids and tannins (Doherty *et al*., 2010; Kigigha *et al*., 2015, 2016; Epidi, 2016; Kalunta, 2017; Kigigha & Kalunta, 2017).

The ~~A~~ntimicrobial (antibacterial and antifungal) properties of *B. coriacea* ~~Wonderful kola~~ seed have been published ~~discovered in~~ numerous papers/articles~~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 of compounds have an impact on the final result of the sensitivity test of plant materials. According to Ibrahim & Fagbonun (2013), methanolic extracts of *B.~~uchholzia~~ coriacea* seed showed a superior efficacy against a wide spectrum of bacteria when compared to ethanol extract. Fresh ~~express~~ extract of *B. coriacea* ~~Wonderfulkola~~ has a greater ~~better~~ effect than methanol and hexane extracts, according to Ezekiel and Onyeoziri (2009). Fresh express extract of *B. coriacea* ~~Wonderful kola~~ seed has greater efficacy, compared to oven dried, uncooked and cooked seed, according to Nwachukwu *et al*. (2014). Methanol is a better solvent for *B. coriacea* leaf extract ~~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 *B. coriacea* ~~Wonderful kola~~ have focused only

Comment [PM5]: One cannot discover "properties" in a study.

Comment [PM6]: Sensitive to what? Please elaborate.

Comment [PM7]: Previously "compared with" were used. Be consistent.

Comment [PM8]: Seed or leaves

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on the fresh seed, bark and leaf of the plant; and no work has been reported on freeze dried leavesf and leaf. so far. Hence, tThis study aimed to determine the antimicrobial efficacy of fresh leaves and seeds, compared with freeze dried leavesf and seed of *B. coriacea*wonderful kola.

Comment [PM10]: What does this mean?

2. MATERIALS AND METHODSMETHODOLOGY

2.1 Plant Collection and Authentication

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

2.2 Preparation of Seed and Leaf samples Extract

The leaves were sorted, washed, chopped and divided into two parts. The first part was blended fresh leaves using an electric blender and refrigerated at 4ºC. The second part was freeze dried leaves, 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 as the leaves. 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.

Comment [PM11]: Please give the name of the freeze drier, supplier's name, city and country.

2.3 Ethanol Extract Preparation

A Satoric AG Gottingen Electronic weighing scale was used to weigh 200 grams of pulverized ~~kola~~ seed. The weighed samples were~~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. T~~ and t~~he filtrate (ethanol) was subjected to ~~recovered using a~~ Soxhlet extraction and subsequently ~~apparatus and was~~ evaporated to dryness using a steam bath at 100~~o~~^{o}C.

2.4 Aqueous Extract Preparation

Two hundred grams (200 g) of the pulverized ~~kola~~ seed was weighed and macerated in 500 ml of distilled water. The mixture~~s~~ was~~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.

2.5 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.

2.6 Test Organisms/isolates?

The microorganisms used were obtained from Department Of Microbiology, Federal University Of Technology, Akure, Ondo State,

Comment [PM12]: Fresh or freeze dried seeds?

Comment [PM13]: What was the percentage ethanol?

Comment [PM14]: Were there replicates or not?

Nigeria. The bacteria include *Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Salmonella typhi, Klebsiella pneumoniae* and Xanthomonas ~~*Xanthiomonas*~~ *oryzae*. These organisms/isolates? were further streaked out on nutrient agar and incubated at 37 ºC for 18 hours respectively. The isolates/organisms? identities were ~~further~~ confirmed using standard biochemical procedures as described by Leber (2016). T~~,~~ ~~t~~he isolates/organisms? were stored on agar slants at 4 ~~o~~ºC until further ~~prior to their~~ use. The fungi used were *Trichoderma harzianum*~~*harzionum*~~, *Fusarium*~~*Fusconium*~~ *oxysporium*, *Aspergillus niger, Aspergillus flavus* and *Penicillium notatum*. These were maintained on malt extract agar.

Comment [PM15]: How were these organisms propagated?

2.7 Screening for Antimicrobial Activities

The process involves the use of test organisms/isolates? to screen for the inhibitory properties of the extracts by measuring the diameters of slants ~~and stored~~ at 4 ºC. A c~~C~~ontrol 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.

Comment [PM16]: Don't understand this statements. Inhibitory zones are usually measured on agar plates.

2.8 Determination of antibacterial activity of the extracts: Nutrient agar was poured into Petri dishes, allowed to set and holes were bored into the agar 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.

2.9 Determination of antifungal activity of the extracts: Nutrient agar was poured into Petri dishes, allowed to set and holes were bored into the agar 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 ºC for 120 h~~ours~~ 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 C~~c~~oncentrations of 500 mg, 250 mg, 125 mg and 50 mg of each extracts was prepared.

3. RESULTS

Results of antimicrobial properties of ethanolic and aqueous extract of fresh dried seeds, fresh leavesf, freeze dried seeds and freeze dried leavesf of *B. coriacea* Wonderful kola are was presented in figures 1-8.

