



## ANTIBACTERIAL AND ANTIOXIDANT ACTIVITY OF *Bacillus* SPECIES ISOLATED FROM FERMENTED *Parkia biglobosa* (IRU) AND *Ricinus communis* (OGIRI)- AFRICAN TRADITIONALLY FERMENTED FOOD CONDIMENTS

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### ABSTRACT

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Fermented food condiments are essential parts of the diet of various cultures in different part of the world. Iru and ogiri are locally produced food condiments in Nigeria made from locust beans (*Parkia biglobosa*) and castor oil seeds (*Ricinus communis*) respectively. An attempt was therefore made in this study to isolate and characterize different *Bacillus* species from the condiments samples as well as investigate the antibacterial and antioxidant properties of some selected *Bacillus* species. Five *Bacillus* species were isolated from the condiment samples using standard methods, *in-vitro* antibacterial activity of *Bacillus* species was carried out using the agar well diffusion method. The broth dilution technique was used to determine the minimum inhibitory concentration and minimum bactericidal concentration of *Bacillus* species while thin layer chromatography technique was employed to identify a metabolite produced by *B. subtilis*. The predominant *Bacillus* species isolated from ogiri were: *B. mycoides* and *B. subtilis*; iru pete: *B. mycoides*, *B. licheniformis* while *B. brevis* and *B. licheniformis* were the predominant *Bacillus* species isolated from iru woro. The results of the antibacterial activity of the selected *Bacillus* species revealed that the following organisms OGA3, IWA3, IWB5, IPI3, OGB3, IPB3 and IPH3 showed the following zones of inhibition against: *Escherichia coli* (0mm, 16mm, 0mm, 13mm, 0mm, 14mm and 0mm); *Corynebacterium diphtheriae* (0mm, 0mm, 0mm, 20mm, 17mm, 0mm and 0mm); *Proteus mirabilis* (0mm, 17mm, 16mm, 20mm, 23mm, 18mm, and 19mm) while *Staphylococcus aureus* and *Klebsiella pneumoniae* showed the same zones of inhibition (0mm, 0mm, 0mm, 0mm, 0mm, and 0mm). IWA3 and IPI3 exhibited MIC of 3.7mg/ml against *E. coli*; IPI3 showed MIC of 2.5mg/ml against *C. diphtheriae*, IWA3 and IWB5 exhibited MIC of 2.5mg/ml against *P. mirabilis*. No MIC was recorded for *S. aureus* and *K. pneumoniae*. Nevertheless, only IWA3 showed minimum bactericidal concentration (MBC) of 3.5mg/ml and 3.7mg/ml against *E.coli* and *P.mirabilis* respectively. OGB3 and IPB5 exhibited 65.80% and 27.52% antioxidant activity while the reducing power was recorded as 0.683 and 0.309. In addition, OGB3 produced a metabolite known as iturin which is a strong anti-fungal agent. Therefore, it can be concluded that the *Bacillus* species isolated from condiment samples can be used as an antimicrobial agent against *P. mirabilis* as they showed higher zones of inhibition than the control antibiotic (gentamycin). In addition, OGB3 (*B. subtilis* isolated from ogiri) can also serve as a potential biopreservative in foods.

**Contribution/Originality:** The paper's primary contribution is finding that metabolites produced by food grade *Bacillus* species are sources of natural bioactive compounds with antibacterial and antioxidant properties which helps to promote the health of individuals consuming foods produced with these microorganisms when employed as starter cultures during fermentation.

## 1. INTRODUCTION

Iru and Ogiri are among the two most popular indigenous traditionally fermented condiments produced from leguminous proteins (Omafuvbe, Falade, Osuntogun, & Adewusi, 2004). Fermented African locust bean (*Parkia biglobosa*) is called iru in Yoruba and dawadawa in Hausa respectively (Odunfa & Oyewole, 1998). Ogiri is obtained by fermenting melon seeds (*Citrullus vulgaris*), fluted pumpkin (*Telferia occidentalis*) and castor oil seeds (*Ricinus communis*) (Omafuvbe et al., 2004) which is mostly consumed among the Igbos. These seeds are used in the production of ogiri-egusi, ogiri-ugu and ogiri-igbo/isi (Achi, 2005; Omafuvbe et al., 2004) which can be used as protein supplements and as a functional ingredients in food preparation. Soetan, Akinrinde, and Adisa (2014) reported that traditional fermented foods contain high nutritive value, better digestibility, flavors, aroma and texture. The substrates used for the fermentation of these condiments harbour diverse microorganisms from the environment which helps to transform the chemical constituents of the raw materials into useful products. The advantages of fermenting the substrates include: enhance nutritive value of the products; enrich bland diets with improved flavor and texture; fortify food products with essential amino acids, health promoting bioactive compounds, vitamins and minerals; degrade undesirable compounds and anti-nutritional factors; impart antioxidant and antimicrobial properties; improve digestibility and stimulate probiotic functions (Ling et al., 2013). Fermentation also results in lower proportion of dry matter in the food condiments and increases the concentration of vitamins, minerals and protein when measured on dry weight basis (Savadogo et al., 2011).

