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INFLUENCE OF WASTE NITROGEN AND CARBON SOURCES ON ANTIMICROBIAL PRODUCTION BY *Pseudomonas fluorescens* ISOLATES FROM SOIL AND WATER IN ZARIA, NIGERIA

ABBA KHALID ADBULLAHI[1,*], JOSEPH OLORUNMOLA EHINMIDU[2], MARYAM ADENIKE SALAUDEEN[3]

1. Department of Pharmaceutics and Industrial Pharmacy, Ahmadu Bello University, Zaria, Nigeria.
2. Department of Pharmaceutical Microbiology, Ahmadu Bello University, Zaria, Nigeria.
3. Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria, Nigeria.

ABSTRACT

Antibiotics are one of the most significant and commercially used secondary metabolites derived from microorganisms in the treatment of an array of infections. This study was aimed at determining the influence of nitrogen sources in combination with waste carbon sources on the production of antimicrobial substances by *Pseudomonas fluorescens*. The inhibitory activity of the antimetabolite from *Pseudomonas fluorescens* was studied against *Escherichia coli* and *Candida albicans* inoculated in an appropriate media. The bacteria inoculum was incubated at 37 °C for 18 h and at 30 °C for the fungal inoculum. Fermentation was carried out using yam peel, cassava peel, and maize shaft as carbon sources and two nitrogen sources (groundnut cake and soya-beans cake). Method of well diffusion was employed in the study of the antibacterial activity of the extract from the fermentation broth. The zones of inhibition against the test organisms (*Escherichia coli* and *Candida albicans*), which is a measure of antimicrobial activity, differed significantly when the sources of nitrogen in the fermentation media was changed. The highest inhibitory zone was recorded for soya-beans cake/cassava peel (10.25±11.84 - 21.00±1.45 mm) against *Escherichia coli* and (20.00±0.00 - 27.25±3.40 mm) against *Candida albicans*. The combination of soya-beans cake/yam peel in the fermentation medium produced maximum antimicrobial activity. This result suggests that the use of appropriate carbon and nitrogen source can increase the efficacy of antibiotics produced by a microorganism.

KEYWORDS: ***Pseudomonas fluorescens*; Antimicrobial activity; Nutrient sources; *Escherichia coli*; *Candida albicans*.**

INTRODUCTION

The study of microorganisms has become increasingly important in recent years because of the production of novel metabolites, which exhibits antibacterial, antiviral, anti-tumour as well as antifungal properties [1]. As such, bacteria in the genus *Pseudomonas* that are widely distributed in nature have become one of the most studied bacterial group. It is estimated that half of the natural isolates in mineral water and soil consist of *Pseudomonas aeruginosa* [2]. *Pseudomonas* are Gram-negative, strictly aerobic, polarly flagellated rod-shaped bacteria. They are aggressive colonizers of the rhizosphere of varied crop plants, and have a broad-spectrum antagonistic activity against plant pathogens, like antibiosis (the production of inhibitory compounds) [3,4], siderophores production (iron-sequestering compounds) [5] and nutrition or site competition [6]. Some species of *Pseudomonas* can also produce levels of hydrogen cyanide that are toxic to certain pathogenic microorganisms [7]. For example, certain fluorescent pseudomonads from soil have been

***Corresponding author:** khalidabba90@yahoo.com; +2348030790928*
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shown to promote plant growth by inhibiting bacteria and fungi that are deleterious to plants. The production of antibiotic substances by some strains has been reported as the major factor in the suppression of many plants roots pathogens [8]. A number of these disease-suppressive antibiotic compounds have been reported and characterized chemically which include N-containing heterocycles such as phenazines, pyro type antibiotics, pyro-compounds, and indole derivatives [9].
Fluorescent pseudomonads are ubiquitous bacteria that are common inhabitants of the rhizosphere, and are the foremost studied group within the *Pseudomonas*. They comprise of *Pseudomonas aeruginosa*, the species type of the genus; *Pseudomonas aureofaciens*, *Pseudomonas chlororaphis*, *Pseudomonas fluorescens* (four biotypes), *Pseudomonas putida* (two biotypes), and therefore the plant pathogenic species *Pseudomonas cichorii* and *Pseudomonas syringae*; the latter includes a large number of nomen species. The production of antibiotic compounds by this species is influenced by different chemical conditions. In 2005, Samuel and colleagues showed that glucose stimulated the production of some antibiotics by a strain of *Pseudomonas aeruginosa*, whereas the production of other antibiotics was inhibited by glucose. The production of other pseudomonad metabolites is also influenced by growth conditions and growth phase. For example, production of active metabolite by *P. fluorescens* started after the exponential growth phase and was greatly affected by temperature [10].