3.1 Fresh dried seeds

The ethanolic extracts of fresh dried seed 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 (figures 1 & 2). From the result of antimicrobial screening it can was found be observed that the ethanolic and aqueous seed extract of *B. coriacea* showed recorded antibacterial activity against the bacterial test isolates/organisms? (except *S.almonella typhi*), with the widest zone best activity recorded against *B. subtilis*. Antifungal activity was also recorded against all fungal isolates/organisms? (except *F.usconium oxysporium*), with the widest zone best activity recorded against *P.enicillium notatum.* The use of Gentamycin (50 mg) as control only showed better antibacterial activity against *E. coli* (10 mm) at a high concentration of (500 mg,) than the and aqueous (5mm) and ethanolic (7mm) extract of fresh seed of *B. coriacea* wonderful kola, while the aqueous and ethanolic extract of fresh seed of wonderful kola at 500 mg

Comment [PM17]: ?

Comment [PM18]: Sometimes "wonderful kola is used and sometimes B. coriacea is used. Be consistent. It would be more suitable to use B. coriacea in a science paper.

high concentration showed better antifungal activity than Fluconazole (50µg/ml) used as control.

3.2 Freeze dried seeds

Also looking at figure 3 & 4, it can be observed that Tthe aqueous and ethanolic extract of freeze dried seed of *B. coriacea* recorded antibacterial activity against all the bacterial test isolates/organisms? (Figures 3 & 4). 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 *K.lebsella pneumoniae*. Also, antifungal activity was recorded against all fungal isolates/organisms?. The best fungal activity of the ethanolic and aqueous extract was recorded against *A.spergillus niger*. The ethanolic extract recorded better antifungal activity than antibacterial activity. The use of Gentamycin (50 mg) as control only showed better antibacterial activity against *B.acillus subtilis* and *S.taphylococcus aureus* at concentration of 50 mg/ml, compared with the aqueous and ethanolic extract of freeze dried 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*.~~

Comment [PM19]: This does not make sense

3.3 Fresh leaves

The aqueous and ethanolic extract of fresh leaf of *B. coriacea* ~~wonderful kola~~ is ~~was~~ presented in figures 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 was found ~~can be observed~~ that the ethanolic and aqueous fresh leaf extract ~~of *B. coriacea*~~ recorded antibacterial activity against the bacterial test isolates/organisms? at different concentrations except for *K.~~lebsella~~ pneumoniae*, which showed antibacterial activity only at 500 mg. The best antibacterial activity was recorded against *X.~~anthiomonas~~ oryzae*. Antifungal activity was also recorded against all fungal isolates/organisms? at different concentrations, with the best activity recorded against *T.~~richoderma~~ harzianum ~~harzonium~~*. The use of Gentamycin (50 mg) as control only showed slightly better antibacterial activity against *B.~~acillus~~ subtilis* and *E.~~scherichia~~ coli* at 50 mg/l concentration, ~~of 50mg/ml~~ compared with the 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).

3.4 Freeze dried leaves

From figures 7 and~~&~~ 8, the aqueous and ethanolic extracts of ~~*B. coriacea*~~ freeze dried leaf showed inhibitory zones ranging from 3-40 mm and …..respectively. It can be seen ~~observed~~ that the aqueous and ethanolic extract of freeze dried leaf ~~of *B. coriacea*~~ recorded antibacterial activity against all the bacterial test isolates/organisms? at different concentrations except for *B. ~~acillus~~ subtilis* which did not show any antibacterial activity at 2 mg. The highest bacterial activity of the ethanolic and aqueous extract was recorded against *E.~~scherichia~~ coli* at a concentration of 500 mg/ml. Also, antifungal activity was recorded against all fungal isolates/organisms?. The highest fungal activity of the ethanolic and aqueous extract was recorded against *P.~~enicillium~~ notatum* (40 mm) 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 (50 mg) as control only showed slightly better antibacterial activity against *B.~~acillus~~ subtilis* at concentration of 50mg/ml, compared with the 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.

Please identify the y axes in figures 1-8. Give the units (mm)