The genus *Bacillus* are Gram-positive, rod-shaped bacteria, belonging to the phylum Firmicutes. Antimicrobial metabolites have been widely documented to be produced among the genera *Streptomyces* and *Bacillus* (Arasu, Duraipandiyan, & Ignacimuthu, 2013). *Bacillus* species are good sources of bioactive compounds, notably antibiotics, therapeutic proteins, enzyme inhibitors and pharmacologically active agents (Huang, Gao, Zheng, & Hao, 2009). Lipopeptides such as surfactin, iturin and fengycin have been reported to be recovered from *Bacillus* species and characterized (Huang et al., 2009). However, most of these bioactive compounds are active against Gram-positive bacteria, some of them have a wide range of bio-activity against Gram-negative and filamentous fungi (Sirtori, Motta, & Brandelli, 2008). In addition, they have also been used as bio-control agents due to their antagonistic and repressive activities against disease causing organisms such as fungi and bacteria (Youcef-Ali et al., 2014).

The search for natural biological agents that have antibacterial and antioxidant activities is a new research trend in the fields of biology, medicine and food sciences (Affy, Romeilah, Sultan, & Hussein, 2012). Antioxidants play important roles in food as a health protecting and promoting constituent. Antioxidant reduce the risk of chronic diseases such as cancer and heart problems. Proteinous food condiments are natural sources of antioxidants such as vitamin C, vitamin E, carotenes, phenolic acids, phytate and phytoestrogens which have the ability to prevent the risk of infections and diseases (Tatiana et al., 2018). This research was therefore designed to investigate the antibacterial and antioxidant activity of *Bacillus* species isolated from spontaneously fermented food condiments.

## 2. MATERIALS AND METHODS

### 2.1. Sample Collection

Thirty (30) samples of fermented food condiments comprising 10 samples of each of Iru woro, Iru pete and Ogiri were obtained from different local markets including Owode, Igbona, Oja-oba and Sasa in Osogbo, Osun State, Nigeria.

### 2.2. Preparation of Samples

One (1g) of each sample was weighed and added into a test tube containing 9ml of Ringer solution. The mixture was shaken thoroughly on rotary shaker to obtain stock solution and 6 folds serial dilutions were made. Exactly 0.1ml of  $10^{-3}$  and  $10^{-5}$  of each diluted samples were introduced into sterile plates and molten nutrient agar was poured and allowed to solidify and the plates were incubated for 24 hours at 37°C.

Distinct colonies obtained after incubation were re-streaked on nutrient agar plates and incubated for 24 hours at 37°C to obtain pure cultures. Pure cultures of the different organisms were sub-cultured and preserved on agar slants and refrigerated at 4°C.

### 2.3. Characterization and Identification of Isolates

#### 2.3.1. Morphological Characterization

The cultural characteristics of the pure isolates on the agar plates were observed by checking the appearance, Gram staining and spore staining reactions.

#### 2.4. Biochemical Characterization

The biochemical characteristics of the pure isolates were observed using citrate, indole, catalase, coagulase, oxidase and sugar fermentation tests (lactose, mannitol, sucrose, maltose and glucose).