Natural products have served and continue to provide useful lead compounds for development into chemotherapeutic agents. Natural products represent the traditional source of new drug candidates. The microbial synthesis of antibiotics has been reported to be regulated by nutrients (such as nitrogen, phosphorous and carbon source), metals, growth rate, feedback control and enzyme inactivation [11].

Conditions of fermentation are known to influence the generation of secondary metabolites. Because of this, the search for new bioactive compounds depends not only on testing microorganisms in places that have not been studied, but also in fermentation conditions, taking into account the possibility that these metabolites act synergistically [12]. Nutrients such as phosphorus, carbon source, and nitrogen have been reported to influence the synthesis of antibiotics by microbes [13]. Of these factors, the role of nitrogen and carbon source on antibiotic production has been viewed and approached from different perspective including both industry and research groups. These perspectives considered the role of nutrients from biochemical, biological, and molecular stand point as critical to the production of inhibitory secondary metabolite. The carbon and nitrogen sources are important constituents which have been reported to have high influence on the degree of potency of antibiotic production by bacteria associated with nematode [14].

Antimicrobial heavy use has both helped people live longer, healthier lives, and it has also resulted in the selection of many of today's modern "superbugs" that are highly drug-resistant. This rise of the super-bug has shown a need for a new round of drug discovery for effective arrest of the emergence of antibiotic super-bug. Current drug development methods have been slow to produce effective new antibiotics as they have primarily focused on modifying existing classes of antibiotics or using genomics to identify new drug targets. Unfortunately, this approach is yielding results too slowly to keep up with the rapid pace of emergence of antibiotic resistant bacteria.

the search for new bioactive compounds depends not only on testing microorganisms in places that have not been studied, but also in fermentation conditions, taking into account the possibility that these metabolites act synergistically. Exploring the possibility of discovering new antimicrobials from bacteria was chosen for this research because bacteria have been at war with each other for survival for approximately 4-5 billion years [11]. This would lead one to think that bacteria might be the best source for finding compounds to kill other bacteria.

The need for new antibiotics from natural waste sources, and new classes of antibiotics, was a major drive in the direction of this research. This study was designed to isolate *Pseudomonas fluorescens* from local sources that can be used to produce antibiotics from versatile media for antibiotic fermentation and to determine the effectiveness of cell freed antibiotic extracts.

MATERIALS AND METHODS

Materials

Culture Media: Sabouraud dextrose agar (65g/L, Oxoid, UK), Mueller Hinton Agar (38g/L, Oxoid, UK)**;** Nutrient Broth (18g/L, Oxoid, UK); Nutrient Agar

(23g/L, Oxoid, UK); Cetrimide Agar (0.03%, Oxoid, UK); Solvents (Distilled water, 0.9% Normal saline); Test organisms (*Escherichia coli, Candida albicans*); Reagents (Lactophenol blue, Oxidase reagent, Hydrogen peroxide, Crystal violet, 70% alcohol, Iodine, Carbon Fusin); Filter paper (Corning Sterile Syringe Filter, pore 0.20 µm, Corning Inc.)

Collection of Samples

Samples were taken randomly from two (2) rice farms and three (3) water pools with the aim of obtaining an extensive collection of *Pseudomonas* isolate from different locations in Zaria for the study. Water samples were obtained by dipping sterile glass bottles in the water to a depth of 10 cm, opened to fill after, and then closed and brought to surface. About 10 g of top soil were also collected into sterile glass bottles. A total of 5 samples were collected; one sample from each of the water pool and one soil sample from each of the rice farms. and the samples were transported at room temperature to the laboratory and kept in an aseptic working area. After collections, the samples were processed within 12 h. In 99 ml of microbes-free distilled water, one gram of soil was suspended. Serial dilutions were prepared and 0.1 ml of the sample was spread on sterile nutrient agar plates. With the aid of needle and syringe, similar procedure was adopted for the pool sample. Only in this case, 1 ml of the pool water samples was mixed with 99 ml of sterile distilled water. Both samples were incubated at 37 °C for 24 h.

Isolation of Pathogenic Bacteria

The availability of pathogenic bacterial and fungal cultures of health significance: *Escherichia coli* and *candida albicans*, were maintained by ensuring their growth in the agar medium and stored at 4 °C as stock. Strains were allowed to multiply twice before use in the experiments.

