Full Length Research Paper

Comparative evaluation of antimicrobial properties and phytochemical composition of *Artocarpus altilis* leaves using ethanol, n-hexane and water

Mbaeyi-Nwaoha, I. E.* and Onwuka, C. P.

Department of Food Science and Technology, University of Nigeria, Nsukka, Enugu State, Nigeria.

Received 30 May, 2014; Accepted 1 September, 2014

This study was undertaken to describe the comparative evaluation of phytochemical constituents and antimicrobial activity of *Artocarpus altilis* leaves in three different solvents (ethanol, n-hexane and water). Qualitative and quantitative phytochemical analysis carried out confirmed the presence of alkaloids, tannins, flavonoids, steroids, reducing sugar, saponins, soluble carbohydrate, hydrogen cyanide and glycoside in all the extracts. The antimicrobial activity was tested using two different methods: Agar well diffusion method and agar dilution method against seven bacterial and three fungal strains. The results showed that antibacterial and antifungal activities were more effective using the agar dilution method with the ethanol extract having the highest inhibitory activity against the microorganisms as compared to the other solvents used (n-hexane and water). At concentrations of 10, 9, 8, 7 and 6 mg/ml, ethanol extract showed total inhibitory activity against all the test microorganisms used. N-hexane extract showed inhibitory activity against all the microorganisms up to a concentration of 8 mg/ml, while the aqueous extract had no activity against the microorganisms at all the concentrations tested (10 to 1 mg/ml). Under the agar diffusion method, the microorganisms were tested at concentrations of 100, 50, 25, 12.5 and 6.25 mg/ml and the method showed very minimal inhibition against all the microorganisms tested with only the ethanol extract showing a little degree of inhibition with an MIC of 3.98 mg/ml against *B. subtilis*. The other solvents (n-hexane and water) showed no activity against the test microorganisms at all the concentrations tested. Anti-nutrient analysis revealed the presence of phytate, oxalate, tannin and cyanide in all the extracts.

**Key words:** Agar diffusion method, anti-nutrient, *Artocarpus altilis* leaves, phytochemical constituents.

**INTRODUCTION**

Over the years, emerging and re-emerging infections and spread of deadly, drug-resistant strains of organisms pose a challenge on the global public health for their treatment. Bacterial resistance to antibiotics and anti-microbials over time has been a major therapeutic problem and the rate at which new antibiotics are now being produced is reducing (Russell, 2002). There is a continuous and urgent need to discover new antimicrobial compounds.

*Corresponding author. E-mail: miphie2003@yahoo.co.uk or ifeoma.mbaeyi-nwaoha@unn.edu.ng.

Author(s) agree that this article remain permanently open access under the terms of the Creative Commons Attribution License 4.0 International License
with diverse chemical structure and novel mechanism of activities for new and re-emerging infectious disease. Therefore, researchers are increasingly looking for new leads to develop better drug against microbial infections. In recent years, secondary plant metabolites (phytochemicals), previously with unknown pharmacological activities, have been extensively investigated as a source of medical agents (Krishnaraju et al., 2005). These natural products can provide unique elements of molecular diversity and biological functionality, which is indispensable for novel drug discovery (Nisbet and Moore, 1997). Thus, it is anticipated that phytochemicals with adequate antibacterial efficacy can be used for the treatment of bacterial infections and illness ranging from sore eyes to sciatica. These plants have the capacity to produce a large number of organic chemicals so called secondary metabolites; which are divided into different categories based on their mechanism of function like chemotherapeutic, bacteriostatic, bactericidal and antimicrobial agent. The World Health Organization also has recognized the importance of traditional medicine and has been active in creating strategies, guidelines and standards for botanical medicines in the search for novel antimicrobial agents in ensuring the safety of man and also reducing losses of food products to microbial spoilage. The widespread use of this plant species in medicine and more recent empirical evidence suggest validity in its value in traditional medicine and potential for modern drug discovery (McINTOCH and Manchew, 1993). Recent research in Indonesia has reported the patenting of phytochemicals isolated from leaf tissue of breadfruit trees for the prevention of stroke and cardiovascular diseases (Sagita, 2009).

Breadfruit (Artocarpus altilis), is a large, round, starchy fruit produced by a tree. It is a species of flowering tree in the Mulberry family of about 50 general and over 1000 species and was introduced into Venezuela in 1789 as a food for the slaves (Rincon, 2004). A. altilis is a long-lived, tropical, evergreen tree and has been cultivated in the Pacific Island for nearly three millennia. The breadfruit is principally grown as a source of carbohydrates and is an important component of agro-forestry systems. Unlike herbaceous starch crops harvested for their vegetative storage tissues, breadfruit is a tree grown for its fruit. In addition to producing abundant, nutritious, tasty fruits, this multipurpose tree provides medicine, construction materials, insect repellent and animal feed (Ragone, 2006). Over thousands of years of cultivation, humans have selected hundreds of cultivars, many of which are seedless and are vegetatively propagated. Seedless cultivars have no seeds but rather contain tiny aborted ovules (Deivanaz and Subhash, 2010). The loss of fertility in breadfruit is due to triploidy (2n=3x=84) (Ragone, 2001). The term ‘breadfruit’ is derived from the Greek words artos (bread) and karpos (fruit). The fruit when cooked or baked effuses a fragrance that is reminiscent of fresh baked bread hence the name (Natural Standard, 2013). It is a common plant in and around town occasionally planted as an ornamental plant in parks and gardens.

Breadfruit has long been a traditional starch crop through Oceania (Melanesia, Micronesia and Polynesia). The prominence of seedless cultivars increases as one travels from New Guinea eastward through Melanesia (where seeded cultivars are common) into Western Polynesia (where few seeded and seedless cultivars are prevalent) and into Eastern Polynesia (where virtually all cultivars are seedless triploids with identical zymotypes). The greatest diversity of seedless cultivars occurs in the eastern Polynesian islands (Society Islands and Marquesas) and Pohnpei and Chuuk in Micronesia (Ragone, 1991). Breadfruit trees grow to a height of about 85 ft (26 m) and bears fruits at seven years and continues to produce for 30 to 40 years, it grows best where annual rainfall is between 1,500 and 3,000 mm (McCoy, 2010). The leaves are thick, large alternate, coriaceous, ovate to oblong, up to 50 cm long, wide and acuminate and are deeply cut into pinnate lobes. All parts of the tree are rich in milky, gummy latex. The fruit is seedless with the surface marked with polygonal faces (Stuartxchange, 2012). The fruits are usually variable in shape, size and surface texture. They are round, oval or oblong ranging from 9 to 20 cm (3.6 – 8 in) wide and more than 30 cm (12 in). The tough skin is composed of five to seven-sided disks. The skin texture varies from smoothly to slightly bumpy or spiny. The colour is light green, yellowish-green or yellow when mature, although one unusual variety has pinkish or orange-brown skin. Research has shown that breadfruit (A. altilis) possesses antibacterial, anti-inflammatory, antidiabetic, antioxidant, and immunomodulatory properties (Kumbhani, 2010). The crop is considered a carbohydrate food source. It is high in starch, and also in fair amounts of vitamin B and C. Studies has also shown presence of anti-nutritional factors such as oxalates, phytic acid, tannin, trypsin inhibitor and haemagglutin (Oladunjoye et al., 2010).