#### 2.5. Antibacterial Activity

The antibacterial activity of the pure isolates against pathogenic bacteria including *Staphylococcus aureus* (NCTC 6571) and *E. coli* (ATCC 25922) obtained from Obafemi Awolowo University, Ile-Ife and *Corynebacterium diphtheria* (ATCC 13813), *Proteus mirabilis* (ATCC 7002) and *Klebsiella pneumonia* (ATCC 43816) obtained from Nigeria Institute of Medical Research (NIMR), Lagos were investigated by using agar-well diffusion method. Standardization of the broth cultures was done according to the method of Cheesbrough (2000) by diluting 1 ml of broth culture to 5ml of nutrient broth and visually comparing the turbidity to that of 0.5 Macfarland turbidity standards after incubating at 37°C for 3-5 hours. Nutrient agar was poured into sterilized Petri-dishes and allowed to solidify for 30 minutes. The test organism was inoculated onto the sterile plates by seeding with sterile swab sticks. Wells of 9mm diameter were aseptically bored using sterile cork borer. On each agar plate, 0.3ml of the concentration was added to the wells and gentamycin was used as a positive control. The plates were then incubated at 37°C for 24hours. Effect of the isolates metabolites was assessed by measuring of zones of inhibition (mm) and then compared with the standard gentamycin (CLSI, 2018).

#### 2.6. Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

##### 2.6.1. MIC

MIC of the pure isolates was determined using the broth dilution method. Test tubes labeled 1-5 were used for each isolate. Each of the test tubes contained 5ml of nutrient broth and 5ml of the appropriate concentration of each isolate was introduced into tube one and mixed thoroughly. 5ml of the content of tube 1 was introduced into tube 2 and the procedure was repeated for the remaining test tubes except tube 5 (control). To each of the test tubes, 0.1 ml of broth cultures of the test organism was added. All the tubes were incubated at 37°C for 18-24 hours, after which they were examined for bacterial growth (Doughari, 2006).

##### 2.6.2. MBC

MBC was determined by selecting the tubes that showed no growth during the MIC determination. One loopful from each of these tubes was sub-cultured onto the surface of culture free nutrient agar plates and incubated for 24hrs at 37°C. The lowest concentration at which no growth was observed on the agar plate was recorded as the MBC.

### 2.7. Antioxidant Activity

#### 2.7. diphenyl- 1- picrylhydrazyl (DPPH) Radical Scavenging Activity Assay

The scavenging effect of selected pure isolates on the free radical 2,2- diphenyl- 1- picrylhydrazyl (DPPH) was measured in accordance with the slightly modified method of Lin. and Chang (2000). A sample (Nutrient broth, 1 mL) and a freshly prepared 2,2- diphenyl- 1- picrylhydrazyl (DPPH) solution (0.2mM, 1m) were mixed. The mixture was vigorously shaken and left to react for 30 minutes in the dark at room temperature, The control sample contained deionized water instead of the sample solution. The scavenged DPPH was then monitored by determining the absorbance at 517 nm using Visible Spectrophotometer (BOSCH 712G). The radical scavenging activity was quantified as units/ml (U/ml) by using the expression.

$$\text{DPPH Scavenged (\%)} = [(A \text{ control} - A \text{ test}) / A \text{ control}] \times 100$$

Where A control is the absorbance of the control reaction and A test is the absorbance in the presence of the sample of the extracts.

### 2.8. Reducing Power Assay

The reducing activity was determined as described by Lin and Yen (1999) with slight modification. A sample (Nutrient broth, 0.5 ml) was mixed with potassium ferricyanide (1%, 0.5 ml) and Phosphate-buffered saline (pH 6.6, 0.5 ml). Subsequently, the mixture was heated at 50°C for 20 min and allowed to cool. Upon cooling, 0.5 ml of 10% trichloroacetic acid (TCA) was added to the mixture and then centrifuged at 3000g for 5 min. The upper layer (1 ml) was mixed with ferric chloride (0.1%, 1 mL) and allowed to react for 10 min. The absorbance of the mixture was obtained at 700 nm by Visible Spectrophotometer (BOSCH 712G).

### 2.9. Thin Layer Chromatography (TLC) Analysis of Fermented *Parkia Biglobosa* (iru) and *Ricinus Communis* (ogiri)

Each of the samples were dissolved in 2ml of distilled methanol to allow the extraction of the *Bacillus* species metabolites in the sample. 2mg of the samples were weighed in a dish and 2ml of distilled methanol were added. The sample solutions were mixed thoroughly until a homogenous mixture was obtained. TLC is a procedure that is used to separate compounds by their rate of movement through a thin layer of silica gel coated on a glass plate. Developing tank: 3ml of distilled N: Hexane, 6ml of distilled ethyl acetate, 9ml of distilled methanol were used based on capillary action of the samples on plate (ratio: 1:2:3) observed under the UV- visible light (Meena, Saha, & Ramashish, 2014).