Isolation of Colonies

Sterile cetrimide agar plates were placed in aseptic work area. A sterile inoculating loop was dipped into the cultured nutrient broth to obtain a loopful of the sample which was allowed to form a colony by streaking on the surface of the solidified agar. The plates were subsequently incubated for 24 h at a temperature of 37 °C to obtain distinctive colonies. Colonies were then sub-cultured on an agar slant and stored for future studies.

Staining and motility evaluation; cultural tests such as nutrient agar, cetrimide agar; biochemical tests (catalase test, oxidase test, starch hydrolysis) and growth at temperatures of 4 °C and 42 °C were all employed to confirm the bacteria isolates.

Culture medium optimization

Six (6) culture media were optimised by using powdered maize shaft, cassava peel and yam peel, to substitute carbon and nitrogen sources in standard medium with the following formulations:

Chemically defined medium (Standard medium) K2HPO4 0.98 g/l, MgSO4 0.40 g/l; CaCO3 0.40 g/l, glucose 20 g/l, NH3NO3 20 g/l.

1. K_2HPO_4 0.98 g/l, MgSO4 0.40 g/l; CaCO3 0.40 g/l, Maize shaft 20 g/l, Groundnut cake 20 g/l.
2. K_2HPO_4 0.98 g/l, MgSO4 0.40 g/l; CaCO3 0.40 g/l, Maize shaft 20 g/l, Soya bean cake 20 g/l.
3. K_2HPO_4 0.98 g/l, MgSO4 0.40 g/l; CaCO3 0.40 g/l, Cassava peel 20 g/l, Groundnut cake 20 g/l.
4. K_2HPO_4 0.98 g/l, MgSO4 0.40 g/l; CaCO3 0.40 g/l, Cassava peel 20 g/l, Soya bean cake 20 g/l.
5. K_2HPO_4 0.98 g/l, MgSO4 0.40 g/l; CaCO3 0.40 g/l, Yam peel 20 g/l, Groundnut cake 20 g/l.
6. K_2HPO_4 0.98 g/l, MgSO4 0.40 g/l; CaCO3 0.40 g/l, Yam peel 20 g/l, Soya bean cake 20 g/l.

Each medium (50 ml) was placed in a volumetric flask (250 ml) and a *Pseudomonas* preculture 1% (v/v) was added. The mixture was incubated at a temperature of 37 °C for 24 h. The supernatant was filtered. This was followed by assay of antimicrobial susceptibility.

Extraction of Secondary Metabolites

The filtrates of *Pseudomonas* were centrifuged at 3500 rotations per minutes for 25 min. The supernatant was thereafter, passed through a bacteriological filter.

Determination of Antimicrobial Activity

Agar well diffusion method was used to determine the minimum inhibitory concentration (MIC). The surfaces of Muller Hinton agar plates were flooded with 2 ml of the standardized overnight culture of the test isolates *Escherichia coli*. The excess was drained off in a disinfectant jar. The agar surface was allowed for 10 min to dry. Wells of 6 mm were bored in the middle of the plate using a cork borer and the base was sealed with molten agar and allowed to cool. The hole was then inoculated with 0.1 ml of

overnight culture of cell-free extract of potential antibacterial *Pseudomonas fluorescens* fermentation broth. The plates were subsequently incubated at for 24 h at temperature of 37 °C, consequent to the observation of a zone of clearing around the wells as a measure of inhibitory activity. The clearing zones diameter was examined for zone of inhibition around each hole and measured with the aid of ruler to the nearest millimetres and recorded. Results were expressed as average of two measurements. Three replicate plates were prepared for each *Pseudomonas fluorescens* fermentation broth culture filtrate and the experiments were repeated twice.

Sterile Sabouraud Dextrose (SDA) agar surface was flooded with 2 ml of the standardized overnight inoculum of *candida albicans*, the excess was drained off in a disinfectant jar and allowed to dry. Using a sterile cork borer, wells of 6 mm were bored on each plate. The base of the plate was sealed with a drop of SDA agar, allowed to cool and 0.1 ml of the cell-free extracts of *Pseudomonas fluorescens* fermentation broth was put in the well. It was allowed a pre-diffusion time of 1 h before incubating for 24 h at 37 °C. The zone of inhibition was measured, after incubation, to the nearest millimetre. Three replicate plates were prepared for each *Pseudomonas fluorescens* fermentation broth culture filtrate and the experiment were repeated twice.