Therefore, this research work is aimed at evaluating the effects of the phytochemical and antimicrobial components of the seedless breadfruit (A. altilis) leaf extracts on some selected microorganisms. The specific objectives of this study include: to produce extracts from the seedless breadfruit leaf using three different extracts (ethanol, hexane and water); to determine the proximate composition and phytochemicals of seedless breadfruit leaf as well as the extracts, and; to evaluate the antimicrobial properties of these leaf extracts on the selected test organisms.

**MATERIALS AND METHODS**

**Plant material**

Fresh seedless breadfruit leaves were obtained from a compound in Ihe-akpu Awka village in Nsukka, Enugu state. Nigeria. The plant was identified by the botanist in Herbarium section, Department of
Test organisms for antimicrobial activities/reagents

Test organisms used for the evaluation of the antimicrobial activity of the leaves were obtained from Pharmaceutical Microbiology Laboratory of the Department of Pharmacy, University of Nigeria, Nsukka. All reagents used were all of analytical quality.

Sample preparation

The fresh seedless breadfruit leaves were sorted, destalked and washed thoroughly in water several times until they become clean and free from debris. The leaves were then drained, spread on trays and oven-dried till the weight became constant. After drying, the dried leaves were milled and then finally stored in an air tight container ready to use (Figure 1).

Extraction of antimicrobial compounds in seedless breadfruit leaves powder

The extraction was carried out using three solvents namely; water (used as control), hexane and ethanol. Cold maceration extraction as described by Anowi et al. (2012) was carried out. One (1) kg of the dried leaf powder was soaked in 2000 ml of analytical ethanol and was allowed to stand for 48 h with intermittent shaking. The suspension was filtered with filter paper and the filtrate (extract) was exposed for evaporation. After complete evaporation of the solvent, the crude ethanolic extract of the plant was recovered and stored at 4°C until used.

Fractionation of the crude extract

Fractionation of the crude extract was carried out adopting the method described by Shahia et al. (2001). Then, 200 g of ethanol crude extract was mixed with 400 g of silica gel until the extract was no longer sticky. The mixture was transferred into a 300 ml quick fit round bottom flask. 1000 ml of n-hexane was added into the bottle and shaken for about 10 min. The fraction was filtered and the mixture was further rinsed three times with 200 ml of the solvent (n-hexane) after which the filtrate became colorless. The filtrate was then oven dried at 40°C. The supernatant rotavapour was used for 10 min at 40°C. After drying, the supernatant was transferred into the 300 ml round bottom flask and treated (fractionated) with 1000 ml of absolute ethanol and also shaken for about 10 min after which it was filtered and the mixture further treated three times with 200 ml of the absolute ethanol. The filtrate was collected and also oven-dried at 40°C. The supernatant was collected and oven-dried at 40°C for about 10 min after which it was transferred into the round bottom flask and then treated with 1000 ml of aqueous solution for about 10 min amidst shaking at intervals. The mixture was filtered and the mixture was further treated three times with 200 ml of aqueous solution. The filtrate was then collected and oven-dried at 40°C. The three different fractions (ethanol, n-hexane, and aqueous fraction) were obtained for evaluation of the antimicrobial activity.

Determination of phytochemicals in seedless breadfruit leaves powder and extracts

Qualitative phytochemical screening of seedless breadfruit leaves powder and its hexane, ethanol, and water soluble extracts was done. Phytochemical screening procedures to be used were adopted from Oloyed (2005). This analysis determined the biologically active compounds that contribute to the flavor, color and other characteristics of seedless breadfruit leaves.

Test for alkaloids

About 0.2 g of each of the samples was boiled with 5 ml of 2% hydrochloric acid on a steam bath for 5 min. The mixtures were allowed to cool and filtered and the filtrates were shared in equal proportions into 3 test tubes and labeled A, B, C. One (1) ml portion of the filtrate was treated with 2 drops of the following reagents, respectively. With Dragendroff's reagent, a red precipitate was shown. With Meyer's reagent, a creamy white colored precipitate indicated the presence of alkaloid (Oloyed, 2005).

Test for flavonoids

About 0.5 g of each of the samples were introduced into 10 ml of ethyl acetate and heated in boiling water for 1 min. The mixtures were then filtered. Four (4) ml of the filtrates was shaken with 1 ml dilute ammonia solution and kept. Formation of a yellow color in the presence of dilute ammonia solution indicated the presence of flavonoids (Oloyed, 2005).

Test for steroids

Approximately 9 ml of ethanol was added into the sample and refluxed for few minutes and then filtered. The filtrate was concentrated to 2.5 ml in a boiling bath, allowed to stand for 1 h and the waxy matter was filtered off. One (1) ml of concentrated sulphuric acid was carefully added to 0.5 ml of the chloroform extracted in a test tube to form a lower layer. A reddish brown interface showed the presence of steroids (Oloyed, 2005).

Test for saponins

About 0.1 g of each of the samples was boiled with 5 ml of distilled water for about 1 h and then filtered. Four (4) ml of the filtrates was shaken with 1 ml dilute ammonia solution and kept. Formation of a yellow color in the presence of dilute ammonia solution indicated the presence of saponins (Oloyed, 2005).
Determination of flavonoids
One gram (1 g) of each of the sample was measured out and macerated with 20 ml of ethyl. The mixture was filtered and then 5 ml of the filtrate was measured into test tubes. To each test tube containing the filtrate, 5 ml of dilute ammonia was added and the mixture was shaken. The upper layers were collected and absorbance was measured at 490 nm.