## 3. RESULTS

### 3.1. Identification of Bacterial Isolates

Table 1 shows the Gram's reaction and biochemical characteristics of bacteria isolated from ogiri. Two *Bacillus* species were isolated and identified as : *B. subtilis*, *B. mycoides*. Other bacteria that were isolated and identified include: *Enterococcus* sp, *Listeria* sp and *Micrococcus* sp. Only the *Bacillus* species were used for further studies.

The Gram's reaction and biochemical characteristics of bacteria isolated from iru pete is presented in Table 2. Three *Bacillus* species were isolated and identified as *B. laterosporus*, *B. licheniformis* and *B. subtilis*. Other bacteria that were isolated and identified include: *Enterococcus* sp, *Listeria* sp and *Micrococcus* sp. Only the *Bacillus* species were used for further studies.

Table 3 shows the Gram's reaction and biochemical characteristics of bacteria isolated from iru pete. Three *Bacillus* species were isolated and identified as: *B. subtilis*, *B. mycoides* and *B. licheniformis*. Other bacteria that were isolated and identified are: *Corynebacterium* sp and *Listeria* sp.

Table-1. Gram's reaction and biochemical characteristics of bacterial isolates from ogiri.

Isolates code	Catalase	Oxidase	Spore	Indole	Coagulase	Citrate	Lactose	Mannitol	Sucrose	Maltose	Glucose	Motility	Gram's reaction	
OG A3	+	-	+	-	-	+	+	+	+	-	+	+	+	<i>B. mycooides</i>
OG A5	+	-	-	-	-	+	+	+	-	+	+	+	+	<i>Enterococcus sp</i>
OG B3	+	-	+	-	-	+	+	+	+	+	+	+	+	<i>B.subtilis</i>
OG B5	+	-	-	-	-	-	+	+	-	+	+	+	+	<i>Listeria sp</i>
OG C3	+	-	+	-	-	+	+	+	+	+	+	+	+	<i>B. subtilis</i>
OG C5	+	-	+	-	-	+	+	+	+	+	+	+	+	<i>B. subtilis</i>
OG D3	+	-	+	-	-	+	+	+	+	+	+	+	+	<i>B. subtilis</i>
OG D5	+	-	-	-	-	+	+	+	-	+	+	+	+	<i>Micrococcus sp</i>
OG E3	+	+	-	-	-	+	+	-	-	-	+	+	+	<i>Listeria sp</i>
OG E5	+	-	+	-	-	+	+	+	+	+	+	+	+	<i>B. subtilis</i>
OG F3	+	-	-	-	-	-	-	-	-	-	+	-	+	<i>Micrococcus sp</i>
OG F5	+	-	-	-	-	-	-	+	+	-	+	-	+	<i>Enterococcus sp</i>
OG G3	+	-	+	-	-	+	+	+	+	+	+	+	+	<i>B. subtilis</i>

Note: KEY: += Growth; -= No growth.

Table-2. Gram's reaction and biochemical characteristics of bacterial isolates from iru pete .

Isolates code	Catalase	Oxidase	Spore	Indole	Coagulase	Citrate	Lactose	Mannitol	Sucrose	Maltose	Glucose	Motility	Gram's reaction	Probable Organisms
IP A3	+	-	+	-	-	+	-	+	+	+	+	+	+	<i>B. subtilis</i>
IP A5	+	-	-	-	-	+	+	+	-	+	+	-	+	<i>Micrococcus sp</i>
IP B3	+	+	-	-	-	+	+	-	-	-	+	+	+	<i>Listeria sp</i>
IP B5	+	-	+	-	-	+	-	+	+	+	+	+	+	<i>B. licheniformis</i>
IP C3	+	-	-	+	-	+	-	+	+	++	+	+	+	<i>B. licheniformis</i>
IP C5	+	+	-	+	-	+	-	+	+	-	+	+	+	<i>Enterococcus sp</i>
IP D3	+	+	-	-	-	+	+	-	-	-	+	+	+	<i>Listeria sp</i>
IP D5	+	+	-	-	-	+	-	+	+	+	+	+	+	<i>B. licheniformis</i>
IP I3	+	-	+	-	-	+	+	+	+	-	+	+	+	<i>B. laterosporus</i>
IP E5	+	+	-	-	-	+	+	-	-	-	+	+	+	<i>Listeria sp</i>
IP H3	+	-	+	-	-	+	+	+	+	+	+	+	+	<i>B. subtilis</i>
IP F5	+	-	-	+	-	+	-	+	+	+	+	+	+	<i>B. licheniformis</i>
IP G3	+	+	-	-	-	+	-	-	-	-	+	-	+	<i>Corynebacterium sp</i>

Note: KEY: += Positive; -= Negative.