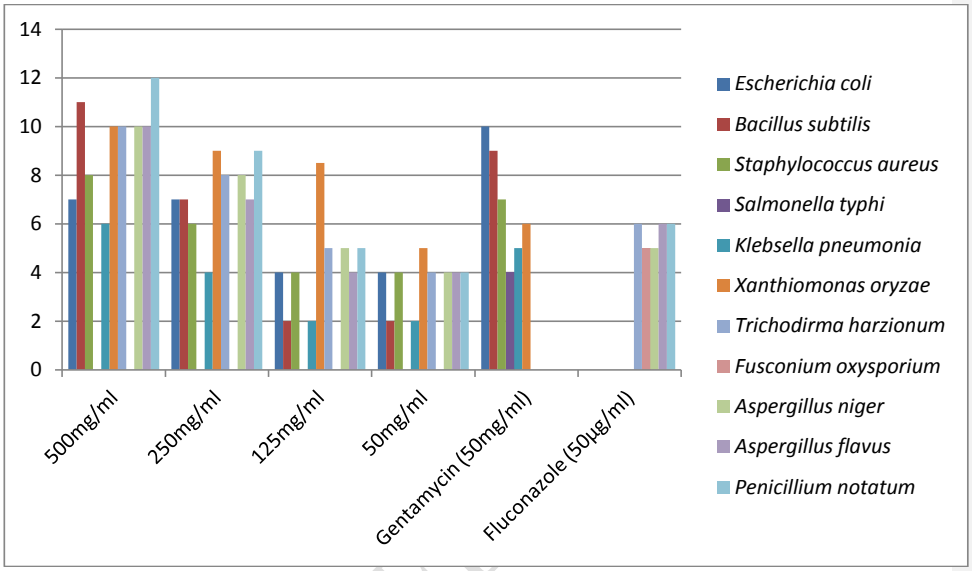
Comment [PM20]: Is this the zone for aqueous extract? Where is the zone for ethanolic extracts?

Comment [PM21]: There is not "2 mg" in either figure 7 or 8.

Comment [PM22]: Be specific

Klebsiella pneumoniae, *Trichoderma harzianum*, *Fusarium oxysporum* and *Xanthomonas* are spelt incorrectly in figures 1-8.

Organism names should be in italics in figure 4.



Fig~~ure~~ 1.~~:~~ Result of antimicrobial screening of ethanolic extract of fresh seed of *B. coriacea* ~~Wonderful kola~~ with z~~Z~~one of inhibition in mm

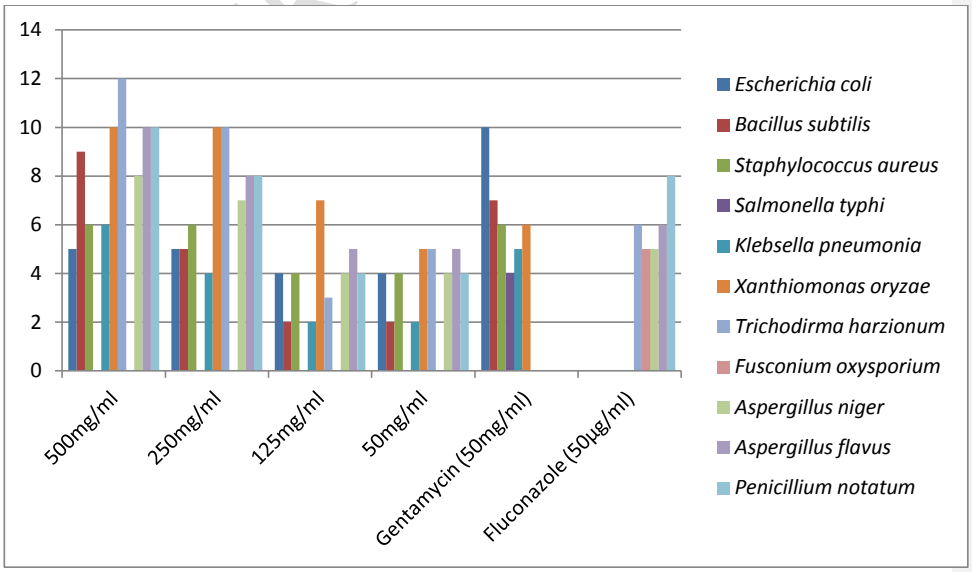


Fig.~~ure~~ 2.~~:~~ Result of antimicrobial screening of aqueous extract of fresh seed of *B. coriacea* ~~Wonderful kola~~ with z~~Z~~one of inhibition in mm