Determination of glycosides
About 1 g of the samples were weighed out and macerated with 50 ml of distilled water. The mixture was filtered and 1 ml of the filtrate was pipetted into test tubes and 2 ml of saturated picric acid was added. The absorbance was then measured at 568 nm.

Determination of steroids
One gram (1 g) of each of the samples was weighed out and macerated with 10 ml of petroleum ether. The suspension was decanted into a beaker and another 10 ml of petroleum ether was added. The supernatant was decanted and mixed with the first. The mixture was evaporated to dryness and 6 ml of ethanol was added. Approximately 2 ml of the mixture was pipetted into a test tube and the mixture was allowed to stand for 30 min, then the absorbance was read at 550 nm.

Determination of alkaloid
About 1 g of the sample was weighed out and macerated with 20 ml of 20% H₂SO₄ in ethanol (1:1). The mixture was filtered and 1 ml of the filtrate was pipetted into test tubes. Five (5) ml of 40% H₂SO₄ and the content of the test tube were mixed properly. The mixture was allowed to stand for 3 h and the absorbance measured at 568 nm.

Quantitative phytochemical analysis of seedless breadfruit leaves powder and its extracts
The quantitative phytochemical analysis was carried out according to the method of Harborne (1973).

Determination of saponin
One gram (1 g) each of the samples was weighed out and macerated with 10 ml of petroleum ether. The suspension was decanted into a beaker and another 10 ml of petroleum ether was added. The supernatant was decanted and mixed with the first. The mixture was evaporated to dryness and 6 ml of ethanol was added. Approximately 2 ml of the mixture was pipetted into a test tube and the mixture was allowed to stand for 30 min, then the absorbance was read at 550 nm.

Determination of reducing sugar
About 1 g of the sample was weighed out and macerated with 2.5 ml of 15% lead acetate was added and the mixture was filtered. Two (2) ml of chloroform was added to the filtrate and the mixture was shaken vigorously. The lower layer was collected and evaporated to dryness. 3 ml of glacial acetic acid was added and the 0.1 ml of 5% ferric chloride and 0.25 ml concentrated H₂SO₄ were added and the mixture was shaken. The mixture was then left to stand in the dark for 2 h and absorbance was measured at 530 nm.

Determination of flavonoids
One gram (1 g) of each of the sample was measured out and macerated with 5 ml of 45% ethanol and boiled for 5 min. The mixtures were cooled and filtered. Then, 3 drops of lead subacetate was added into 1 ml of each filtrate. A gelatinous precipitate was observed indicating the presence of flavonoids (Oloyed, 2005).

Determination of glycosides
Two grams (2 g) of each of the samples was mixed with 30 ml of distilled water and they were heated for 5 min on a water bath, filtered and used as follows: into 5 ml of the filtrates was added 0.2 ml of Fehling solution A and Fehling solution B until they turn alkaline and heated in a water bath for 2 min. A light blue coloration was observed (instead of brick red precipitate) which indicates the presence of glycosides (Oloyed, 2005).

Determination of carbohydrates (Molisch test)
About 0.1 g of each of the sample was boiled with 2 ml of distilled water and filtered. To the filtrates, few drops of naphthol solution in ethanol (Molisch’s reagent) were added. Concentrated sulphuric acid in a Pasteur pipette was then gently poured down the side of each of the test tubes to form a lower layer. A purple interfacial ring indicated the presence of carbohydrates (Oloyed, 2005).

Test for carbohydrates (Molisch test)
Approximately 1 g each of the samples were weighed out and macerated with 20 ml of distilled water. The mixture was filtered and 2 ml of the filtrate was measured into test tubes. To each test tube containing the filtrate, 5 ml of dilute ammonia was added and the mixture was shaken. The upper layers were collected and absorbance was measured at 550 nm.
gram of the test sample was weighed out into a conical flask and 10 ml of water was added. The mixture was shaken at 5 min interval for 30 min and filtered to get the extract. About 2.5 ml of the supernatant was transferred into a 50 ml flask. 1 ml of Follin-Denis reagent was then added in to the flask followed by 2.5 ml of saturated Na₂CO₃ solution. The absorbance was read at 720 nm spectrophotometrically after 90 min and incubated at room temperature.

\[
\text{Tannin (\%) = } \frac{\text{An} \times \text{C} \times 100 \times \text{Vf}}{\text{As} \times 1 \times \text{Va}}
\]

Where, \(\text{As} = \) absorbance of test sample; \(\text{As} = \) absorbance of standard solution; \(\text{C} = \) Concentration of standard solution; \(\text{W} = \) Weight of sample used; \(\text{Vf} = \) Total volume of extract; \(\text{Va} = \) Volume of extract analyzed.

**Determination of phytate**

Phytate content was determined according to the method of Pearson (1976). Approximately 0.5 g of sample was weighed out into a 500 ml flat bottom flask, placed in a shaker and extracted with 100 ml of 2.4% hydrochloric acid for one hour at 25°C. After this, the mixture was decanted and filtered. 5 ml of the filtrate was diluted to 25 ml with distilled water and 10 ml of it was collected into a flask and 15 ml of 0.1 M sodium chloride was added to it. The mixture was passed through No.1 Whatman filter paper to elute inorganic phosphorus and 15 ml of 0.7 M sodium chloride was to elute phytate. The absorbance was read at 520 nm.

**Determination of oxalate**

The determination of oxalates was carried out by the titration method of AOAC (2010). Two grams (2 g) of sample was suspended in a mixture of 190 ml of distilled water and 10 ml of HCl in a 250 ml volumetric flask and digested for one hour at 100°C, cooled and made up to 100 ml with distilled water. The digest was then filtered through Whatman No. 1 Filter paper using a suction pump. A duplicate proportion of 125 ml of the filtrate was measured into 250 ml beaker and four (4) drops of methyl red indicator added into each beaker. Concentrated NH₄OH or NH₃ solution was added drop wise until the test solution changed from its salmon pink color to a faint yellow colour (pH 4 - 4.5). Each portion was heated up to 90°C and 10 ml of 5% CaCl₂ was added while being stirred constantly. After heating, it was cooled and left over night at 5°C. The supernatant was decanted and the precipitate completely distilled in 10 ml of 20% (v/v) H₂SO₄ solution. At this point, the filtrate resulting from digestion of 2 g of the sample was combined and made up to 300 ml. Aliquots (125 ml) of the filtrate was heated until near boiling and then titrated against 0.05 M standard KMnO₄ solution to a faint pink color. Oxalic acid content was calculated using the formula:

\[
\text{Oxalate (mg/100 g)} = \frac{T \times (\text{Vme}) \times 10^5}{\text{ME} \times \text{Mf}}
\]

Where; \(T = \) Volume of KMnO₄; \(\text{Vme} = \) Volume – mass equivalent (1 ml of 0.05 M KMnO₄ solution is equivalent to 0.00225 g anhydrous oxalic acid); \(\text{Df} = \) The dilution factor; \(\text{ME} = \) molar equivalent of KMnO₄ in oxalic acid (KMnO₄ redox reaction is 5); \(\text{Mf} = \) mass of sample used.