Figure 1 shows the percentage susceptibility pattern of the isolated *Bacillus* species metabolites against test organisms. *P.mirabilis* was 43% resistant and 57% susceptible, *E. coli* and *C. diphtheria* were 86% resistant and 14% susceptible, while *S. aureus* and *K. pneumoniae* were 100% resistant and not susceptible to the isolated *Bacillus* species.

Table-3. Gram's reaction and biochemical characteristics of bacterial isolates from iru woro.

Isolates code	Catalase	Oxidase	Spore	Indole	Coagulase	Citrate	Lactose	Mannitol	Sucrose	Maltose	Glucose	Motility	Gram's reaction	Probable organisms
IW A3	+	-	+	-	-	+	+	+	+	+	+	+	+	<i>B. subtilis</i>
IW A5	+	+	+	-	-	+	-	+	+	+	+	+	+	<i>B. licheniformis</i>
IW B3	+	+	-	-	-	+	+		-	-	+	+	+	<i>Listeria sp</i>
IW B5	+	-	+	-	-	+	+	-	+	-	+	+	+	<i>B. mycooides</i>
IW C3	+	+	-	-	-	+	-	+	-	-	+	-	+	<i>Corynebacterium sp</i>
IW C5	+	+	+	-	-	+	+	-	+	-	+	+	+	<i>B. mycooides</i>
IW D3	+	+	-	-	-	+	+	+	-	-	+	+	+	<i>Listeria sp</i>
IW D5	+	+	-	-	-	+	-	-	-	-	+	-	+	<i>Corynebacterium sp</i>
IW E3	+	+	-	-	-	+	-	-	-	-	+	-	+	<i>Corynebacterium sp</i>
IW E5	+	+	-	-	-	+	+	-	-	-	+	+	+	<i>Listeria sp</i>
IW F3	+	-	+	-	-	+	-	-	+	+	+	+	+	<i>B. licheniformis</i>
IW F5	+	-	+	-	-	+	-	+	+	+	+	+	+	<i>B. licheniformis</i>
IW G3	+	+	-	-	-	+	-	-	-	-	+	-	+	<i>Corynebacterium sp</i>

Note: KEY: += Growth; -= No growth

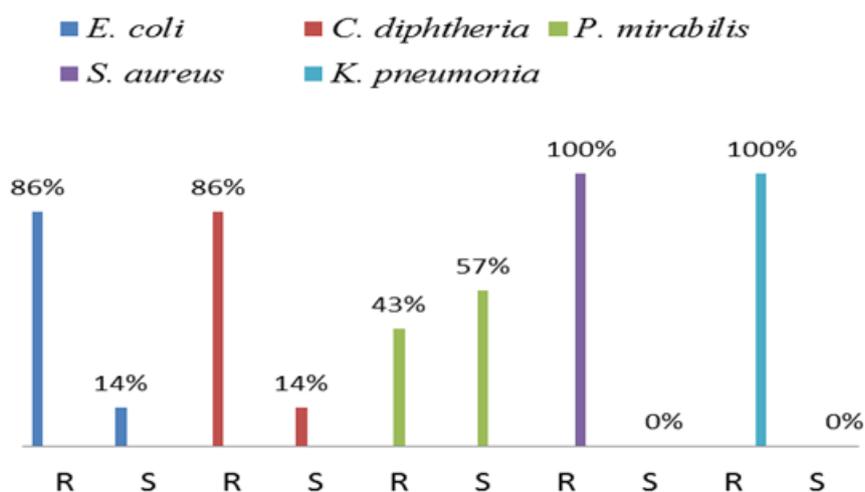


Figure-1. Percentage susceptibility pattern of Bacillus species metabolites against test organisms. Note: Key: R= Resistance S= Susceptible

The zones of inhibition produced by Bacillus species metabolites against test organisms during agar well diffusion method is presented in Table 4. It was observed that OG B3 (*B. subtilis*) had the highest zone of inhibition of 23 mm against *P. mirabilis* and IP I3 (*B. laterosporus*) had the least zone of inhibition (13 mm) against *E. coli*.

Table-4. Zones of inhibition produced by Bacillus species metabolites against test organisms during the agar well diffusion method.

