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Phytochemical and Antimicrobial Properties of Anunuebe (*Okoubaka aubrevillei*) Fruit

Sylvia O. Anyadoh-Nwadike[1], *Edith N. Alisa, Peter C. Okeke[2], Christopher O. Alisa[3]
[1]Department of Biotechnology, Federal University of Technology, P.M.B 1526 Owerri, Nigeria
[2]Entomology Unit, Opec Research Consult, Awka, Anambra, Nigeria
[3]Department of Chemistry, Federal University of Technology, P.M.B 1526 Owerri, Nigeria

ABSTRACT: *The increase in the development and spread of resistance against antimicrobials in recent times have spurred a renewed interest in the study of medicinal plants as alternative sources of antimicrobial treatments that are effective, natural, less-toxic and affordable. Medicinal plants owe their activity to their phytochemical constituents, particularly, their secondary metabolites. This highlights the need to study these plants with the view to identify the phytochemicals that confer on them their unique medicinal properties. Although many plants have been extensively studied, there are still a number of plants that are used in the therapeutic treatment of ailment whose phytochemical constituents have not been determined. Of such plants is Anunuebe (O. aubrevillei), which is a medicinal plant that is commonly used among locals throughout its areas of distribution for the treatment of ailments. This study was therefore conducted to determine the phytochemical and antimicrobial properties of Anunuebe fruit. The phytochemical parameters of the aqueous and ethanolic extracts were assessed using standard procedures. Agar well diffusion method was used to ascertain antimicrobial activities of the different fractions – ethyl acetate, butanol, aqueous and crude – of the extracts against four common human pathogenic microorganisms; Staphylococcus aureus (Gram-positive bacteria), Pseudomonas aeruginosa, and Escherichia coli (Gram-negative bacteria) and Candida albicans (fungi). The qualitative analysis showed the presence of alkaloids, saponins, tannins, flavonoids, terpenoids, cardiac glycosides, proteins, carbohydrates, and reducing sugars in the aqueous and ethanolic extracts of the fruit. The antimicrobial test showed that although ethyl acetate, butanol, aqueous and crude fractions of Anunuebe (O. aubrevillei) fruit extract had activity against at least one of the test organisms, the best activity was observed in the ethyl acetate fraction followed by the crude extract which had activity against all the test organisms. Candida albicans was susceptible to all the fractions, while E. coli was susceptible to only the ethyl acetate fraction. This study showed that the fruit extracts had broad-spectrum antimicrobial and antifungal activities that is dependent on the extraction solvent and concentration used. Furthermore, it can be effectively used in traditional medicine for the treatment of microorganism that infect humans.*

KEYWORDS*: Okoubaka aubrevillei*; phytochemicals; antimicrobial activity; medicinal plants

INTRODUCTION

The use of medicinal plants in traditional medicine, for the treatment of various diseases, has been an age long practice in Africa and other developing countries (WHO, 2002; Okoye, Uzor, Onyeto,

& Okereke, 2014), where they still remain an integral part of the primary health care system (Kumar, Moorthy, Vinodhini, & Punitha, 2013). They are also used in complementary and alternative medicine in developed countries (WHO, 2002). These medicinal plants, often prepared in different forms such as herbal extracts and concoctions for the treatment of different diseases, owe their medicinal activity to their diverse phytochemical constituents (Linga Rao, Savithramma, & Ankanna, 2011), particularly, their secondary metabolites which include alkaloids, steroids, saponins, flavonoids, tannins etc. (Kumar *et al*., 2013). As a result of their diverse secondary metabolites, most of the pharmaceuticals currently being used in the treatment of diseases are derived from plants (Gupta & Birdi, 2017). There has been a renewed scientific interest in medicinal plants in recent years (Manimekalai, Sivakumari, Ashok, & Rajesh, 2016). This may be attributed to the fact that, in addition to their efficacy, medicinal plants are readily available and accessible at affordable rates within the locality (Okoye *et al*., 2014).

With the increase in the development and spread of resistance against antimicrobial drugs in recent times, there is a need for the development of alternatives (Manandhar, Luitel, & Dahal, 2019) that are effective, natural, non-toxic and affordable. There are diverse secondary metabolites in extracts of medicinal plants as a result, they could be used as alternate sources of antimicrobials and resistance modifying agents. This is premised on the fact that, beyond killing the pathogen, they also affect key events in the pathogenic process such that the ability of the microorganism to develop resistance to botanicals is reduced (Gupta & Birdi, 2017). Although experiences from the traditional use of medicinal plants have demonstrated their efficacy and safety in the treatment of different diseases and ailments, a comprehensive research on their phytochemical constituents and antimicrobial properties is still needed to provide additional information on their medicinal potentials, as source for new drugs. The phytochemical compounds identified can also be used to compare the amount of bioactive principles that are present in different plant parts. Such comparison may also be extended to plant species among populations of different regions where the climatic conditions are different (Uddin, Rauf, Rehman, & Qaisar, 2011).