**Determination of cyanide**

Cyanide content was determined according to the method of AOAC (2010). Five grams of the sample was weighed into a conical flask and 50 ml of distilled water was added to it and the solution was allowed to stand overnight and then filtered. 1 ml of the sample filtrate was measured into a test tube and 4 ml of alkaline picrate was added and it was allowed to stand for 5 min. The absorbance was read at 490 nm. The reading was taken with the reagent blank at zero.

**Determination of antimicrobial properties of seedless breadfruit leaves powder**

The antimicrobial properties of the seedless breadfruit leaves powder were determined using two different methods: Agar Well Diffusion technique described by Wan et al. (1998) and Agar Dilution technique as described by Wiegand et al. (2008).

**Test microorganisms used**

The test microorganisms used for the evaluation of the antimicrobial potency of the leaf extracts were clinical isolates of *Bacillus subtilis*, *Klebsiella pneumonia*, *Shigella dysenteriae*, *Proteus mirabilis*, *Staphylococcus aureus*, *Echerichia coli*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Candida albicans* and *Aspergillus niger*.

**Standardization of the test organisms**

The test microorganisms used were standardized using 0.5% MacFaland Turbid Equivalent. A 1% v/v solution of sulphuric acid was prepared by adding 1 ml of concentrated sulphuric acid to 99 ml of water and 1% w/v solution of barium chloride was prepared by dissolving 0.5 MacFarland equivalent standard prepared by adding 0.6 ml of barium chloride solution to 99.4 ml of sulphuric acid solution.

**Preparation of nutrient agar**

The media was prepared following the manufacturer’s instruction (Oxoid, 1982). 28 g of dehydrated nutrient agar powder was suspended in 1000 ml of distilled water and was allowed to soak. The suspension was then dissolved by heating in a water bath at 100°C. Then, 20 ml volume each of the molten agar was dispensed into bijou bottles and autoclaved at 121°C for 15 min. The sterile molten nutrient agar was allowed to cool to 40°C.

**Preparation of sabouraud dextrose agar (SDA)**

The media were prepared according to the manufacturer’s instructions. SDA powder (63 g) was suspended in 1000 ml of distilled and allowed to soak. The suspension was then dispensed into autoclave at 121°C for 15 min. It was then allowed to cool to 40°C according to Oxoid (1982).

**Preparation of agar slants**

A 2.8 g quantity of nutrient agar and 3.8 g of Mueller Hinton agar was each dissolved in 100 ml of distilled water by heating over flame. 5 ml volume each of the molten agar was then dispensed into macAnthony bottles and sterilized at 121°C for 15 min. The sterile molten agar was kept in a slanting position to gel.

**Preparation of culture**

A 3 ml volume of sterile nutrient broth was prepared and the test
Table 1. Qualitative phytochemical composition of seedless breadfruit leaves using ethanol, n-hexane and water extracts.

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>Red sug</th>
<th>Sap</th>
<th>Alk</th>
<th>Sol CHO</th>
<th>Steroid</th>
<th>HCN</th>
<th>Gly</th>
<th>Flav</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water extract</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>Ethanol</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>n-hexane</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>SBLP</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>+++</td>
</tr>
</tbody>
</table>

Key; + = present in low concentration; ++ = present in moderate concentration; +++ = present in high concentration; Red sug = Reducing sugar; Sap = Saponin; Alk = Alkaloid; Sol CHO = Soluble carbohydrate; HCN = Hydrogen cyanide; Gly = Glycoside; Flav = Flavonoid; SBLP = Seedless breadfruit leaves powder.

organism was inoculated into the nutrient broth aseptically under flame at 37°C for 24 h.

Agar well diffusion technique

The molten agar on cooling to about 40-47°C after sterilization was aseptically poured into sterile plates. Then, 0.1 ml of sterile water and 0.1 ml of the diluents was transferred into the center of a sterile Petri-dish. Also, 20 ml of the sterile molten agar was then added and the dish was swirled to mix its content. The plates were allowed to stand for 30 min so that solidification can take place. Six holes were then bored on each plate using a 6 mm diameter cork borer. Each hole was for a concentration of the breadfruit leave extract. The holes were labeled 1 to 6 to representing 100, 50, 25, 12, 6.25 and 3.125 mg/ml concentration of the three breadfruit leave extracts, respectively. Four drops of each concentration was dropped in corresponding holes and the plates were then kept undisturbed for 15 min so as to allow the extracts to diffuse properly and dry to a considerable level before incubation. The measurements (in millilitres) of the zone of inhibitions of the extracts against the test organisms were taken and recorded.

Determination of minimal inhibitory concentration (MIC) using agar dilution technique

The MIC of the extracts of the extracts was determined using agar dilution technique. A 50 g of the extract was dissolved in 10 ml of sterile water, thus obtaining 50 mg/ml stock solution, using the arithmetic method of dilution:

\[ C_1V_1 = C_2V_2. \]

Where; \( C_1 \) = initial concentration; \( C_2 \) = final concentration; \( V_1 \) = initial volume; \( V_2 \) = final volume.

Concentrations (10, 9, 8, 7, 6, 5, 4, 3, 2 and 1 mg/ml) were obtained from the stock by seeding (4.0, 3.6, 3.2, 2.8, 2.4, 2.0, 1.6, 1.2, 0.8 and 0.4 ml) aliquots of the stock in their corresponding volumes (16, 16.4, 16.8, 17.2, 17.6, 18.0, 18.4, 18.8, 19.2 and 19.6 ml) of pre-sterilized Mueller Hinton agar at a temperature of 40°C. The media were then poured into sterile Petri dishes and allowed to solidify. The surfaces of the media were allowed to dry and streaked with 24 h old cultures of the test microorganisms. The plates were later incubated in an incubator at 37°C for 24 h, observations were made and it was further incubated for another 24 h, after which they were examined for the presence or absence of growth. The MIC was taken as the lowest concentration that prevented microbial growth.