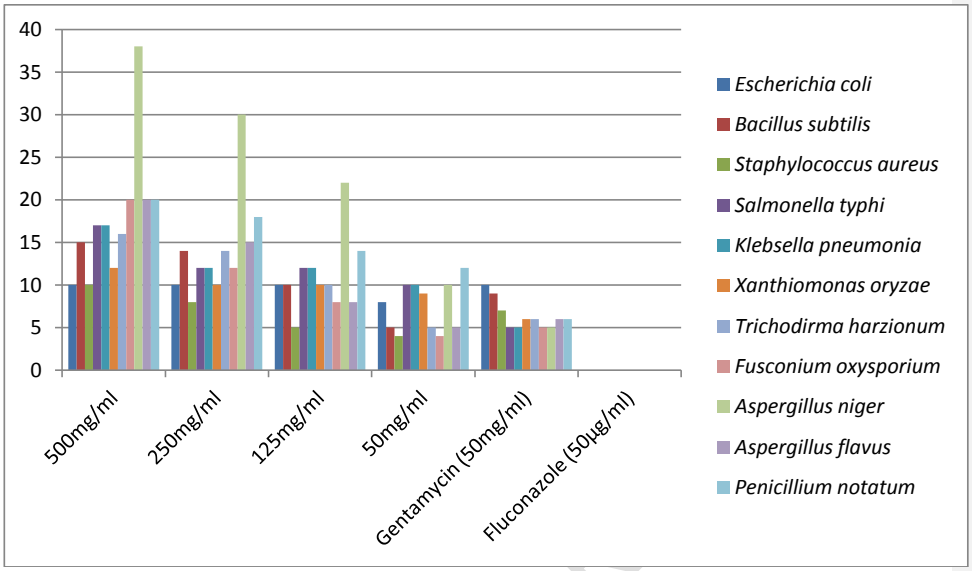


Fig.~~ure~~ 3.~~:~~ Result of antimicrobial screening of ethanolic extract of freeze dried seed of *B. coriacea*~~Wonderful kola~~ with z~~Z~~one of inhibition in mm

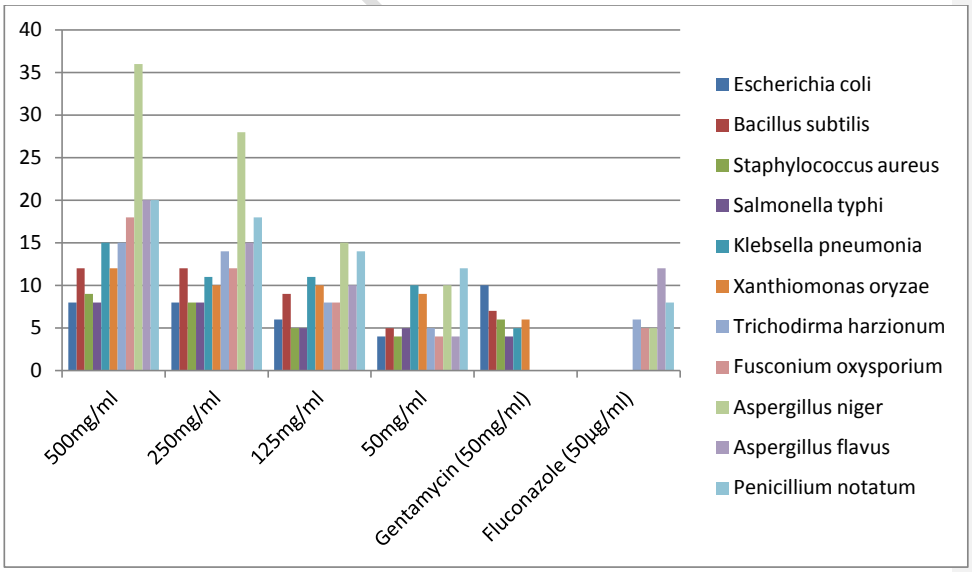


Fig.~~ure~~ 4.: Result of antimicrobial screening of aqueous extract of freeze dried seed of *B. coriacea* ~~Wonderful kola~~ with z~~Z~~one of inhibition in mm

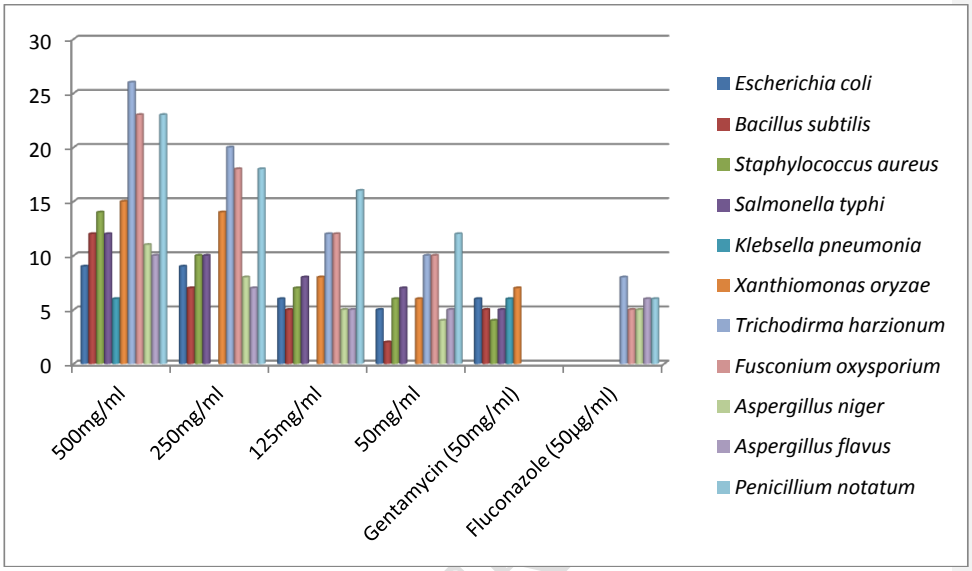


Fig.~~ure~~ 5.: Result of antimicrobial screening of ethanolic extract of fresh leaf of *B. coriacea* ~~Wonderful kola~~ with z~~Z~~one of inhibition in mm