Although a number of studies have been carried out on many medicinal plants in Africa (Kutalek & Prinz, 2005; Linga Rao *et al*., 2011; Mahomoodally, 2013), there remains quite a number of plants still to be fully explored for their medicinal properties. *Okoubaka aubrevillei* is a medicinal plant that is commonly used among locals throughout its areas of distribution. In West Africa *Okoubaka aubrevillei* bark and seeds are widely used for medicine (Borokini, Rivers, & Wheeler, 2015). It has also been used in the treatment of skin problems, including those caused by syphilis and leprosy (Bagot, 2015). The treatment is done by washing with, or bathing in a macerate or infusion of the bark in water. External application of bark preparations is also practiced to counter poisoning. Bark macerate is drunk to cure tachycardia and is taken as a vapour bath or as nose drops to cure oedema. In a compress it is used to disperse hematomas (Achukwu *et al*., 2018). Despite the numerous medicinal potential of the *O. aubrevillei* plant, there is still a paucity of research on the plant (Borokini, 2015), especially the fruit.

The use of the bark for various medicinal purposes have been widely reported in different studies (Borokini, 2015). However, the wanton destruction of the plant as a result of the uncontrolled

exploitation of the bark is a major conservation concern for *O. aubrevillei*, as a sharp decline in the population of the tree declined over the years (Borokini, 2015). This therefore necessitates exploring alternative ways of harnessing the plants medicinal potential without the direct destruction of the plant through scarring, debarking, Bagot, (2015) and felling of the tree (T. Borokini, 2014). The *O. aubrevillei* fruits may be a suitable alternative, hence, the need for scientific validation of this fruit in order to determine its phytochemical properties and the extent of its antimicrobial potentials. In view of this, the aim of this study was to isolate, identify and analyze the qualitative and quantitative phytochemical constituents, and the antimicrobial properties of *O. aubrevillei* fruit.

METHODS

Collection of Plant Materials
The fruits of *Okoubaka aubrevillei* were obtained from Eke Awka market in Anambra State, in Nigeria. The samples were identified by an expert at tree crops and tropical Ecological Centre at no 7 Dona Drive Independence Layout Enugu Nigeria.

Preparation of Fruit Extracts
Thoroughly washed mature fruits of *Okoubaka aubrevillei* and leaves of *Pychnobotrya nitida* were shade dried and then powdered using an electric blender. Afterwards, 50 g of the powder were filled in the thimble and extracted successively with ethanol using a Soxhlet extractor. All the extracts were concentrated using rotary flash evaporator and preserved at 5˚C in airtight bottles until required for further use. Cold maceration was also used to obtain some extracts from both the *Okoubaka aubrevillei* and the *Pychnobotrya nitida*.

Liquid-Liquid extraction: this was carried out using the methods described by (Irina & Barbulescu, 2011). In principle, the liquid-liquid extraction is used to isolate a compound from a mixture based on the immiscibility of two liquids due to their different polarity. To obtain the N-hexane, ethyl acetate, and butanol fractions of the plant extracts, the liquid-liquid extraction was performed in a separatory funnel. The extraction solvent was prepared by dissolving about 40 g of the previously concentrated plant extract in 200 ml of water and poured into the separatory funnel, while ensuring that the Teflon stopcock was closed first. To this, 400 ml of N-hexane was added before shaking the funnel. While holding the cap and the separatory funnel securely, the funnel was inverted and the Teflon stop cork opened to reduce any pressure that had built up. The shaking and venting steps were repeated several times. The separatory funnel was, afterwards, allowed to rest for some minutes while in an upright position on a ring clamp. The phases formed were collected by removing the cap from the separatory funnel and draining the two phases into separate beakers. The aqueous phase was poured back into the funnel and another 400 ml of normal hexane was added to it to repeat the same process for about three times until the solution appeared clear, indicating that the metabolites had been extracted. The above procedure was then repeated for ethyl acetate and butanol to obtain their fractions. Whatever that was left in the aqueous base became the aqueous fraction of the extract.

Qualitative Analysis of the Phytochemical Constituents of Okoubaka aubrevillei Fruit Extract
The phytochemical constituents present in the aqueous and ethanol extracts of *Okoubaka aubrevillei* fruit were identified using standard procedures described by Sofowora (1993) and Harborne & Harborne (1973), with slight modifications.
Test for Alkaloids: Two milliliter of Wagner reagent was added to a little portion of the filtrate of the plant sample. The production of a reddish-brown precipitate indicated the presence of alkaloids.

Test for Tannins: A few drops of 0.1% ferric chloride ($FeCl_3$) was added to the plant extract and observed for greenish brown or blue-black colouration, which indicated the presence of tannins.
Test for Saponins: About 2 g of the powdered sample was boiled in 20 ml of distilled water in a water bath and filtered. Ten milliliters of the filtrate were mixed with 5 ml of distilled water and shaken vigorously on a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion which indicated the presence of saponins.

Test for Flavonoids: Few drops of 1% aluminum solution ($AlCl_3$) were added to a portion of each plant extract. A yellow colouration indicated the presence of flavonoids.
Test for Steroids: Acetic anhydride was added to a portion of each plant extract with H_2SO_4. The formation of a blue or green ring indicated the presence of steroids.

Test for Terpenoids: Five milliliters of each extract was mixed in 2 ml of chloroform, and 3 ml of concentrated H_2SO_4 was carefully added to form a layer. A reddish-brown ring of the interface indicated the presence of terpenoids.