Data analysis and experimental design

The proximate and anti-nutrient composition was analyzed using one-way analysis of variance (ANOVA) based on completely randomized design; mean separation will by Duncan’s New Multiple Range Test. The antimicrobial testing was statistically analyzed using a 3-factorial or split plot in completely randomized design (CRD).

RESULTS

Phytochemical composition of seedless breadfruit leaves powder (SBLP) using ethanol, n-hexane and water extracts

Qualitative and quantitative phytochemical composition of seedless breadfruit leaves powder and ethanol, n-hexane and water extracts are shown in Tables 1 and 2. The result confirmed the presence of reducing sugar, saponins, alkaloids, soluble carbohydrate, steroid, hydrogen cyanide, glycosides, flavonoids and tannins (Tables 1 and 2, respectively).

The seedless breadfruit leaves had the highest amount of reducing sugar (2.15±0.005 mg/100g) while the n-hexane extract contained the highest amount of reducing sugar out of the three extracts. Ethanol extract was observed to contain 1.77 ± 0.00 mg/100g of reducing and finally the water extract had the least reducing sugar value of 1.45 ± 0.00 mg/100g.

The amount of saponin was observed to be the highest in the seedless breadfruit leaves with a value of 2.37 ± 0.01 mg/100g. Water extract had the least amount of saponin among all the extracts with a value of 2.20±0.005 mg/100g. The ethanol extract had a value of 2.21±0.00 mg/100g of saponin while the n-hexane extract had a value of 2.22±0.00 mg/100g.

The amount of saponin was observed to be the highest in the seedless breadfruit leaves with a value of 2.37 ± 0.01 mg/100g. Water extract had the least amount of saponin among all the extracts with a value of 2.20±0.005 mg/100g. The ethanol extract had a value of 2.21±0.00 mg/100g of saponin while the n-hexane extract had a value of 2.22±0.00 mg/100g.

The seedless breadfruit leaves had the highest amount of alkaloid with a value of 3.35±0.02 mg/100g. The ethanol extract had the highest alkaloid content (2.99±0.05 mg/100 g) out of the three extracts used. The ethanol extract contained 2.52±0.00 mg/100g while the water extract contained 2.12±0.00 mg/100 mg.

The steroid content of the seedless breadfruit leaves
was 1.94±0.005 mg/100 g and this value was higher than the values recorded in the other samples. Water extract had the highest steroid content with a value of 1.92±0.00 mg/100 g of all the extracts. Ethanol extract had a steroid value of 1.72±0.00 mg/100 g while n-hexane had a value of 1.89±0.01 mg/100 g.

The seedless breadfruit leaves had the highest glycol-side content (2.83±0.005 mg/100 g). The water extract had a glycoside content of 2.37±0.002 mg/100 g, hence contained the highest amount of glycosides as compared to the other extracts. This was followed by ethyl alcohol extract which had a value of 2.32±0.01 mg/100 g and the n-hexane extract which had a glycoside content value of 2.25±0.01 mg/100 g.

The seedless breadfruit leaves had the highest amount of soluble carbohydrate with a value of 0.86±0.02 mg/100 g while the water extract had the highest amount of soluble carbohydrate out of the extracts with a value of 0.79±0.005 mg/100 g. This was followed by the n-hexane extract which had a value of 0.71±0.002 mg/100 g and the ethanol extract whose value was 0.69±0.05 mg/100 g.

The seedless breadfruit leaves had the highest content of flavonoids (3.94±0.01 mg/100 g) among all the samples. This was followed by the value of 3.79±0.01 mg/100g recorded for ethanol extract. The n-hexane extract had a value of 3.31±0.00 mg/100g and the water extract had the least value of 3.11±0.01 mg/100 g.

**Antimicrobial activity of seedless breadfruit**

**Antimicrobial activity of seedless breadfruit leaves extracts using the agar diffusion technique (ADT)**

From the Tables 5 - 7, it is observed that the ethanol extract has the highest inhibitory activity against the microorganisms as compared to the other solvents used (n-hexane and water). At concentrations of 10, 9, 8, 7 and 6 (mg/ml), it showed total inhibitory activity against all the test microorganisms used. However, the minimal inhibitory concentration (MIC) for *Pseudomonas aeruginosa* was observed at 6 mg/ml, followed by *Candida albicans* *Aspergillus niger*, *Shigella dysentariae* and *Klebsiella pneumonia* at concentration of 5 mg/ml and then *B. subtilis*, *P. mirabilis*, and *E. coli* at concentration level of 3 mg/ml. Finally, all the test organisms showed activity at the concentration level of 1 mg/ml. The n-hexane fraction had a minimal inhibitory concentration of 8 mg/ml for *P. aeruginosa* and *A. niger*. At the concentration level of 3 mg/ml, it was observed that all the test organisms became resistant. The aqueous fraction had no inhibitory activity against all the test organisms at all the concentration levels.

**Anti-nutrient content of seedless breadfruit leaves (SBLP) powder and its extracts**

Test for anti-nutrient was carried out on the seedless breadfruit leaves and the extracts in order to determine the anti-nutrient present and also to quantify the level at which they are present. The analysis confirmed the presence of phytate, oxalate, cyanide and tannin. Table 8 shows the anti-nutrient content of the seedless breadfruit leaves powder and the water, n-hexane and ethanol extracts.