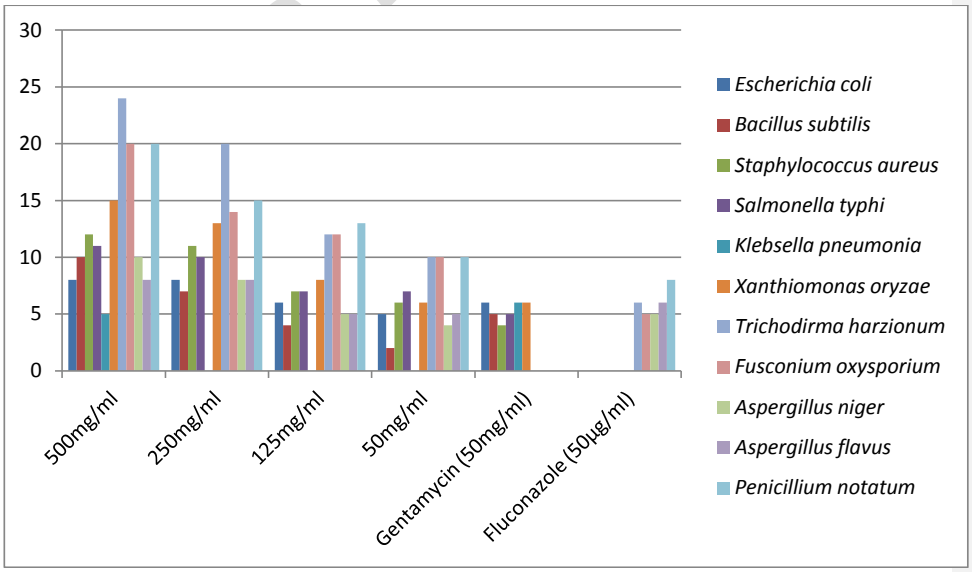


Fig.~~ure~~ 6.: Result of antimicrobial screening of aqueous extract of fresh leaf of *B. coriacea* ~~Wonderful kola~~ with z~~Z~~one of inhibition in mm

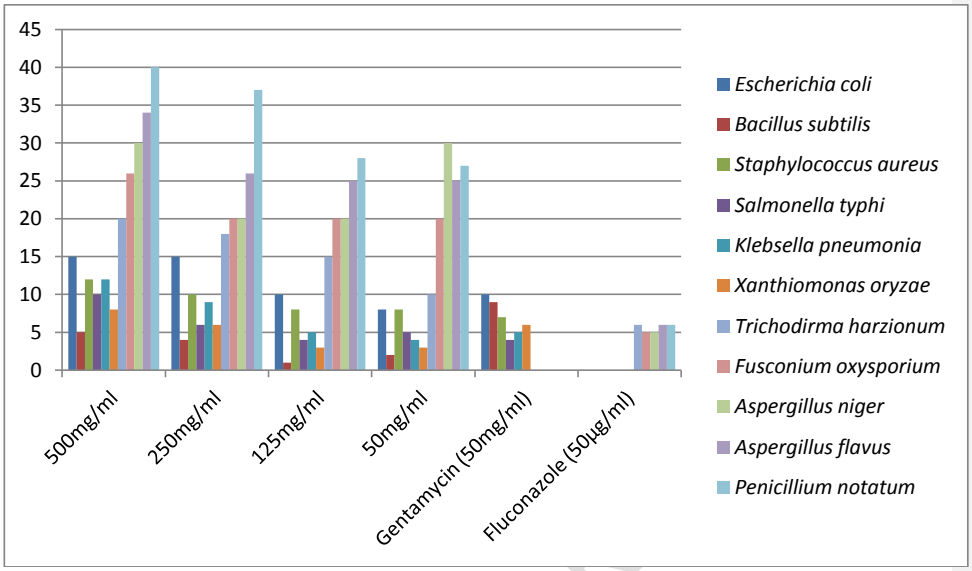


Fig.~~ure~~ 7.: Result of antimicrobial screening of ethanolic extract of freeze dried leaf of *B. coriacea* ~~Wonderful kola~~ with z~~Z~~one of inhibition in mm