Test for Cardiac Glycosides: An aliquot of 5 ml of each extract was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. Then 1 ml of concentrated H_2SO_4 was added. A reddish-brown ring of the interface indicated a deoxy sugar characteristic of cardenolides.

Test for Proteins: Two drops of Million's reagent were added to a little portion of the extract in a test tube. The formation of a white precipitate indicated the presence of proteins.

Test for Carbohydrate: To a little portion of the extract in a test tube, 2 ml of Molisch's reagent was added. While being maintained in a slanted position, 2 ml of concentrated H_2SO_4 was added to the test tube. A deep violet or purple colour at the interface indicated the presence of carbohydrate.

Test for Reducing Sugar: Benedict solution was added to a portion of the extract and heated in a water bath. Equal volumes of Fehling A and Fehling B solutions were mixed together and added to the mixture. A change in colour from deep blue to yellow, brown or red indicated the presence of reducing sugar.

Quantitative Analysis of Phytochemical Constituents of Okoubaka aubrevillei Fruit

Alkaloids Determination: The quantitative determination of alkaloids was done according to the method described by Harborne and Harborne (1973). About 200 cm^3 of 10% acetic acid in ethanol was added to 5 g of the plant extract in a 250 cm^3 beaker and allowed to stand for 4 hours. The extract was concentrated on a water bath to one-quarter of the original volume. Fifteen drops of concentrated ammonium hydroxide were added to the extract until the precipitation was completed. After 3 hours of mixture sedimentation, the supernatant was discarded and the precipitate was washed with 20 cm^3 of 0.1 m of ammonium hydroxide and then filtered using Gem filter paper (12.5 cm). It was then weighed using electronic weighing balance (model B-218) and the residue dried in an oven. The percentage alkaloid was determined using the formula;
% Alkaloid = (weight of alkaloid)/ (weight of sample) × 100/1

Tannins Determination: The tannin content was determined using Folin Denis reagent as described by (Nangia-Makker, Ochieng, Christman, & Raz, 1993). A standard calibration curve was prepared and the absorbance (A) against concentration of tannins at specific wavelength was established as follows; Aliquots of 0.05 ml, 0.2 ml and 0.5 ml of the extracts were pipetted into test tubes and the volumes were made up to 1.00 ml using distilled water. To each of the test tubes, 2.5 ml of sodium carbonate reagent was added and shaken. The absorbance was recorded at 725 nm after 40 minutes. The amount of total phenols was calculated as tannic acid equivalent from the standard curve using the formula below.
Tannic acid (mg/100g) = (C × extract volume × 100)/ (Aliquot volume × weight of sample)
Where C = Concentration of tannic acid read off the graph.

Saponins Determination: Saponins quantitative determination was done using the method reported by (Ejikeme, Ezeonu, & Eboatu, 2014; Obadoni & Ochuko, 2002). A 100 cm^3 of 20% aqueous ethanol was added to 20 g of the extract in a 250 cm^3 conical flask. The mixture was heated over a hot water bath for 4 hours with continuous stirring at a temperature of 55 ˚C. The residue of the mixture was re-extracted with another 100 cm^3 of 20% aqueous ethanol after filtration and was heated for 4 hours at a constant temperature of 55 ˚C with constant stirring. The combined extract was evaporated to 40 cm^3 over water bath at 90 ˚C. About 20 cm^3 of diethyl ether was added to the concentrate in a 250 cm^3 separator funnel and vigorously agitated. From the resulting layers formed, the aqueous layer was recovered while the ether layer was discarded. This purification process was repeated twice. About 60 cm^3 of N-butanol was added and extracted twice with 10 cm^3 of 5% sodium chloride. After discarding the sodium chloride layer, the remaining solution was heated on a water bath for 30 minutes after which the solution was transferred into a crucible and was dried in an oven to a constant weight. The saponin content was calculated as a percentage using the formula. % Saponin = (weight of saponin)/ (weight of sample) × 100/1

Flavonoids Determination: This was determined using the method described by Harborne and Harborne (1973). Following the method, 5 g of sample was boiled in 100 ml of 2 M HCl solution for 40 minutes. It was allowed to cool to room temperature before being filtered through Whatman filter paper (Number 42). The flavonoids in the extract was precipitated by dropwise addition of concentrated ethyl acetate until in excess. The flavonoids precipitate recovered was oven dried and

the weight of the flavonoids determined. The percentage flavonoid was calculated using the formula below.

% Flavonoid = (weight of flavonoid)/ (weight of sample) × 100/1

Evaluation of Antimicrobial Activities of Okoubaka aubrevillei Fruit Extract

Confirmation of Test Organisms

Four standard human pathogenic microorganisms namely, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Candida albicans* were used as test organisms. These were standard laboratory cultures whose susceptibility on commonly used antibiotics was already established. *Staphylococcus aureus* represented Gram-positive bacteria while *Escherichia coli*,

Pseudomonas aeruginosa, represented Gram-negative bacteria, and *Candida albicans* represented the fungi. These test organisms were collected from the Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmaceutical Sciences in Nnamdi Azikiwe University, Awka. They were further reconfirmed by sub culturing and subjecting pure isolates to specific pure culture identification techniques (Ugwu, Edeani, Ejikeugwu, Okezie, & Ejiofor, 2017).