Statistical analysis

All data were Analysed (Mixed-design Analysis of Variance followed by Dunnett Posthoc test) using SPSS version 20 and presented as mean standard deviation on tables.

RESULTS

The morphological features of the colonies including sizes, shapes, colour and microscopic features of the cells were used to characterize the isolates. Staining tests and microscopic analysis revealed the presence of *Pseudomonas species* on all the samples collected (Table 1).

The result demonstrated the different biochemical tests performed to ascertain the identity of the *Pseudomonas specie* isolates. The genus of the *pseudomonas species* was identified as *fluorescens* using biochemical and temperature tests (Table 2).

The result showed that the activity of the antibiotic producing bacteria differs in the different media. For example, the order of cell free extract activities from the different antibiotic producing bacteria was WP1 displayed 22.00±4.76 mm zone of inhibition in cassava medium > RF2 showed 18.25±1.26 mm from yam peel medium > RF1 exhibited 16.75±2.36 mm from maize shaft medium > WP2 inhibited *E. coli* with 13.05±1.63 mm from chemically defined medium.

Furthermore, this result showed that cassava supplemented medium with WP1 produced the most active antimetabolite with 22.00±4.76 mm zone of inhibition with groundnut cake as the nitrogen source. This result below showed that the carbon source introduced to the medium has significant influence on the activity of the cell free extract from the different media.

This result showed RF1 produced better potent antimetabolite with zone of inhibition of 21.50±1.73 mm and 21.00±1.15 mm from yam and cassava peel medium respectively. It also shown that WP1 produced antibiotics that displayed 18.25±6.40 mm zone of inhibition of test *E. coli* from maize shaft medium.

Results showed that cell free extract from test media fermented by *Pseudomonas specie* displayed significant antifungal activities. The result from this investigation showed that WP2 produced potent antifungal agent with 30.00±0.00 mm zone of inhibition from yam peel with groundnut cake (nitrogen source). RF1 and WP2 using carbon source of cassava peel and maize shaft produced potent antifungal agent with 20.00±4.08 mm and 20.50±5.20 mm zone of inhibition respectively against *Candida albicans.*

Results showed that when the three supplemented test carbon sources were used with soya-bean cake (nitrogen source), the cell free extract from the fermentation broth displayed significant antifungal activity.

Observation from the antibiotic producing bacteria, showed that RF2 and WP1 synthesizes potent antifungal secondary metabolite with 27.75±2.06 mm zone of inhibition with yam peel medium and maize shaft medium respectively and RF1 with 27.25±3.40 mm zone of inhibition with cassava peel carbon source. The order of antifungal activity was cassava peel medium > yam peel medium > maize shaft medium > chemically defined medium.

Figure 1 showed a high production of zone of inhibition of the *Pseudomonas fluorescens* isolates when grown in nutritionally substituted fermentation broth. The average zone of inhibition of test *E. coli* by cell free extract from different carbon sources was yam peel medium (17.94 mm) > cassava peel medium (15.65 mm) > maize shaft medium (13.50 mm) > chemically defined medium (8.50 mm); while that of test organism *C. albicans* was cassava peel medium (24.70 mm) > yam peel medium (22.55 mm)

> maize shaft medium (19.70 mm) > chemically define medium (14.70 mm.

In Figure 2, the average zone of inhibition of test *E. coli* by cell free extract from different fermentation broth was maize shaft (12.94 mm) > cassava peel (12.35 mm) > yam peel (11.05 mm). While that of test organism *C. albicans* was yam peel (22.90 mm) > maize shaft (15.95 mm) > cassava peel (14.65 mm). The result showed that the carbon sources introduced in to the medium has a significant influence on the activity of cell free extract in the different media. The observed inhibitory activity of cell free extract from these three carbon sources against *C. albicans* were better than those obtained in *E. coli.*

DISCUSSION

Two (2) soil samples and three (3) water pool samples, collected from different locations in Sabon-Gari local government of Kaduna State in Nigeria, were screened for their potential source of antibiotics active against test microorganisms. The 5 bacterial isolates recovered from the soil and pool water were Gram-negative, oxidase positive, catalase positive and grew at 4 °C and 42 °C temperatures. All the isolates were observed to produce inhibitory substances against two test microorganisms.