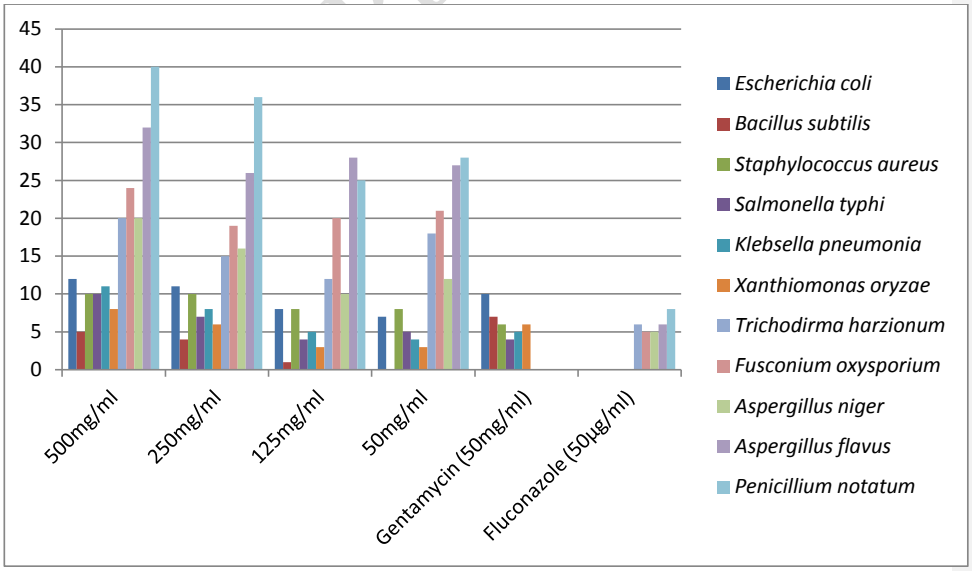


Fig.~~ure~~ 8.: Result of antimicrobial screening of aqueous of freeze dried leaf of *B. coriacea* ~~Wonderful kola~~ with z~~Z~~one of inhibition in mm

Fig.~~ure~~ 9.~~:~~ The pictures of *Buchholzia coriacea* tree, leaves and seeds.

The tables1-4 need editing. Spacing and font are inconsistent

Table 1.~~:~~ Fresh seed of *Buchholzia coriacea* ~~Wonderful cola~~ with z~~Z~~one of inhibition in mm at different concentration in mg/ml.

S/N	Microorganism	500mg	250mg	125mg	50mg	Control
	Bacteria					Gentamycin
1	E. coli Eth	7 mm	7 mm	4 mm	4 mm	10 mm
	Aq	5 mm	5 m	4 mm	4 mm	10 mm
2	B. subtilis Eth	11mm	7 mm	2 mm	2 mm	9 m m
	Aq	9 mm	5 mm	2 mm	2 mm	7 m m
3	S. aureus Eth	8 mm	6 mm	4 mm	4 mm	7 m m
	Aq	6 mm	6 mm	4 mm	4 mm	6 m m
4	S. typhi Eth	-	-	-	-	4 m m
	Aq	-	-	-	-	4 m m

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5	K. pneumoniae Eth	6 mm	4 mm	2 mm	2 mm	5 m m
	Aq	6 mm	4 mm	2 mm	2 mm	5 m m
6	X. oryzae Eth	10mm	-9mm	8.5mm	5 mm	6 m m
	Aq	10mm	10mm	-7mm	5 mm	6 m m
	FUNGI					**Fluconazole**
1	T. harziaonium Eth	10mm	8 mm	4 mm	5 mm	6 m m
	Aq	12mm	10mm	3 mm	5 mm	6 m m
2	F. oxysporium Eth	-	-	-	-	5 m m
	Aq	-	-	-	-	5 m m
3	A. niger Eth	10mm	8 mm	5 mm	4 mm	5 m m
	Aq	7 mm	8 mm	4 mm	4 mm	5 m m
4	A. flavus Eth	10mm	7 mm	5 mm	4 mm	6 m m
	Aq	10mm	8 mm	5 mm	5 mm	6 m m
5	P. notatum Eth	12mm	9 mm	4 mm	5 mm	6 m m
	Aq	10mm	8 mm	4 mm	5mm	8 m m

Control ; 50mg/ml

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Table 2 : Freeze dried seed of *Buchholzia coriacea* ~~Wonderful cola~~ with z~~Z~~one of inhibition at different concentration in mm

s/n	Microorganism	500mg	250mg	125mg	50mg	Control
	Bacteria					Gentamycin
1	E. coli	10mm	10mm	10mm	8mm	10mm
		8mm	8mm	6mm	4mm	10mm
2	B. subtilis	15mm	14mm	10mm	5mm	9mm
		12mm	12mm	9mm	5mm	7mm
3	S. aureus	10mm	8mm	5mm	4mm	7mm
		9mm	8mm	5mm	4mm	6mm
4	S. typhi	10mm	10mm	7mm	6mm	4mm
		8mm	8mm	5mm	5mm	4mm
5	K. pneumoniae	17mm	12mm	12mm	10mm	5mm
		15mm	11mm	11mm	10mm	5mm
6	X. oryzae	12mm	10mm	10mm	9mm	6mm
		12mm	10mm	10mm	9mm	6mm
	Fungi					Fluconmazole
1	T. harziaonium	16mm	14mm	10mm	5mm	6mm
		15mm	14mm	8mm	5mm	6mm
2	F. oxysporium	20mm	12mm	8mm	4mm	5mm
		18mm	12mm	8mm	4mm	5mm
3	A. niger	38mm	30mm	22mm	10mm	5mm
		36mm	28mm	15mm	10mm	5mm
4	A. flavus	20mm	15mm	8mm	5mm	6mm
		20mm	5mm	10mm	4mm	6mm
5	P. notatum	20mm	19mm	14mm	12mm	6mm
		20mm	19mm	14mm	12mm	8mm