To reconfirm them, the isolates were characterized by subjecting them to morphological (colonial morphology), physiological and biochemical tests. The morphological characterization was done by examining and recording the isolated colonies based on the type of growth, elevation, size, colour, margin edge, consistency, opacity, and change in medium. To characterize the isolates physiologically, the Gram staining technique described by Ann, Smith & Marise, (2018) of immersion oil was added to the slide and viewed under the microscope, Gram-positive cells stained purple while Gram-negative cells stained pink. The results obtained were recorded. To further identify the organisms, the following biochemical tests were carried out as described by Talaiekhozani, Alaee, & Mohanadoss, (2015) Catalase test, Coagulase test, Indole test and Oxidase test.

Antibiotic Susceptibility Testing

For Conventional Commercial Antibiotics

Antibiotic susceptibility of pure culture of the confirmed isolates was performed on the sensitivity test agar (Mueller Hinton Agar), by the standard Kirby Bauer disc diffusion method.Standardized overnight culture of each isolate was prepared by inoculating each isolate into 3 ml sterile nutrient broths in test tubes and incubated at 37 ˚C for 24 hours and adjusted to the 0.5 McFarland turbidity standard. The Mueller Hinton Agar used was prepared, based on the manufacturer's specifications and sterilized by autoclaving at 15 psi, 121 ˚C, for 15 minutes. After autoclaving, it was left to cool to 50 ˚C before being poured into flat bottom petri dishes on a horizontal surface and allowed to solidify. The standard inocula were respectively swabbed under aseptic conditions on the appropriately labelled solidified Mueller Hinton Agar plates and allowed to soak for about 5 minutes. After this, the antibiotic discs were aseptically placed on the surface of the media and

pressed down gently to lap with the media. The plates were incubated at 37 ˚C for 24 hours. At the end of 24 hours, the inhibition zones created by each antibiotic against each isolate were measured and recorded as Inhibition Zone Diameter (IZD). This was performed in triplicates for each antibiotic per isolate and the average was obtained.

Preparation of Extracts for Evaluation

Stock concentrations of each of the extract were made by weighing 50 mg each of crude extract and fractions respectively into sterile beakers. About 2 ml of Dimethyl sulfoxide (DMSO - organic diluent) was added into each of the samples and reconstituted properly. This gave a stock concentration of 25 mg/mL of each extract. Thereafter, twofold serial-dilutions were made from each of the stock concentrations to get graded concentrations (12.5 mg/mL, 6.25 mg/mL, and 3.125 mg/mL) of each of the crude extract and fractions.

Determination of Antimicrobial Activity

The antimicrobial assay for crude extracts and fractions was carried out using the agar well diffusion assay as described by (Ugwu et al., 2017) with slight modifications. The antimicrobial activity of the extracts of *Okoubaka aubrevillei* fruit was then tested against the four test organisms.

The bacterial suspensions were adjusted to 0.5 McFarland turbidity standard and inoculated onto previously sterilized Mueller-Hinton Agar (MHA) plates (90 mm in diameter). A sterile cork-borer was used to make five wells (8 mm in diameter) on each of the MHA plates. Aliquots of 80 µl of each extract dilutions, reconstituted in DMSO at concentrations of 50 mg/mL, 25 mg/mL, 12.5 mg/mL, 6.25 mg/mL, and 3.13 mg/mL were applied in each of the wells in the culture plates previously seeded with the test organisms. Ciprofloxacin single disc (10 µg) served as the positive control against the test organisms as the test isolates were known ciprofloxacin susceptible strains. The cultures were incubated at 37 ˚C for 24 hours. The antimicrobial potential for each extract was determined by measuring the diameter of zone of inhibition around each well (excluding the diameter of the well). The test was respectively carried out in triplicate for the crude and fractions. Each of the samples were tested against all the test isolates.

Statistical Analysis

The data obtained were subjected to descriptive statistical analysis such as mean and standard deviation for the replicates. Global validation of linear model assumption (GVLMA) test was used to check for violation of the linear model assumptions. For data that satisfy the linear model assumptions, the one-way analysis of variance was used to compare the mean inhibition zone diameter for the different concentrations and the various extraction solvents. Significant difference was determined at 5% probability level. All statistical analyses were done using R software (version 4.0.2).

RESULTS

Qualitative Analysis of Phytochemical Constituents of Okoubaka aubrevillei Fruit Extract:
The phytochemical screening of *Okoubaka aubrevillei* fruit extract showed the presence of alkaloids, tannins, saponins, flavonoids, terpenoids, cardiac glycosides, proteins, carbohydrates and reducing sugar in both the aqueous and ethanol extracts, while steroid was absent. Among the secondary metabolites that were observed in the aqueous extract of *Okoubaka aubrevillei* fruit, saponin terpenoids, cardiac glycoside, carbohydrate and reducing sugar were heavily present. In the ethanol extract, only terpenoid, cardiac glycoside, protein, and reducing sugar were heavily present (Table 1).