The chemotherapeutic application of rhizosphere and aquatic microorganisms, which are the emerging sources of diverse chemical compounds, have not been adequately studied. This study identified five (i.e., 100%) antibiotic-producing bacteria, recovered from both rice rhizosphere and pool water sources in Sabon-Gari L.G.A of Kaduna State, Nigeria. Many similar studies have been done elsewhere. For example, in 2020, Risa and colleagues reported that approximately 6% of the 205 isolates of bacteria from various marine sources, produced antibiotics [15]. In 2018, Davis and colleagues did a similar study in which they reported that more than 50 % of the bacterial isolates from different soil samples produced activity against at least two of the six tested microbes [16]. In another study with samples from the Amazon Basin, about 70 % antibiotics-producing bacteria were isolated in the [17] while in 2018, Danilovich and colleagues isolated almost 100% antibiotic-producing microbes from over 6000 different colony morphotypes in Antarctic seawater samples [18]. Table 3 of the antibacterial activity of cell-free extract from *Pseudomonas fluorescens* fermentation broth shows that the carbon source substituted in the medium has significant influence on the activity of the cell-free extract in the different media.

The result of the inhibition of the *C. albicans* by cell free extracts from soya-beans cake medium supports the best antibiotic production, as observed from the maximum zone of inhibition that was recorded. The average zone of inhibition by the cell free extracts from the different test media of waste carbon was cassava peel medium > yam peel > maize shaft > chemically defined medium, although a distinct result was obtained when groundnut and yam peel were used together. All the three waste carbon sources have shown promising in support of antibiotic production of the bacterial isolate. This further demonstrate that the antibiotic producing bacteria synthesises potent antifungal antimetabolite that is effective against the test organism (*C. albicans*).

The antibacterial activity of all the *pseudomonas* isolates against *E. coli* showed a significantly (p<0.05) increased in the presence of various carbon sources when compared to the standard culture medium (Table 3). Furthermore, cassava supplemented medium with WP1 produced the most active antimetabolite with 22.00±0.00 mm zone of inhibition when groundnut cake was the source of nitrogen in the fermentation broth. The antibacterial activity of the isolates was significantly (p<0.05) enhanced when soyabeans cake replaced groundnut cake as the nitrogen source (Table 4).

In all the culture media, the antifungal activity of *Pseudomonas* isolate against *C. albicans* from pool water (WP-2) had comparable (p>0.05) antifungal efficacy. However, in comparison to the standard medium, the zone of inhibition of *Pseudomonas fluorescens* from all the other sources was dramatically increased (p<0.05) when groundnut cake (nitrogen source) and yam peel (carbon source) was used (table 5.0). When soyabeans was used to replace groundnut cake as the nitrogen source, there was also a significant improvement in the antifungal activity of all the isolates in the presence of cassava and yam peel (Table 6).

The antimicrobial activity of the *Pseudomonas fluorescens* against *E. coli* and *C. albicans* was significantly high in the other culture medium compared to standard medium when soyabeans cake was used as the nitrogen source (Figure 1). However, with maize shaft, cassava peel and yam peel as carbon source, changing the nitrogen source to groundnut cake caused a significant decrease in the antifungal activity of *Pseudomonas fluorescens* whilst maintaining the antibacterial effect.

Table 1: Chemical and microscopic characteristics of *Pseudomonas specie* isolates from different sources

Sample source	Cultural observation on cetrimide agar	Gram staining	Inference
farm A	Yellowish green colony	Red Gram-negative rod with elongated sides and curved edges	*Pseudomonas specie*
Pool A	Green colony	Red Gram-negative rod with elongated sides and curved edges	*Pseudomonas specie*
Pool B	Green colony	Red Gram-negative rod with elongated sides and curved edges	*Pseudomonas specie*
Pool C	Green colony	Red Gram-negative rod with elongated sides and curved edges	*Pseudomonas specie*
Farm B	Brown colony	Red Gram-negative rod with elongated sides and curved edges	*Pseudomonas specie*

Table 2: Biochemical characteristics of *Pseudomonas specie* isolates from different sources

Oxidase test	Catalase test	Temperature variation		Inference
		4 °C	42 °C	
+++	+++	+-+	+++	*P. fluorescens*
+++	+++	---	+++	*P. fluorescens*
+++	+++	-+-	+++	*P. fluorescens*
++-	+++	---	+++	*P. fluorescens*
+++	+++	---	+++	*P. fluorescens*

Key: - signifies no growth, and + signifies present of growth of *Pseudomonas specie* isolates.