Test Organism	OG A3	IW A3	IW B5	IP I3	OG B3	IP B5	IP H3	GT(30µg)
<i>E. coli</i>	0	16 mm	0	13 mm	0	14 mm	0	19 mm
<i>C. diphtheriae</i>	0	0	0	20 mm	17 mm	0	0	22 mm
<i>P. mirabilis</i>	0	17 mm	16 mm	20 mm	23 mm	18 mm	19 mm	18 mm
<i>S. aureus</i>	0	0	0	0	0	0	0	20 mm
<i>K. pneumoniae</i>	0	0	0	0	0	0	0	17 mm

Note: KEY: GT: Gentamycin, OG: Ogiri, IW: Iru woro, IP: Iru pete.

Table 5 shows the minimum inhibitory concentration at which the *Bacillus* species isolated from iru woro, iru pete and ogiri inhibited the growth of test organisms. IW A3, IW B5 and IP I3 showed the MIC at which they inhibited the test organisms. OG A3 did not show any zone of inhibition.

Table-5. Minimum inhibitory concentration of *Bacillus* species against test organisms at 530 nm.

Test organisms	OG A3				IW A3				IW B5				IP I3 (10 <sup>-1</sup> )			
	35	25	15	10	37	30	25	15	36	24	16	10	38	25	18	11 (mg/ml)
<i>E. coli</i>	+	+	+	+	-	+	+	+	+	+	+	+	-	+	+	+
<i>C. diphtheriae</i>	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+
<i>P. mirabilis</i>	+	+	+	+	-	+	+	+	-	+	+	+	+	+	+	+
<i>S. aureus</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<i>K. Pneumoniae</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<i>E. coli</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Note: KEY: + = Growth - = No growth.

The minimum bactericidal concentration at which the *Bacillus* species metabolites isolated from iru woro, iru pete and ogiri produced substances that killed test organisms is presented in Table 6. It was observed that IW A3 showed the MBC at which it killed the test organisms but OG A3, IP I3 and IW B5 did not produce any metabolite that could kill the test organisms.

Table-6. Minimum bactericidal concentration of *Bacillus* species metabolites against test organisms at 530nm.

Test organisms	OG A3				IW A3				IW B5				IP I3 (10 <sup>-1</sup> )			
	37	30	25	15	35	25	20	15	36	24	16	10	38	25	18	11 (mg/ml)
<i>E. coli</i>	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+
<i>C. diphtheriae</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<i>P. mirabilis</i>	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+
<i>S. aureus</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<i>K. Pneumoniae</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
<i>E. coli</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Note: KEY: + = Growth - = No growth.

Table 7 shows the radical scavenging activity using 2,2- diphenyl- 1- picrylhydrazyl (DPPH) at which OG B3 present 65.80% and IP B5 present 27.52% antioxidant properties that acted against the activity of free radicals. OG B3 produced a higher antioxidant than IP B5.

Table-7. Reading for DPPH at 517 nm from the visible spectrophotometer.

Sample	Absorbance 1	Absorbance 2	Absorbance 3	Mean	DPPH Scavenged %
OG B3	0.250	0.252	0.251	0.251	65.80
IP B5	0.530	0.533	0.535	0.532	27.52
Control	0.730	0.740	0.731	0.734	0

Note: KEY: OG B3 - *Bacillus subtilis*, IPB5 - *Bacillus licheniformis*, Control: Deionized water.

The reducing power (i.e., the rate at which the *Bacillus spp* metabolites acted against the activity of free radicals) with OG B3 having a higher reducing power than other *Bacillus* species is presented in Table 8.

Table-8. Reading for reducing power at 700 nm from the visible spectrophotometer.

Sample	Absorbance 1	Absorbance 2	Absorbance 3	Mean
OG B3	0.680	0.689	0.679	0.683
IP B5	0.313	0.299	0.314	0.309
Control	0.113	0.114	0.112	0.113

Note: KEY: OG B3 - *Bacillus subtilis*, IPB5 - *Bacillus licheniformis*, Control: Deionized water.

Figure 2 shows the TLC of extracted lipopeptide by *Bacillus subtilis* OG B3 which produced a blue violet colour, indicating the presence of iturin as compared to the standard obtained from literature (Meena et al., 2014). Iturin is a cyclic antibiotic that can be used as a biocontrol and antifungal agent.