Table 1: Qualitative phytochemical analysis of aqueous and ethanol extracts of *O. aubrevillei* fruit extracts

Secondary metabolites	*Okoubaka aubrevillei* extracts	
	Aqueous	Ethanol
Alkaloid	+	+
Saponin	++	+
Tannin	+	+
Flavonoids	+	+
Steroid	-	-
Terpenoid	++	++
Cardiac glycoside	++	++
Protein	+	++
Carbohydrate	++	+
Reducing sugar	++	++

Present: +; Heavily present: ++; Absent: -

Quantitative Analysis of Phytochemical Constituents of Okoubaka aubrevillei Fruit Extracts
The quantitative analysis of phytochemical constituents of *Okoubaka aubrevillei* fruit extract was determined for alkaloids, saponins, tannins and flavonoids. In *O. aubrevillei* fruit extract, the concentrations of the secondary metabolites were observed to be 5.20%, 33.80%, 1741.82 mg/100 g, and 3.47% in alkaloids, saponins, tannins and flavonoids, respectively (Table 2).

Table 2. Quantitative phytochemical constituents of *Okoubaka aubrevillei* fruit expressed as percentage

Constituent	*Okoubaka aubrevillei*
Alkaloids (%)	5.20 ± 0.20
Saponins (%)	33.80 ± 0.66
Tannins (mg/100 g)	1741.82 ± 45.7
Flavonoids (%)	3.47 ± 0.12

Values are mean of three replicate determinations (n = 3) ± standard deviations.

Evaluation of Antimicrobial Activities of Okoubaka aubrevillei Fruit Extract.
Effects of Different Concentrations of O. aubrevillei on Each of the Test Organisms
Figure 1 shows that *O. aubrevillei* extract had activity against all the test organism at concentrations of 200 mg/mL and 100 mg/mL. At concentrations of 50 mg/mL and 25 mg/mL, activity was observed against only 3 organisms; *S. aureus*, *Ps. aeruginosa*, and *C. albicans*, while at the lowest concentration of 12.5 mg/mL, only *S. aureus* and *C. albicans* were inhibited. The positive control had activity against *E. coli* and *C. albicans*, but not *S. aureus* and *Ps. aeruginosa*. For all the different concentrations of *O. aubrevillei* extract, the highest inhibition zone was against *C. albicans*, which compares well with the positive control where the highest inhibition zone was also against *C. albicans*.

The observed difference in the inhibition effect of *O. aubrevillei* extract against each of the test organisms was statistically significant ($P < 0.007$) at the different concentrations. At concentrations of 200 mg/mL and 100 mg/mL, the inhibition zone diameter of *O. aubrevillei* extract was significantly ($P < 0.05$) higher than that of the control, which had no inhibition against *S. aureus*. The inhibition zone diameter of the control against *E. coli* was significantly ($P < 0.05$) higher than those of 50 mg/mL, 25 mg/mL and 12.5 mg/mL concentrations of *O. aubrevillei* extract, but not those of 200 mg/mL and 100 mg/mL. Against *Ps. aeruginosa* the inhibition zone diameter of 200 mg/mL of *O. aubrevillei* extract was significantly higher than that of 25 mg/mL ($P < 0.005$), as well as those of 12.5 mg/mL and the control ($P < 0.001$); both of which had no inhibition effect. That of 100 mg/mL was significantly higher than that of 25 mg/mL ($P < 0.04$), as well as those of 12.5 mg/mL and the control ($P < 0.002$); both of which had no inhibition effect. Also, that of 50 mg/mL was significantly higher than those of 12.5 mg/mL and the control ($P < 0.02$); both of which had no inhibition effect., the observed difference in the inhibition effect of *O. aubrevillei* was statistically significant ($P < 0.001$) at the different concentrations. Against *C. albicans*, the inhibition zone diameter of the plant extract at 200 mg/mL, 100 mg/mL, and 50 mg/mL were significantly ($P < 0.05$) higher that of 12.5 mg/mL, which had no inhibition effect. Similarly, the inhibition zone diameter of the control was significantly ($P < 0.05$) higher that of 12.5 mg/mL of the plant extract, but not those of 200 mg/mL, 100 mg/mL, and 50 mg/mL (Figure 1).

While the control had inhibition activity against only two of the test organisms; *E. coli* and *C. albicans*, the plant extract had inhibition against all the test organisms at concentrations of 200 mg/mL and 100 mg/mL.