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Table 3 : Fresh leaf of *Buchholzia coriacea* ~~Wonderful cola~~ with z~~Z~~one of inhibition at different concentration in mm

s/n	Microorganism	500mg	250mg	12mg	50mg	Control
	Bacteria					Gentamycin
1	E. coli Eth	9mm	9 m m	6 m m	5 m m	6 m m
	Aq	8mm	8 m m	6 m m	5 m m	6 m m
2	B. subtilis	12mm	7 m m	5 m m	2 m m	5 m m
		10mm	7 m m	4 m m	2 m m	5 m m
3	S. aureus	14mm	10 m m	7 m m	6 m m	4 m m
		12mm	11 m m	7 m m	6 m m	4 m m
4	S. typhi	12mm	10 m m	8 m m	7 m m	5 m m
		11mm	10 m m	7 m m	7 m m	5 m m
5	K. pneumoniae	6mm	-	-	-	6 m m
		5mm	-	-	-	6 m m
6	X. oryzae	15mm	14 m m	8 m m	6 m m	7 m m
		15mm	13 m m	8 m m	6 m m	6 m m
	Fungi					Fluconazole
1	T. harziao~~i~~nium	26mm	20 m m	12mm	10mm	8 m m
		24mm	20 m m	12mm	10mm	6 m m
2	F. oxysporium	23mm	18 m m	12mm	10mm	5 m m
		20mm	14 m m	12mm	10mm	5 m m
3	A. niger	11mm	8 m m	5 m m	4 m m	5 m m
		10mm	8 m m	5 m m	4 m m	5 m m
4	A. flavus	10mm	7 m m	5 m m	5 m m	6 m m
		8mm	8 m m	5 m m	5 m m	6 m m
5	P. otatum	23mm	18 m m	17mm	12mm	6 m m
		20mm	15 m m	13mm	10mm	8 m m

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Table 4: Freeze dried leaf of *Buchholzia coriacea* ~~Wonderful cola~~ with z~~Z~~one of inhibition at different concentration in mm

s/n	Microorganism	500 mg	250mg	125 mg	50 mg	Control
	Bacteria					**Gentamycin**
1	E. coli	15mm	15mm	10mm	8mm	10 mm
		12mm	11mm	8 mm	7mm	10 mm
2	B. subtilis	5mm	4 mm	1 mm	-	9 mm
		5mm	4 mm	1 mm	-	7 mm
3	S. aureus	12mm	10mm	8 mm	8	7 mm
		10mm	10mm	8 mm	8	6 mm
4	S. typhi	10mm	6 mm	4 mm	5mm	4 mm
		10mm	7 mm	4 mm	5mm	4 mm
5	K. pneumoniae	12mm	9 mm	5 mm	4mm	5 mm
		11mm	8 mm	5 mm	4mm	5 mm
6	X. oryzae	8mm	6 mm	3mm	3mm	6 mm
		8mm	6 mm	3mm	3mm	6 mm
	Fungi					**Fluconazole**
1	T. harzianum~~harzionum~~	20mm	18mm	15mm	10mm	6 mm
		20mm	15mm	12mm	1.9mm	6 mm
2	F. oxysporium	26mm	20mm	20mm	30mm	5 mm
		24mm	19mm	20mm	21mm	5 mm
3	A. niger	30mm	20mm	20mm	20mm	5 mm
		20mm	16mm	10mm	12mm	5 mm
4	A. flavus	34mm	26mm	25mm	26mm	6 mm
		32mm	26mm	28mm	27mm	6 mm
5	P. notatum	40mm	37mm	27mm	28mm	6 mm
		40mm	36mm	25mm	28mm	8 mm

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4. 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 *B. coriacea,* ~~wonderful kola~~ compared with its freeze dried leaves~~f~~ 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/organisms? (*B. subtilis*, *E. coli*, *S. aureus*, *K. pneumoniae* and *X. oryzae*). Antifungal activity was also recorded against *A. niger*, *A. flavus*, *T. harzianum*~~*harzionum*~~ and *P. notatum*. These results are ~~This observation is~~ in agreement with previous studies which have ~~variously~~ shown that *B. coriacea* ~~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 hexane and methanol extracts of fresh *B. coricea* ~~kola~~, ~~hexane, and methanol extracts of *B. coricea*~~ on various food borne pathogens (*E.*~~*scherichia*~~ *coli*, *Enterococcus faecalis*, *S.*~~*taphylococcus*~~ *aureus*, *Trichoderma viride*, and *A.*~~*spergillus*~~ *niger*) was studied by Ezekiel and Onyeoziri (2009). The fresh *B. coriacea* ~~kola~~ showed inhibitory zones with the test bacteria: *E. coli* (62 mm), *E. faecalis* (40 mm) and *S. aureus* (50 mm). The growth of the two test fungi, i.e. *T. viride* and *A. niger* was completely inhibited. According to Umeokoli *et al*. (2016), ~~the~~ aqueous seed extract of *B. coriacea* has antibacterial

Comment [PM23]: Which part of kola? Seed. or leaves?