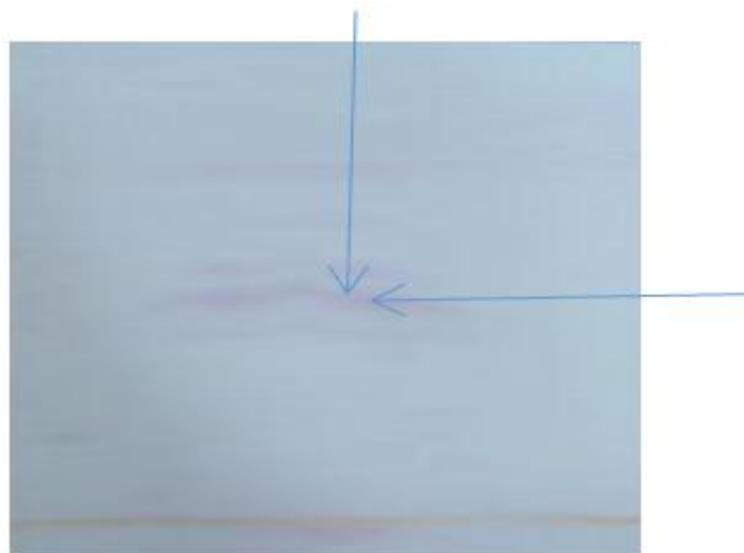


Figure-2. Thin layer chromatography sheet which indicates the presence of iturin.  
Note: Key: purple lines indicate the presence of iturin.

#### 4. DISCUSSION

The result of this study provides information on the different types of bacteria, with emphasis on *Bacillus* species isolated from locally fermented condiments, antibacterial activity of the *Bacillus* species against test pathogens implicated in nosocomial infections and susceptibility pattern of the *Bacillus* species metabolites against the test organisms. Minimum inhibitory concentration and minimum bactericidal concentration was also determined. Antioxidant activity of selected *Bacillus* strains and the identification of *B. subtilis* metabolite using the thin layer chromatography method was also carried out. The different types of microorganisms isolated from ogiri includes: *B. mycoides*, *Enterococcus* sp, *B. subtilis*, *Micrococcus* sp and *Listeria* sp; iru pete: *Enterococcus* sp, *B. licheniformis*, *Listeria* sp and *B. laterosporus*; iru woro: *B. licheniformis*, *B. mycoides*, *Listeria* sp and *Corynebacterium* sp. Several researcher have reported that *Bacillus* species are the most predominant organisms involved in the fermentation of condiments (Achi, 2005; Afify et al., 2012; Soetan et al., 2014; Tatiana et al., 2018). However, other species of bacteria isolated from the condiment samples might due to the contamination of the utensils and water used for the fermentation process. Several authors have confirmed that *B. subtilis* is the most predominant *Bacillus* strain isolated from traditionally fermented condiments (Adebayo, 2018; Lawal, Oso, Sanni, & Olatunji, 2011; Oguntoyinbo et al., 2010). However, the results obtained from this work is in agrees with previous reports as *B. mycoides* and *B. subtilis* was more prevalent in ogiri, *B. subtilis* and *B. licheniformis* in iru pete while *B. subtilis*, *B. mycoides* and *B. licheniformis* was more predominant in iru woro. However, *B. subtilis* was the most predominant of all the *Bacillus* species isolated

The antibacterial susceptibility pattern of the isolated *Bacillus* species against test pathogens; *Escherichia coli*, *Corynebacterium diphtheria*, *Proteus mirabilis*, *Staphylococcus aureus*, *Klebsiella pneumoniae* was determined using the