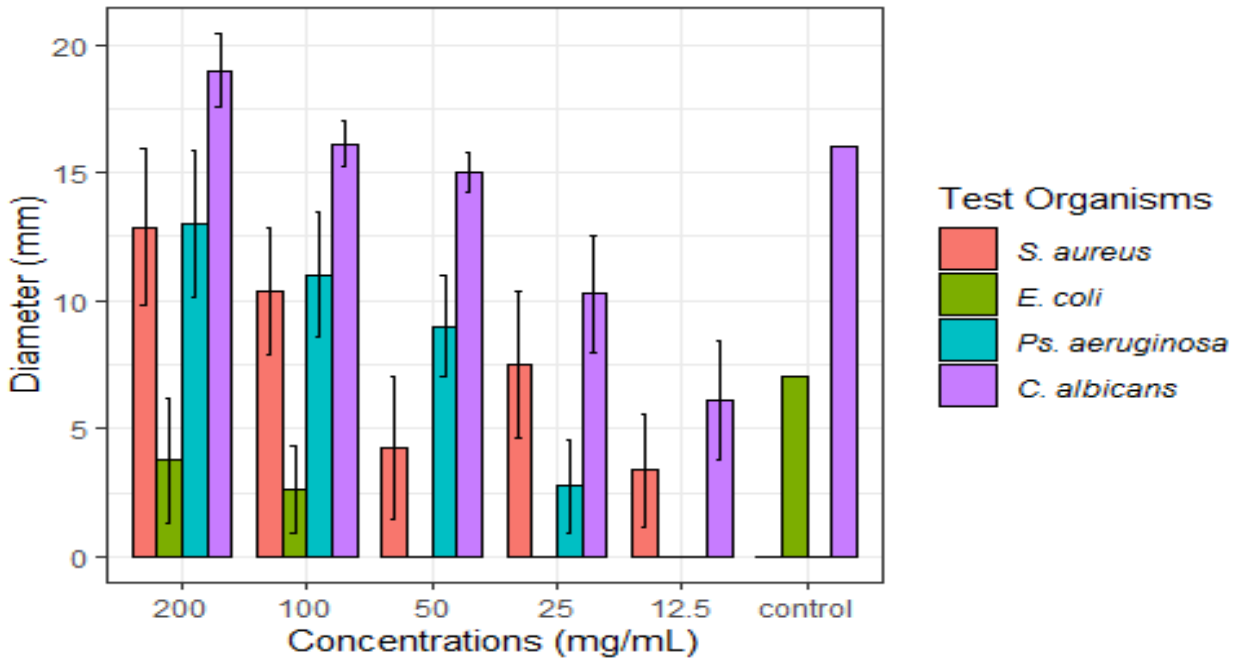

Figure 1. Effect of different concentrations of *O. aubrevillei* on the inhibition zone diameter of the test organisms

Effects of Different Extraction Solvent of O. aubrevillei Fruit Extract on Each of the Test Organisms: Figure 2 shows that against *S. aureus*, the highest (14.00 mm) inhibition zone diameter was observed in the aqueous extract of *O. aubrevillei* fruit, followed by the crude extract (3.67 mm), while the least was observed in the butanol extract; which showed no inhibition zone. The observed difference in the inhibition zone diameter of the different extraction solvents was statistically significant ($P < 0.001$). Particularly, the inhibition zone diameter of the aqueous extract was significantly ($P < 0.001$) higher than those of the crude extract and the butanol extract. The inhibition zone diameter of the ethyl acetate extract against *S. aureus* was also significantly ($P = 0.01$) higher than that of the butanol extract.

The effects of the extraction solvents of *O. aubrevillei* fruit extract on the inhibition of *E. coli* showed that the highest (5.42 mm) inhibition zone diameter was observed in the ethyl acetate extract followed by those of the butanol, aqueous and crude extracts (1.17 mm). The observed difference was not significantly different ($P > 0.05$).

Against *Ps. aeruginosa*, the highest (8.58 mm) inhibition zone diameter was observed in the crude extract of *O. aubrevillei* fruit followed by the ethyl acetate extract (7.67 mm) and then the butanol extract (7.58 mm), while the least was the aqueous extract. The observed difference was statistically significant ($P = 0.009$). Particularly, the inhibition zone of the ethyl acetate and the butanol extracts were significantly ($P = 0.04$) higher than that of the aqueous extract. Also, the inhibition zone diameter of the crude extract was significantly ($P = 0.002$) higher.

The highest (17.08 mm) inhibition zone diameter of the extraction solvents of *O. aubrevillei* against *C. albicans* was observed in the ethyl acetate extract of *O. aubrevillei* fruit followed by the aqueous extract (14.50 mm), then butanol extract (14.08 mm), while the least (9.33 mm) was observed in the crude extract. The observed difference was statistically significant (P = 0.01), particularly, the inhibition zone diameter of the ethyl acetate was significantly (P = 0.01) higher than that of the crude extract.

From the inhibition zone diameter of each of the test organisms, as shown in figure 3, the result shows that for each of the extraction solvents, *O. aubrevillei* fruit extract gave the highest activity against *C. albicans*, while the lowest activity was against *E. coli*. Only the ethyl acetate and crude extracts had inhibition activity against all the test organisms. More so, comparing the inhibition zone diameter of the ethyl aetate and crude extracts against *S. aureus*, *E. coli* and *C. albicans*, the result showed that the ethyl acetate solvents of the plant extract had a better inhibition activity.