The phytate content of the seedless breadfruit leaves was the highest (1.55±0.00 mg/100 g). The water extract had the highest level of phytate out of all the extracts with the other extracts used for the analysis. The minimum inhibition concentration of the ethanol extract against *B. subtilis* is shown in Table 4 and it was deduced to be 3.98 mg/ml (Figure 2).
Table 3. Antimicrobial activity of seedless breadfruit leaf extracts (ethanol, n-hexane and water extracts) using the agar well diffusion technique

<table>
<thead>
<tr>
<th>Extract conc. (mg/ml)</th>
<th>B. sub</th>
<th>Shig</th>
<th>Kleb.</th>
<th>P. mir</th>
<th>S. aureus</th>
<th>E. coli</th>
<th>Sal</th>
<th>C. alb.</th>
<th>A. niger</th>
<th>Ps.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>16.90 ±0.14</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
</tr>
<tr>
<td>50</td>
<td>14.15 ±0.21</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
</tr>
<tr>
<td>25</td>
<td>11.20 ±0.28</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
</tr>
<tr>
<td>12.5</td>
<td>9.00 ±0.00</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
</tr>
<tr>
<td>6.25</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
</tr>
<tr>
<td>n-hexane</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
</tr>
<tr>
<td>50</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
</tr>
<tr>
<td>25</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
</tr>
<tr>
<td>12.5</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
</tr>
<tr>
<td>6.25</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
</tr>
<tr>
<td>Aqueous</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
</tr>
<tr>
<td>50</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
</tr>
<tr>
<td>25</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
</tr>
<tr>
<td>12.5</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
</tr>
<tr>
<td>6.25</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
</tr>
</tbody>
</table>

Values are means ± standard deviation of duplicate readings. Samples having the same superscript within the column are not significantly different (p<0.05). B. sub = Bacillus subtilis; Kleb = Klebsiella pneumonia; Shig = Shigella dysenteriae; P. mir = Proteus mirabilis; S. aureus = Staphylococcus aureus; E. coli = Escherichia coli; Sal = Salmonella typhi; C. alb. = Candida albicans; A. niger = Aspergillus niger; Ps = Pseudomonas aeruginosa; NI = No Inhibition; Conc = Concentration.

Table 4. Determination of the minimum inhibition concentration (MIC) of the ethanol extract against Bacillus subtilis.

<table>
<thead>
<tr>
<th>Concentrations (mg/ml)</th>
<th>IZD (mm)</th>
<th>Log of Conc (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>17</td>
<td>2.0</td>
</tr>
<tr>
<td>50</td>
<td>14</td>
<td>1.7</td>
</tr>
<tr>
<td>25</td>
<td>11</td>
<td>1.4</td>
</tr>
<tr>
<td>12.5</td>
<td>9</td>
<td>1.1</td>
</tr>
<tr>
<td>6.25</td>
<td>-</td>
<td>0.8</td>
</tr>
</tbody>
</table>

Key: IZD = Inhibition zone diameter; Conc = Concentration.

Figure 2. The minimum inhibition concentration of the ethanol extract against B. subtilis. Taking the antilog of 0.6; Antilog of 0.6 = 3.98. Therefore the minimum inhibition concentration of the ethanol extract against B. subtilis is 3.98 mg/ml.
Table 5. Antimicrobial activity of ethanol extract from seedless breadfruit leaves using agar dilution technique.

<table>
<thead>
<tr>
<th>Conc (mg/ml)</th>
<th>B. sub</th>
<th>Shig</th>
<th>Kleb</th>
<th>P. mir</th>
<th>S. aureus</th>
<th>E. coli</th>
<th>C. alb</th>
<th>A. niger</th>
<th>Ps</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>1</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Key: + = Growth; - = No growth
B. sub = Bacillus subtilis; Kleb = Klebsiella pneumonia; Shig = Shigella dysenteriae; P. mir = Proteus mirabilis; S. aureus = Staphylococcus aureus; E. coli = Escherichia coli; Sal = Salmonella typhi; C. alb = Candida albicans; A. niger = Aspergillus niger; Ps = Pseudomonas aeruginosa; Conc = Concentration

Table 6. Antimicrobial activity of n-hexane extract of seedless breadfruit leaves using agar dilution technique.

<table>
<thead>
<tr>
<th>Conc (mg/ml)</th>
<th>B. sub</th>
<th>Shig</th>
<th>Kleb</th>
<th>P. mir</th>
<th>S. aureus</th>
<th>E. coli</th>
<th>Sal</th>
<th>C. alb</th>
<th>A. niger</th>
<th>Ps</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>1</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Keys; + = Growth; - = No growth
B. sub = Bacillus subtilis; Kleb = Klebsiella pneumonia; Shig = Shigella dysenteriae; P. mir = Proteus mirabilis; S. aureus = Staphylococcus aureus; E. coli = Escherichia coli; Sal = Salmonella typhi; C. alb = Candida albicans; A. niger = Aspergillus niger; Ps = Pseudomonas aeruginosa; Conc = concentration.

Table 7. Antimicrobial activity of aqueous (water) extract of seedless breadfruit leaves using agar dilution technique.

<table>
<thead>
<tr>
<th>Conc (mg/ml)</th>
<th>B. sub</th>
<th>Shig</th>
<th>Kleb</th>
<th>P. mir</th>
<th>S. aureus</th>
<th>E. coli</th>
<th>Sal</th>
<th>C. alb</th>
<th>A. niger</th>
<th>Ps</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>1</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Key; + = Growth; - = No growth
B. sub = Bacillus subtilis; Shig = Shigella; Kleb = Klebsiella pneumonia; P. mir = Proteus mirabilis; S. aureus = Staphylococcus aureus; E. coli = Escherichia coli; Sal = Salmonella typhi; C. alb = Candida albicans; A. niger = Aspergillus niger; Ps = Pseudomonas aeruginosa; Conc = concentration.
Table 8. Anti-nutrient content of seedless breadfruit leaves powder (SBLP) and its extracts.

<table>
<thead>
<tr>
<th>Anti-nutrients</th>
<th>Phytate (mg/100 g)</th>
<th>Oxalate (mg/100 g)</th>
<th>Tannin (mg/100 g)</th>
<th>Cyanide (mg/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water extract</td>
<td>1.46±0.00</td>
<td>2.12±0.00</td>
<td>4.95±0.00</td>
<td>0.05±0.00</td>
</tr>
<tr>
<td>n-hexane</td>
<td>0.72±0.00</td>
<td>0.59±0.00</td>
<td>4.22±0.00</td>
<td>0.04±0.01</td>
</tr>
<tr>
<td>Ethanol</td>
<td>0.69±0.00</td>
<td>0.57±0.00</td>
<td>4.63±0.00</td>
<td>0.04±0.01</td>
</tr>
<tr>
<td>SBLP</td>
<td>1.55±0.00</td>
<td>2.20±0.01</td>
<td>5.75±0.00</td>
<td>0.06±0.00</td>
</tr>
</tbody>
</table>

Values are mean ± standard mean deviation of triplicate readings. Values having the same superscript on the same column are not significantly different (p<0.05)
SBLP = Seedless breadfruit leaves powder

a value of 1.46±0.00 mg/100 g. This was followed by the n-hexane extract with a value of 0.72±0.00 mg/100 g and finally the ethanol having a value of 0.69±0.00 mg/100 g. The oxalate content of the seedless breadfruit leaves was highest with a value of 2.20±0.01 mg/100 g. Water extract was observed to have the highest level of oxalate with a value of 2.12±0.00 mg/100 g out of all the extracts. This was followed by the n-hexane extract having a value of 0.59±0.00 mg/100 g and the ethanol extract having the least oxalate content value of 0.57±0.00 mg/100 g.