agar- well diffusion method. *E.coli* was susceptible to *Bacillus* isolates: IWA3, IPI3 and IPB5 showing following zones of inhibition 16mm, 13mm and 14mm respectively. *C. diphtheriae* was susceptible to IPI3 and OGB3 with the corresponding zones of inhibition: 20mm and 17mm respectively. *S. aureus* and *K.pneumoniae* were resistant to all the *Bacillus* isolates. However, *P. mirabilis* was susceptible to all the selected *Bacillus* isolates: IWA3, IWB5, IPI3, OGB3, IPB5 and IPH3 having the corresponding zones of inhibition: 17mm, 16mm, 20mm, 23mm, 18mm and 19mm respectively. The highest antibacterial activity was demonstrated against *P. mirabilis* whose control (gentamycin) showed 18mm zone of inhibition. This suggests that OGB3 is a potential antimicrobial agent which comparatively has better potency than gentamycin. Kumar, Thippeswamy, and Shivakumar (2015) had earlier reported the ability of many *Bacillus* strains to produce highly potent antibiotics. This is conformity with the results obtained from this work as the *Bacillus* species demonstrated high antibacterial activity against nosocomial implicated pathogens. Kuta (2008) reported 5mm and 6mm *Bacillus* species zones of inhibition against *S. aureus* and *K. pneumoniae* respectively. Kumar et al. (2015) also documented 20mm zones of inhibition against *S. aureus* and *K. pneumoniae*. However, no antibacterial activity was recorded for *S. aureus* and *K.pneumoniae* as the two organisms were resistant to all the *Bacillus* species. The results of the minimum inhibitory concentration showed that IWA3 and IPI3 exhibited MIC of 3.7mg/ml and 3.8mg/ml against *E.coli* respectively; IPI3 showed MIC of 2.5mg/ml and 3.8mg/ml against *C. diphtheriae*; IWA3 and IWB5 showed 2.5mg/ml and 3.6mg/ml against *P.mirabilis* respectively. However, no MIC record was recorded for *S.aureus* and *K. pneumoniae*. In addition, only IWA3 showed MBC of 3.5mg/ml and 3.7mg/ml against *E. coli* and *P.mirabilis* respectively. Shine, Rejiniemon, Khaled, Alharbi, and Mothana (2015) had earlier documented similar results.

Antioxidants are substances such as thiols and ascorbic acid (Vitamin C) produced by microorganisms such as *B. subtilis*, *B. pumilus* and *B. licheniformis* that can prevent or slow down chemical reactions that may damage cells caused by free radicals such as: nitric oxide ( $O_2$ ), nitrous oxide and peroxnitrite. These antioxidants can also be used in food preservation to increase shelf life. DPPH free radical scavenging is one of the mostly used methods in antioxidative assay. This method was employed to determine the antioxidant activity of OGB3 and IPB5 crude extracts by measuring the change in absorbance at 517 nm. OGB3 (*B. subtilis* isolated from ogiri) had higher antioxidative activity (65.80%) than IPB5 (*B. licheniformis* isolated from iru pete) (27.52%). This results is in line with the the report of several authors that have documented similar results (Afify et al., 2012; Katekan, Arunee, & Ekachai, 2011; Kumar et al., 2015; Lin & Yen, 1999). Katekan et al. (2011) recorded antioxidant activities of 65.65% and 62.12% from naturally fermented *Thua Nao* ( a Thailand fermented soybean condiment). However, other bacteria such as *Lactobacillus rhamnosus*, *Lactobacillus reuteria* (ATCC 20016) and *Bifidobacterium breve* (ATCC 15700) have been documented by Afify et al. (2012) to demonstrate very high antioxidant activity with the corresponding values: 91.71%, 96.74% and 86.99% respectively. (Shine et al., 2015) also reported an antioxidant activity of 67.33% from *B. amyloliquifaciens* isolated from a silage. Members of the genus *Bacillus* have been reported to produce lipopeptides that demonstrates antagonistic effect against food borne pathogens, pathogens implicated in nosocomial infections as well as plant pathogens. Different lipopeptides such as iturin, fengycin and surfactin have been produced by *B. subtilis*, *B. amyloliquifaciens*, *B. pumilus* and other members of the *Bacillus* species. The thin layer chromatography method was employed in this work to extract a lipopeptide from OGB3 (*B. subtilis* isolated from ogiri). After extraction, the lipopeptide was identified as iturin. Baruzzi, Quintieri, Morea, and Caputo (2011) had earlier reported that iturin produced by *B. subtilis* possesses strong anti-fungal activity against a wide range of plant fungal pathogens such as *Fusarium graminearum*, *Rhizoctonia solani*, *Aspergillus flavus* and post harvest pathogens such as *Botritis cinerea* and *Penicillium expansum*. Pyoung, Ryu, Kim, and Chi (2010) and Youcef-Ali et al. (2014) also documented the production of iturin and other lipopeptides such as pumilin, lichenysins and subtilosin from *B. subtilis*, *B. mojavensis* and *B. pumilus* which exhibited strong anti-fungal, anti-parasitic and antibacterial activity against pathogenic microorganisms.

From the results obtained from this work, it can be concluded that *B. subtilis* (OGB3) can produce a potential antimicrobial agent that can be used against microorganisms implicated in nosocomial infections. However, it can also be used as a starter culture for the production of fermentable food products. In addition, the lipopeptides produced can be employed as an anti-fungal agent in plants and also as a biopreservative in foods.

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