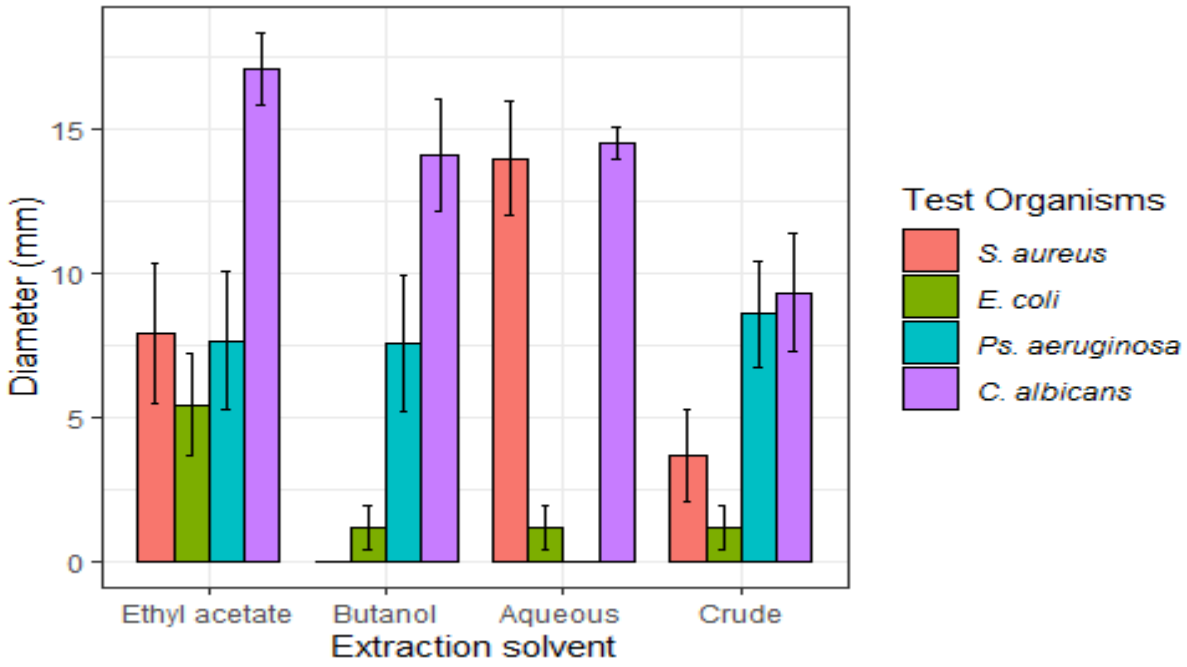

Figure 2. Effect of different extraction solvents of *O. aubrevillei* on the inhibition zone diameter of the test organisms

Determination of Minimum Inhibitory Concentration (MIC)

Among the different extraction solvents of *O. aubrevillei* fruits, the ethyl acetate and crude extracts showed strong antimicrobial activity, therefore, the MIC for both extracts was determined for all the test organisms. For the ethyl acetate extracts, the MIC of *O. aubrevillei* fruit against *S. aureus*, *E. coli*, *Ps. aeruginosa*, *C. albicans* were 100 mg/mL, 100 mg/mL, 50 mg/mL and 25 mg/mL, respectively; while for the crude extracts, it was 100 mg/mL, 25 mg/mL, 25 mg/mL and 100 mg/mL. For the butanol extract of *O. aubrevillei* fruit extract, MIC was determined for only *Ps.*

aeruginosa and *C. albicans* (100 mg/mL and 25 mg/mL, respectively). While for the aqueous extract, MIC was determined for only *S. aureus* and *C. albicans* (100 mg/mL and 25 mg/mL, respectively) (Table 3).

Table 3: Minimum inhibitory concentrations (MIC) according of the plant extracts against the Test Organisms

Extraction solvent	Concentration (%)			
	S. aureus	*E. coli*	*Ps. aeruginosa*	*C. albicans*
Ethyl acetate	100	100	50	25
Butanol	nd	nd	100	50
Aqueous	25	nd	nd	25
Crude	100	25	25	100

nd = Not determined

DISCUSSION

Phytochemical Constituents of Okoubaka aubrevillei Fruit Extract

In the qualitative phytochemical analysis, *Okoubaka aubrevillei* fruit extract showed the presence of alkaloids, tannins, saponins, flavonoids, terpenoids, cardiac glycosides, proteins, carbohydrate and reducing sugar in both the aqueous and ethanolic extract, while steroids was absent in both extracts. These phytocompounds are important bioactive compounds that are effective in the treatment of different diseases and illnesses (Kavit, Patel, & Jain, 2013).

Quantitative analysis of *O. aubrevillei* fruit extract showed that the plant contains large quantity of tannins, which is a phenolic compound of high molecular weight that are very useful in medicine and in the manufacturing industry. In medicine, tannins are used as astringents in the treatment of gastrointestinal disorders such as diarrhoea (Ashok & Upadhyaya, 2012; Labu, Laboni, Mamun, & Howlader, 2015). They also have bacterial and antioxidant effect, as such, tannins-containing plants are effective in inhibiting cancerous growth (Yildirim & Kutlu, 2015). In view of this, the observation of tannins in large quantity in the *O. aubrevillei* fruit extract presents it as a potential source of antioxidants and astringents in traditional medicine and also as candidates for drug production in same line. Despite the medicinal potentials of the plant on account of its high tannins content, there is need to take into account the observation that the intake of high tannins content slows down the absorption of some minerals such as calcium and iron, leading to osteoporosis anaemia according to Ricardo-da-Silva *et al*. (1991), it is therefore advisable to complement tannins intake with high phenolic intake. This should also be noted if being considered for standardization and commercialization as drug form.