The level of tannin observed in the seedless breadfruit leaves was highest with a value of 5.75±0.00 mg/100 g. The n-hexane had the least concentration value of 4.22±0.00 mg/100 g. Ethanol extract had a value of 4.63±0.00 mg/100 g while water extract had a tannin content value of 4.22±0.00 mg/100 g.

The cyanide content of the seedless breadfruit leaves and its extracts were observed to be low. The cyanide content of the seedless breadfruit leaves was observed to be 0.06±0.00 mg/100 g. This was higher than the values observed for all the extracts. The water extract had the highest cyanide content out of all the extracts with a value of 0.05±0.00 mg/100 g. The ethanol and n-hexane extract both had a cyanide content value of 0.04±0.01 mg/100 g.

DISCUSSION
Phytochemical composition of seedless breadfruit leaves powder (SBLP) using ethanol, n-hexane and water extracts

Earlier reports showed the presence of these bioactive components in the Moraceae family (Usman et al., 2000). Qualitative analysis indicated the presence of the phytochemicals and the level at which they are present in the various samples while quantitative analysis quantified the phytochemicals already identified in the qualitative analysis.

The amount of reducing sugar found in seedless breadfruit leaves powder is lower than the values reported by Salam et al. (2011) for some leafy plants such as Chenopodium album and Stellaria media (8.15 mg/100 g and 22.65 mg/100 g). It is also lower than the range of values obtained for reducing sugar (5.90±0.92 mg/100 g, 4.94±0.73 mg/100 g, 8.11±1.10 mg/100 g, 6.62±0.97 mg/100 g) reported by Mohammed et al. (2010) for Morus alba, Morus nigra, Morus laevigata (white) and Morus laevigata (black), respectively.

The saponin content obtained in seedless breadfruit leaves agreed with the saponin value of 2.2±0.00 mg/100 g of Vernonia amygdalina (bitter leaf) reported by Nwaoguikpe (2010). The results obtained however contrasted with the values reported for various leafy vegetables consumed in Nigeria namely V. amygdalina (bitterleaf) (0.12±0.02mg/100 g), Corchorus olitorius (ewedu) (0.13±0.01 mg/100 g), Talinum triangulare (water leaf) (0.12±0.01 mg/100 g), Solanum monacarnum (garden egg) (0.28±0.01 mg/100 g), Telfaririn occidentalis (pumpkin leaf) (0.14±0.02 mg/100 g), Abiemorchus esculentus (okro) (0.24±0.13 mg/100 g), Manihot esculenta (cassava leaves) (0.25±0.11 mg/100 g) and Amaranthus hybridus (green leaves) (0.20±0.01 mg/100 g) as reported by Agbaire (2012).

The alkaloid values observed in all the samples were higher than the range of values of 0.62±0.46 mg/100 g, 0.88±0.46 mg/100 g, 0.99±0.55 mg/100 g, 0.81±0.47 mg/100 g, 1.81±0.34 mg/100 g, 1.19±0.89 mg/100 g, 1.68±1.28 mg/100 g and 1.28±0.84 mg/100 g for “Utazi”, “Nturukpa”, “Nhanwu”, “Ugu”, “Onogbo”, “Oha”, “Ahihara” and “Okazi”, respectively reported by Onyeka and Nwambeke (2007).

The steroid content obtained for all the extracts were higher than the range of values (0.07±0.01 mg/100 g to 0.27±0.01 mg/100 g) for common leafy vegetables consumed in the Eastern part of Nigeria as reported by Onyeka and Nwambeke (2007).

The flavonoid values obtained for all the samples was higher than the range of values (0.03±0.01 mg/100 g to 0.22±0.06 mg/100 g) reported by Onyeka and Nwamkwe (2007) for common leaves such as “utazi”, “nturukpa”, “nhanwu”, “ugu”, “onogbu”, “oha”, “aahihara” and “okazi” consumed in the Eastern part of Nigeria.

The exhibited antimicrobial properties of the A. altitlis could be attributed to the presence of bioactive components such as saponins, steroids, alkaloids, tannins and flavonoids in the plant. Tomas-Barberan et al. (1990) reported that the presence of these compounds might have potentiated or complimented the saponins in the
antimicrobial activities in the plant where both alkaloids and alkaloids were present. These bioactive components have also been reported to have antimicrobial and medicinal effect medicinal effect (Iniaghe et al., 2009).

**Antimicrobial activity of seedless breadfruit**

The antimicrobial activity of seedless breadfruit using agar diffusion technique corresponds with the zone of inhibition range of 16.5 to 9 mm reported by Fakruddin et al. (2012) against Gram positive *B. subtilis* using the same solvent (ethanol). Antimicrobial activity of seedless breadfruit leaves extracts using agar dilution technique showed that the ethanol extract had a higher inhibitory activity against the test organisms used for the analysis than the n-hexane and water extracts as compared to the two methods used for the antimicrobial analysis, agar dilution technique proved to be a better method as compared to the agar diffusion technique in the present study. It was observed to produce inhibitory activity against all the test organisms used at concentration levels as low as 6 and 8 mg/ml for the ethanol and n-hexane fractions respectively. Whereas in the agar diffusion technique, inhibitory activity was very low with only the ethanol extract showing a degree of inhibition against *B. subtilis* at concentration level of 100, 50, 25 and 12.5 mg/ml. The low inhibitory activity of the extracts used in the agar diffusion technique could probably be as a result of the very low and diffusibility of the bioactive components present in the leaf.