Table 3: Inhibitory activity of cell free extract from *Pseudomonas fluorescens* fermentation broth of three waste carbon sources with groundnut cake against *E. coli* isolates at 37 °C for 18 h

	Mean Zones of inhibition (mm)			
Sources of Pf	Standard medium (SM)	SM substituted with maize shaft	SM substituted with cassava peel	SM substituted with yam peel
RF-1	5.25 ± 6.08	16.75 ± 2.36*	8.50 ± 10.11	8.00 ± 9.27
RF-2	6.75 ± 7.80	8.30 ± 0.00	6.00 ± 6.98	18.25 ± 1.26*
WP-1	13.75 ± 1.26	12.25 ± 10.75	22.00 ± 4.76*	6.00 ± 6.98
WP-2	11.00 ± 0.82	13.05 ± 1.63	8.00 ± 9.27	7.75 ± 8.96
WP-3	5.75 ± 6.65	12.75 ± 1.50	16.25 ± 1.50*	15.25 ± 4.03

Data was analysed using Mixed-design Analysis of Variance followed by Dunnett Posthoc test. Zones of inhibition are expressed as means ± SD. * is $p < 0.05$ compared to the standard medium. Key: Pf = *Pseudomonas fluorescens*, RF = Rice farm, WP = Water pool and SD = Standard deviation.

Table 4: Inhibitory activity of cell free extract from *Pseudomonas fluorescens* fermentation broth of three carbon sources with soya beans cake against *E. coli* isolates at 37 °C for 18 h

	Mean Zones of inhibition (mm)		
Sources of Pf	**Maize shaft**	**Cassava peel**	**Yam peel**
RF-1	14.00 ± 1.15	21.00 ± 1.15*	21.50 ± 1.73*
RF-2	13.50 ± 2.08	10.25 ± 11.84	17.25 ± 2.05*
WP-1	18.25 ± 6.40*	15.25 ± 2.06	18.75 ± 5.05*
WP-2	8.25 ± 9.60	17.50 ± 5.50	0.00 ± 0.00
WP-3	0.00 ± 0.00	14.25 ± 2.99	14.25 ± 0.50

Data was analysed using Mixed-design Analysis of Variance followed by Dunnett Posthoc test. Zones of inhibition are expressed as means ± SD. * is $p < 0.05$ compared to the standard medium. Key: Pf = *Pseudomonas fluorescens*, RF = Rice farm, WP = Water pool and SD = Standard deviation.

Table 5: Inhibitory activity of cell free extract from *Pseudomonas fluorescens* fermentation broth of three waste carbon sources with groundnut against *C. albicans* isolates at 30 °C for 18 h

	Mean Zones of inhibition (mm)			
Sources of Pf	**Standard medium**	**Maize shaft**	**Cassava peel**	**Yam peel**
RF-1	14.00 ±1.15	20.50 ±5.20	13.50 ±1.73	23.50 ±4.04*
RF-2	16.00 ±1.15	14.00 ±1.15	13.00 ±0.58	10.75 ±12.47
WP-1	14.75 ±1.41	14.50 ±0.58	12.25 ±1.39	25.25 ±6.08*
WP-2	13.50 ±1.73	17.00 ±3.46	20.00 ±4.08	30.00 ±0.00*
WP-3	15.25 ±1.71	13.75 ±1.50	15.00 ±1.63	25.00 ±0.00*

Data was analysed using Mixed-design Analysis of Variance followed by Dunnett Posthoc test. Zones of inhibition are expressed as means ± SD. * is $p < 0.05$ compared to the standard medium. Key: Pf = *Pseudomonas fluorescens*, RF = Rice farm, WP = Water pool and SD = Standard deviation.

Table 6: Inhibitory activity of cell free extract from *Pseudomonas fluorescens* fermentation broth of three waste carbon sources with soya-beans cake against *C. albicans* isolates at 30 °C for 18 h

	Zones of inhibition (mm)		
Sources of Pf	**Maize shaft**	**Cassava peel**	**Yam peel**
RF-1	20.00 ± 0.0	27.25 ± 3.40*	23.50 ± 0.58*
RF-2	18.50 ±1.73	26.50 ± 2.89*	27.75 ± 2.06*
WP-1	27.75 ± 2.06*	22.75 ± 3.77*	24.50 ± 3.31*
WP-2	12.75±14.73	27.00 ±2.45*	14.25 ± 16.46
WP-3	19.50 ±1.29	20.00 ±0.00	22.75 ± 2.06*

Data was analysed using Mixed-design Analysis of Variance followed by Dunnett Posthoc test. Zones of inhibition are expressed as means ± SD. * is $p < 0.05$ compared to the standard medium. Key: Pf = *Pseudomonas fluorescens*, RF = Rice farm, WP = Water pool and SD = Standard deviation.