Alkaloids were present in both *O. aubrevillei* fruits extract. This is in agreement with previous studies by Yaqoob, Nawchoo, and Ahmad (2016), who observed the presence of alkaloids in fruit extracts of *Ferula jaeschkeara*. Alkaloids are known to be the major constituents of antibacterial,

local anaesthetic, anticancer and also analgesic drugs (Ukoha, Cemaluk, Nnamdi, & Madus, 2011; Kuete, 2014). Its presence in the plant extract makes it suitable for use as a sedative and an antimicrobial agent. Both flavonoids and saponins are antioxidant, antileukemic, anticancer, antiaging and antibacterial (Sharma, 2006). The presence of flavonoids in the *O. aubrevillei* fruit extract is in agreement with the findings of Kumar and Pandey (2013), who reported that flavonoids are important bioactive compound which are usually contained in fruits and vegetables. *Antimicrobial Properties of Okoubaka aubrevillei Fruit*.

The screening of antimicrobial activity of plant extracts is a step towards determining the potential of the plants as sources of antimicrobial compounds that are effective against human pathogenic microorganisms (Joshi *et al.*, 2011). The present study therefore evaluated the antimicrobial properties of *Okoubaka aubrevillei* fruit extracts against the test organisms using different concentrations of the ethyl acetate, butanol, N-hexane, aqueous and crude extract of the plants.
The study also showed that the activity of the plant extracts is concentration dependent as the inhibition zone diameter increases with increasing concentrations of the extract. This agrees with the other previous studies (Agbafor & Nwachukwu, 2011; Arekemase *et al*., 2011; Durai *et al*., 2016). At the highest concentration of 200 mg/ml and at 100 mg/ml both plant extracts had activity against all the test organisms. At these concentrations the plant extract performed better than the control which had activity against only *E. coli* and *C. albicans*. Considering the fact that at high concentrations phenolic compounds denature the proteins present in microbial cells (Cendrowski, Krasniewska, Przybył, Zielinska, & Kalisz, 2020), it is therefore possible that the phenolic compounds present in the plant extract might have also contributed to the strong antimicrobial activity of the plant extract at higher concentrations. In both the different concentrations of the plant extracts and the control, *C. albicans* was the most susceptible organism. Thus, *O. aubrevillei* fruit extract can be effectively used against fungal infections.

In line with the study by Kumar *et al.* (2013) who observed that the activities of organisms towards metabolites varies greatly depending on the extracting solvent, the present study also showed that the antimicrobial activity of the *O. aubrevillei* fruit extracts varied with the different extraction solvents. The strongest antimicrobial activity was observed in the ethyl acetate solvent followed by the crude extract. The strong antimicrobial activity of the ethyl acetate solvent in the present study contradicts the findings of a previous study investigating the antimicrobial activity of different extracts of *Magnifera indica*, in which Vaghasiya & Chanda (2010) observed the least antimicrobial activity in the ethyl aetate extracts. The observation of the present study is probably due to the presence of more bioactive compounds in the ethyl acetate extract of *O. aubrevillei* fruit, as different bioactive compounds are usually extracted by different solvents based on their chemical properties and characterizations (Panphut, Budsabun, & Sangsuriya, 2020).

The highest inhibition zone was observed in the ethyl acetate extract of *O. aubrevillei* fruit against *C. albicans*, while the butanol extract had no activity against *S. aureus*, likewise the aqueous extract which had no activity against *Ps. aeruginosa*. Although in a previous study by Durai, Balamuniappan, & Geetha (2016), the aqueous extracts of *S. macrophylla* was shown to have strong antimicrobial activity against *S. aureus*, and *E. coli*, the present study showed a strong

activity of the aqueous extract on only *S. aureus,* but not *E. coli*. It also did not show any activity against *Ps. aeruginosa*. With the exception of the aqueous extract which had no activity against *Ps. aeruginosa,* the different solvents used showed antibacterial activity against both the Gram-positive and Gram-negative bacteria, an indication of the presence of broad spectrum antibiotic compounds in the plant extracts.

The study also showed that the fungi; *C. albicans* was more susceptible to than the bacteria; *E. coli, S. aerus*, and *Ps. aeruginosa*. This observation is, however, contrary to that of a previous study by Vaghasiya & Chanda (2010), in which the plant extract used in the study did not show any activity against the all the fungal strains studied. The findings of this study may be an indication of the presence of broad-spectrum antimicrobial compounds in the plant extract.
Okoubaka aubrevillei was observed to be almost completely insoluble in nonpolar solvent, such as N-hexane but soluble in semi-polar and polar solvents such as ethyl acetate and butanol. This probably explains why no metabolite was extracted from *O. aubrevillei* fruit extract using N-hexane as the solvent.

CONCLUSION

The results obtained from this study shows that plants can be effectively used as a botanical alternative source of medication. *Okoubaka aubrevillei* fruit has been found in this work to contain many medically important phytocompounds, including large quantity of alkaloids and tannins. This finding is suggestive of its high medicinal potentials. The antimicrobial analysis of the extract suggests that it can be used as antibiotics, as well as broad-spectrum antibiotics, since it inhibited the growth of Gram-positive bacterium, Gram-negative bacteria and Fungus. *Okoubaka aubrevillei* fruit extracts being readily available and efficacious should be further explored for standardization and commercialization as conventional drugs.

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