Also, it could be observed that the ethanol extract had a higher inhibitory activity against the test organisms used for the analysis than the n-hexane and water extracts and this was in agreement with the report by Dash et al. (2011) that ethanol extracts of *Centella asiatica* had higher antimicrobial activities than n-hexane and water extracts. The results obtained also agrees with that of Vasugi et al. (2012) who reported that methanol and ethanol extracts of *Artocarpus altillis* showed high significant antibacterial and antifungal activities than aqueous extract. In the present study, with reference to the agar dilution technique, the ethanol and n-hexane extracts of the *A. altillis* leaves exhibited high inhibitory effect on all the test microorganisms used in the study and thus justifies the reports of its use by local communities for managing microbial infections in wounds, tooth ache and skin infections, ear infections and some intestinal infections (Ragone, 1997).

The antimicrobial study showed that the bacterial strains tested were more susceptible to the ethanol and n-hexane extract than the fungal strains. *P. aeruginosa* and *A. niger* which are both moulds were the first microorganisms to shows signs of resistance in the ethanol and n-hexane extract. The result was in agreement with the report of Lopolito et al. (2007) that moulds are more resistant than yeast and considerably more resistant than vegetative bacteria. It was also observed that the Gram-positive bacteria (*B. subtilis* and *S. aureus*) were the most inhibited of the test microorganisms in the ethanol and n-hexane extract. This result agreed with the report of Staden and Rube (1997) that Gram-positive bacteria are better inhibited than Gram-positive bacteria. The antimicrobial activities of the *A. altillis* could be attributed to the presence of bioactive compounds.

**Anti-nutrient content of seedless breadfruit leaves (SBLP) powder and its extracts**

The phytate content of all the samples were observed to be lower than those reported for some leafy edible vegetables of Southern Nigeria. The values include 13.00±0.40 mg/100 g, 6.11±0.22 mg/100 g, 5.39±0.22 mg/100 g, 4.12±0.45 mg/100 g for scent leaf, lemon grass, cassava leaf and green leaf, respectively (Agbaire, 2012).

In contrast to the test raw material, the oxalate range of values (0.09±0.01 mg/100 g to 1.00±0.22 mg/100 g) was reported by Agbaire (2012) for common leafy vegetables consumed in Southern Nigeria and observed to be lower than the value obtained for the seedless breadfruit leaves.

The tannin content obtained for all the samples were observed to be higher than those reported by Agbaire (2012) for some selected leafy vegetable. These include 0.26±0.03 mg/100 g (scent leaf), 0.12±0.02 mg/100 g (lemon grass), 0.20±0.01 mg/100 g (cassava leaf), and 0.19±0.01 mg/100 g (green leaf). Also, the values were found to contrast with the values reported by Onyeka and Nwambeke (2007) as they are also found to be higher than the range of values (0.08±0.05 mg/100 g to 1.68±1.28 mg/100 g) for some commonly consumed leafy vegetables in the Eastern part of Nigeria.

In contrast to the obtained cyanide content, Okoli et al. (2003) reported that the amount of hydrogen cyanide found in bitter leaf was 6.40 mg/100 g and this is higher than the values reported for both the seedless breadfruit powder and its extracts. The values were however in line with the values reported by Agbaire (2012) for selected leaves in Southern Nigeria, these include 0.13±0.03 mg/100 g (scent leaf), 0.23±0.01 mg/100 g (cassava leaf), 0.10±0.01 mg/100 g (green leaf) but was found to be lower than the 6.09±0.01 mg/100 g value reported for lemon grass.

From the results obtained on anti-nutrient composition, it is observed that the seedless breadfruit leaves contained the highest amount of anti-nutrients identified in the samples, while the ethanol extract contained the least anti-nutrients of all the samples. This could probably be due to the fact that the identified anti-nutrients in the leaves had the least solubility in ethanol leading to a lower yield as compared to the seedless breadfruit leaves in its intact state.

Earlier reports indicated that tannins act on the membrane of the microorganisms and shows the antibacterial
activity by precipitating the microbial protein (Rajesh et al., 2010; Qudsia et al. 2009; Hisonari et al., 2001) and flavonoids exhibit antibacterial activity by inhibition of the nucleic acid synthesis and cytoplasmic membrane function of the pathogen. Saponins also show antifungal, antibacterial and antiprotozoan effects (Morisaki et al., 1995). Studies have also shown that phytochemicals are present in all plant parts and that these present differ according to the type of extracting solvent used (Tijjani et al., 2009) hence this account for the differences observed in the content of phytochemicals in the ethanol, n-hexane and water extracts. The results from Table 8 confirmed the presence of anti-nutrients such as tannins, phytate, cyanide and oxalate. Phytates and oxalates are reported to bind to minerals such as calcium, magnesium, copper, iron and zinc hence making these minerals unavailable for absorption in the intestine. Tannins chelate metals such as iron and zinc and reduce the absorption of these nutrients. They also inhibit digestive enzymes and precipitate proteins (Bruno, 2010). However, processing such as cooking can destroy or reduce the effects of these anti-nutrients permitting the body to absorb the nutrient from them (Decker, 2011).

Conclusion

This research confirmed that the seedless breadfruit leaves extracts possess antimicrobial activity. The antimicrobial property of the seedless breadfruit leaves extracts can be attributed to the phytochemicals identified in the samples and the levels at which they were found to occur. Out of the two methods of antimicrobial analyses used (agar diffusion technique and agar dilution technique) which were compared in order to test for the antimicrobial properties of the samples, it was observed that the agar dilution technique produced a more effective antimicrobial activity as compared to the agar diffusion method. For the two methods compared, the ethanol extract was observed to have the highest inhibition against the microorganisms tested. This was followed by the n-hexane extract and finally the water extract had the least inhibition against the test organisms. With the low cost of the plant and its abundance especially in the rural parts, the leaves can be readily exploited for its potential in the production of medicinal herbs and food preservatives especially for the poor people in the rural areas where modern facilities are not readily available.

With the continuous development of resistant microorganisms to already developed antibiotics overtime, it is recommended that the use of natural extracts of the seedless breadfruit should be highly advocated since they contain chemicals which are similar to anti-bacterial agent and are also considered safer than the use of synthetic antibiotic drugs which sometimes may lead to one or more complications in the body. It is also recommended that since the ethanol extract had the highest inhibition against the test organisms used, research should be carried out using other solvents in the same family with ethanol such as methanol, pro-panol, butanol and acetone in order to ascertain the level of their potency against pathogenic and spoilage organisms. Finally, it is recommended that more research work should be carried out on the seedless breadfruit leaves extracts as the inert potentials of the plant are not exhaustive in this research.

Conflict of Interests

The authors have not declared any conflict of interests.

REFERENCES