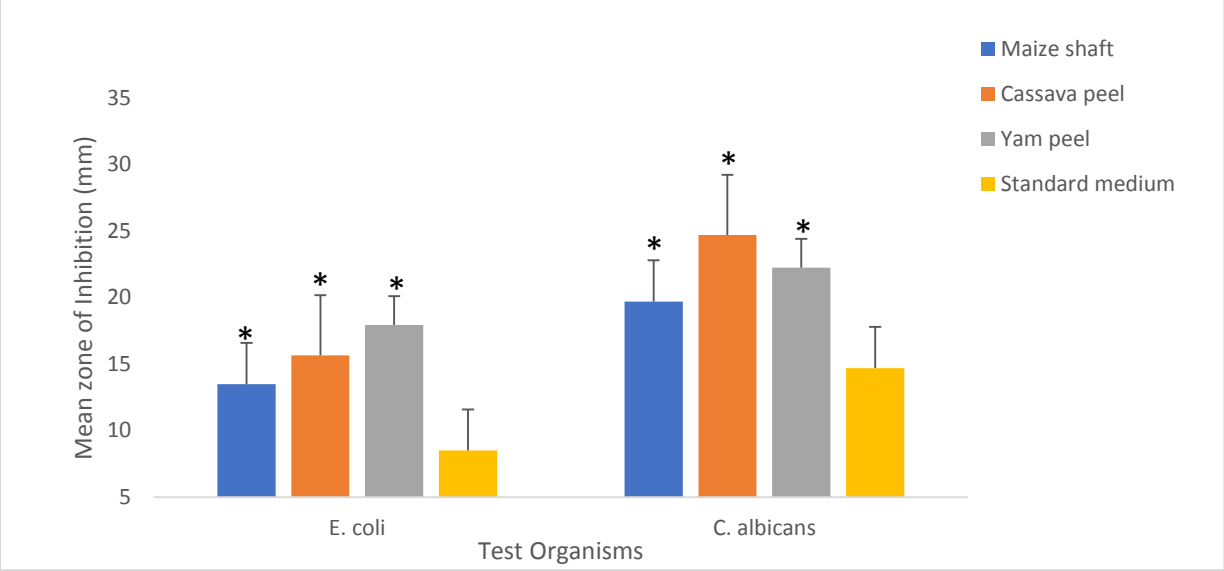

Figure 1: Effect of three waste carbon sources with soya-beans cake on cell free extract from *Pseudomonas fluorescens* fermentation broth against *E. coli* and *C. albicans.*
Data for the zone of inhibition for each organism was analysed using One-way Analysis of Variance followed by Dunnett Posthoc test. * is $p < 0.05$ compared to the standard medium.