Comment [PM24]: Seeds or leaves?

activity against all of the bacterial test isolates/organisms? (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 evident ~~seen~~ in the methanol seed extract of *B. coriacea* against all of the bacterial test isolates/organisms?, as well as antifungal activity against *C.~~andida~~ albicans* and *A.~~spergillus~~ niger*. The methanol extract had better~~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/isolates? (*B. subtilis, E. coli,* S. *typhi* , *K. pneumoniae, 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 an ~~the~~ ethanol extracts of *B. coriacea* showed inhibitory zones ranging from 14–27 mm with all test organisms/isolates? (*Pseudomonas spp., E. coli, S. aureus, Klesiella sp., Streptococcus sp.,* and *C.~~andida~~ albicans*), while an~~the~~ aqueous extract of *B. coriacea* showed inhibitory zones of 2-14 mm (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) treated isolates/organisms? with n-hexane, methanol, and chloroform leaf

Comment [PM25]: Already mentioned

extracts of *B. coriacea*~~,~~ and showed ~~it elicited~~ slight ~~modest~~ antibacterial activities against ~~the test isolates with~~ *E. coli*, *S.~~taphylococcus~~ aureus*, *Shigella* sp. ~~*species*~~, *K.~~lebsiella~~ pneumoniae*, and *B.~~acillus~~ subtilis* ~~susceptible~~. According to Okoli *et al*. (2010), certain ~~extracting~~ solvents can cause variations in components who have an affinity for…..spice ~~extractive components~~, which can affect antibacterial activity. *S. aureus*, *E. coli*, *S. typhii*, *P. aeruginosa*, *C.~~andida~~ 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/isolates? 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*e, *X. oryzae* and *S. aureus*). It ~~it was exposed to and it~~ completely inhibited the growth of *T. harzianum~~harzionum~~*, *F. oxysporium*, *A. niger*, *A. flavus* and *P. notatum*. When Ezekiel and Onyeoziri (2009) investigated the effect of n-hexane and methanol of fresh extract of *B. coriacea*~~kola, hexane, and methanol extracts of B. coricea~~ on several food-borne pathogens (*E.~~sherichia~~ coli*, *E.~~nterococcus~~ faecalis*, *S.~~taphylococcus~~ aureus*, *T.~~richoderma~~ viride* and *A.~~spergillus~~ niger*), they found ~~a~~ similar results. The heat applied during drying process may account for the dried leaf extracts of *B. coriacea* having a lower inhibitory activity than the frozen seed and freeze dry leaf

Comment [PM26]: Inappropriate word.

Comment [PM27]:

Comment [PM28]: This section must be rewritten. It does not make sense.

Comment [PM29]: What is this?

Comment [PM30]: Rewrite this section

Comment [PM31]: ? Be consistent. Isolates/organisms?

Comment [PM32]: Seeds or leaves?

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 the freeze drying process~~processing~~, the rehydrated product is of ~~has~~ good quality as most of the bioactive compounds have ~~has~~ been preserved which could explain why freeze dried seeds and freeze dried~~dry~~ leaves~~f~~ had a better inhibitory impact on the test organisms/isolates? than other drying processes employed in other studies reported.

Improvement ~~Changes~~ in the inhibitory impact of freeze dried seed and freeze dried leaf on the test organisms/isolates? could potentially be attributable to differences in ~~the~~ solvent~~s'~~ 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 used ~~employed~~ in the study (Izah *et al*., 2018). The physiology, metabolism, nutrition requirements, and biochemistry of the microbial isolates/organisms? may also have an impact on the sensitivity of an extract to ~~and~~ organisms/isolates? (Kigigha *et al*., 2016; Epidi *et al*., 2016). Variations in sensitivity could be caused by the age and type of

Comment [PM33]: Rephrase this section. The authors did not compare methods.

Comment [PM34]: Not sure if this is applicable to this work.

Comment [PM35]: Elaborate on "sensitivity"

Comment [PM36]: Elaborate/rephrase

plant extracts used employed, as well as environmental factors (Kigigha *et al*., 2016; Epidi *et al*., 2016).

5. 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/isolates?, 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/isolates?, 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 processes 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.

Comment [PM37]: This is an extension of the discussion.

Comment [PM38]: This was not proven in this study. It is speculation.

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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