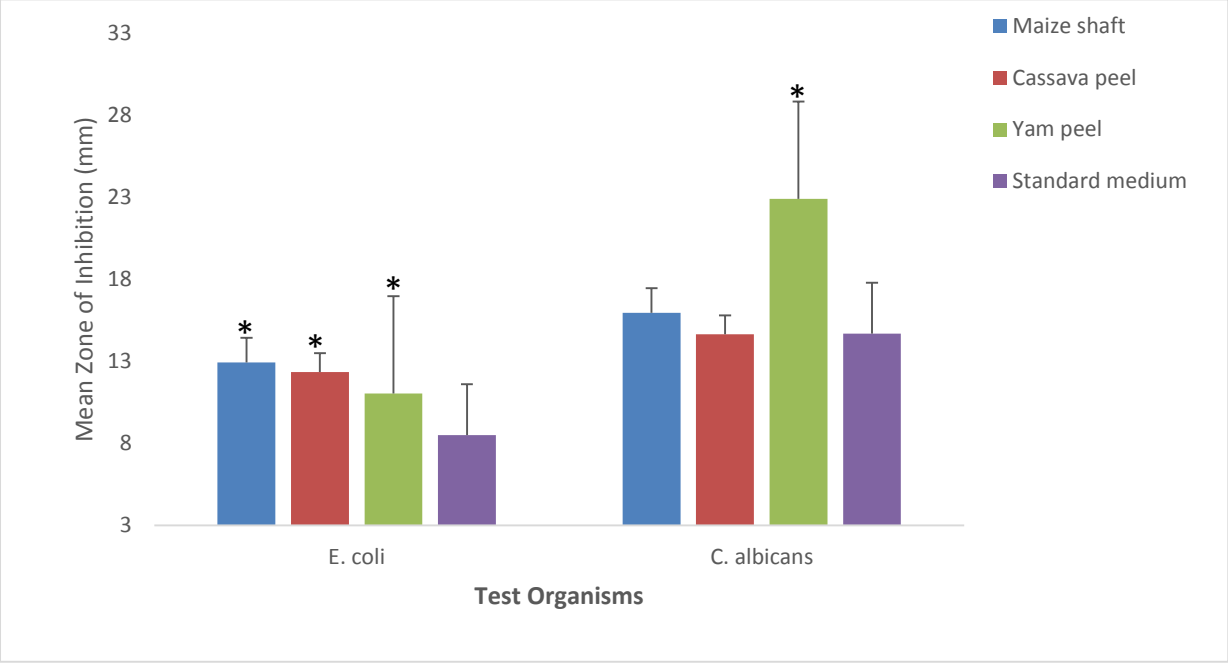

Figure 2: Effect of three waste carbon sources with groundnut cake on cell free extract from *Pseudomonas fluorescens* fermentation broth against *E. coli* and *C. albicans*
Data for the zone of inhibition for each organism was analysed using One-way Analysis of Variance followed by Dunnett Posthoc test. * is $p < 0.05$ compared to the standard medium.

The comparatively high zones of inhibition produced by the isolates RF1 in this study may be attributed to the nature of the soil environment: the local manure is often composed of a variety of waste materials, and agrochemicals. Microbes need to survive and maintain their niche under these unfavourable situations, and as such, produce active metabolite as a survival strategy. Antibiotics production by *Pseudomonas fluorescens* in the variety of carbon and nitrogen sources used was not surprising as *Pseudomonas species* are metabolically very active and excellent scavengers. The availability of nutrients largely determines the production of secondary metabolites in the medium [11]. For this reason, it is possible for different species of *Pseudomonas* to produce secondary metabolites of varying composition during their growth. Nitrogen is also an important micronutrients that play major role in the production of secondary metabolites by microorganisms [19]. The yield of the antimicrobial activity (zone of inhibition) against the test organisms (*E. coli* and *C. albicans*) differed significantly when the nitrogen sources in the fermentation media was changed. The highest inhibitory zone was recorded for soya-beans cake/cassava peel (21.00±1.15 mm) against *E. coli* and 27.25±3.40 mm against *C. albicans*. The result of this study is similar to the findings of Polanski-Cordovano and colleagues in 2013, in which the production CL0145A, an extracellular antibiotic by *P. fluorescens,* was greatly influenced by the carbon, amino acids and vitamins content of the nutrient medium [20]. Similarly, in 2004, Siddiqui and Shaukat determined that the precursors of fatty acid, carbon source and nitrogen source all affected the nematocidal activity of IE-6S from *P. aeruginosa* and CHA0 from *P. fluorescens* [21].

During growth of bacteria, carbon sources are known to specifically influence the acidification of growth medium, and this may indirectly affect control of biological activities and antibiotic production. However, changes such as pH changes were not observed in the medium amendments used in this study. In addition to carbon and nitrogen sources in the nutrient medium, other factors also influence production of secondary metabolites by microbes, with the composition of the fermentation medium being one of the most important factor [22].

Pseudomonas fluorescens strains used in the study inhibited both bacteria and fungal organisms, indicating that its antibiotic is not due solely to the production of a fluorescent siderophore. But possibly due to other phloglucinol derivatives or structurally related compounds that are produced by the bacteria [23,24].

This study demostrates that *Pseudomonas fluorescens* isolates using different carbohydrate and nitrogen source as sole source of nutrition produced significant antimetabolites products against *E. coli* and *C. albicans.* It also evidenced that nitrogen source has a significant effect on antimicrobial metabolite production. Groundnut/cassava peel was observed to be the best medium for the production of antibiotic compared to the other medium including the standard chemically defined medium.

CONCLUSION

In searching for new antibiotics, relatively simple and rapid methods have been developed for screening microorganisms for antibiotic producing ability. Thus, irrespective of the isolates, yam peel and cassava peel (carbon sources), as well as soya-beans cake (nitrogen source) have been found to support an increased yield of antimicrobial production.

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