Evaluation of Phytochemical Composition and Antimicrobial Activity of Sweet Potato (Ipomoea batatas) Leaf

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Abstract: Sweet Potato Leave (SPL) powder and its peptone, ethanol and water extracts were subjected to proximate, phytochemical, anti-nutrient and antimicrobial analysis. The results of proximate analysis revealed the presence of high carbohydrate (43.97±0.019-56.47±0.009%) and crude protein (16.497±0.003-28.163±0.003%). The sweet potato leaves powder and the extracts also contain moisture (3.603±0.004-16.921±0.008%), ash (5.963±0.004-10.414±0.007%), fat (0.208±0.007-14.284±0.002%) and fibre (0.244±0.003-2.764±0.003%), respectively. The phytochemical screening revealed the presence of bioactive compounds in the SPL powder and its extracts namely; tannins, alkaloids, steroids, glycosides, saponins, flavonoids and soluble carbohydrates. The SPL powder had the highest content of these bioactive compounds with alkaloid having the highest value of 3.784±0.007mg/100g, followed by flavonoid (3.357±0.006mg/100g) and steroid had the lowest value (0.393±0.003mg/100g). The extracts had highest content of flavonoid ranging between 2.762±0.008 and 3.355±0.004 mg/100g and least content of steroid ranging between 0.375±0.002 and 0.375±0.003mg/100g. The anti-nutrient analysis indicated the content of very high amount of oxalate ranging between 1.684±0.004 and 6.254±0.004% in the four samples. They contained lower amounts of phytate (3.897±0.003 to 5.933±0.003mg/100g), cyanide (0.353±0.003 to 1.444±0.004mg/100g) and tannin (5.527±0.002 to 9.010±0.002mg/100g). The antimicrobial activity of the peptone, water and ethanol extracts was tested against Escherichia coli, Salmonella typhi, Staphylococcus aureus, Aspergillus niger, Penicillium spp., Pseudomonas aeruginosa and Klebsiella pneumonia. The water extract showed the best antimicrobial activity by inhibiting the growth of all the organisms except E. coli and Penicillium spp at different concentrations of the extract. While the ethanol and peptone extracts only had a minimal activity against Salmonella typhi.

Key words: Anti-nutrients, antimicrobial activity, bioactive compounds ethanol extract, peptone extracts, phytochemical screening, sweet potato leave powder water extract

INTRODUCTION
Sweet potato (Ipomoea batatas) is a vegetable from the tropics that is full of nutrition. There have also been nutritive evaluations performed on sweet potato leaves. Research has proven that sweet potatoes are very healthy and can be used as a long-term strategy to improve the nutrition (vitamin A status) of children in developing countries. Sweet potato is a tuberous-rooted perennial that is cultivated for its orange-fleshed edible tubers and is generally grown as an annual (Kemper, 2009). Flesh color can be creamy white, yellow-orange, orange and purple, although orange fleshed are preferred for their vitamin A content. Their skin colors come in creamy white, yellow, tan reddish purple and red. The herbaceous part of the plant above the ground dies back each year. The stems form a slender running vine up to four meters long, with ovate-cordate leaves borne on long petioles. Depending on the varieties, leaf color can range from green to purple and leaf shape can be angular or lobed. Sweet potato is in the convolvulaceae family, the same family as the morning glory. Flowers can be white or purple and are usually single (Duke, 1998). Today, the plant is grown throughout the tropics, subtropics and warm temperate zones of both hemispheres. The primary or main commercial producers of sweet potato are China, Indonesia, Vietnam, Japan, India, Tanzania and Uganda (Schrank, 2010).

Not only are sweet potatoes harvested for their fleshy tubers but their leaves can be harvested for food as well. There is a new sweet potato cultivar ‘Suioh’ utilized as greens (Ishiguro et al., 2004). Like the tubers, sweet potato leaves contain several nutrients and is commonly eaten in some African countries. Ease and economics are important in the acceptance of a vegetable in one’s diet and since this vegetable is fairly cheap, families can increase consumption without hurting their budgets. Creative uses for this crop are currently being developed. One way of consuming sweet potatoes is through fermenting beverages with lactic acid bacterial strains and yeasts that display probiotic characteristics. Sweet potato lacto-juice can be an alternative to

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consumers with intolerance to milk protein. Sweet potatoes are and will increasingly be a popular way to keep humans healthy. The roots, leaves and vines are used for livestock feeding, as fresh or dried material, or after fermentation into silage (Collaboration for Agriculture and Rural Development, 2010). Leaves have been evaluated to show appreciable amounts of zinc, potassium, sodium, manganese, calcium, magnesium, iron, vitamin C and A and fiber (Antia et al., 2006). They have relatively high protein content (25-30% of dry matter) compared to other leafy vegetables. The leaves also have higher levels of polyphenols than any other commercial vegetables. Polyphenols have many physiological functions such as cancer-fighting properties (Ishiguro et al., 2004). There are low levels of anti-nutrients and values can be reduced by cooking the leaves. Since the leaves contain a substantial amount of nutrients, they should be recommended in diets to contribute to health requirements (Antia et al., 2009). The leaves contain low levels of cyanide, phytic acid and tannins. Oxalate content of the leaves was found to be pretty high (Anon., 2011a). Sweet potato leaves contain significant amount of beta-carotene but bioavailability is certain to be much lower than for their roots. They are rich in unique phytonutrients, including polysaccharide-related molecules called batatins and batatosides (Low et al., 2009).

However, microorganisms have been a major problem to man from time being. Though microbes have been utilized in many ways to man’s advantage, their negative roles or adverse effects cannot be over looked. Many measures have been implemented to tackle these microorganisms, among which is the production of antibiotics and antimicrobials from various plant extracts. These antibiotic and antimicrobial plant extracts have been effective against microorganisms over the years. But the emergence of human pathogenic and other food spoilage microorganisms that are resistant to major classes of antibiotics and antimicrobials have increased in recent years, due to the indiscriminate use of these substances and this has become a major problem to man. It has led to many cases of pathogenicity and food spoilage jeopardizing consumers’ safety and leading to losses. Therefore, research for development of new antimicrobial agents from plant extracts containing certain phytochemicals is an urgent need in order to ensure consumer safety and reduce food losses due to spoilage.

The main aim of this research work was to evaluate the phytochemical and antimicrobial components of the sweet potato leaf powder and its extracts against some selected microorganisms. The specific objectives of this study were to produce extracts from sweet potato leaf using three different extracts (ethanol, peptone and water); to determine the proximate composition and bioactive components (phytochemicals) of sweet potato leaf as well as the extracts and; to evaluate the antimicrobial properties of these leaf extracts on the test organisms.

**MATERIALS AND METHODS**

Three kilograms (3kg) of fresh sweet potato leaves were obtained from Umudimohule village in Nsukka, Enugu State, Nigeria. Test organisms used for the antimicrobial activity were *Escherichia coli*, *Salmonella typhi*, *Aspergillus niger* and *Penicillum* spp. from Pharmaceutical Microbiology Laboratory and *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella pneumonia* obtained from Microbiology laboratory of Faculty of Veterinary Medicine both in University of Nigeria Nsukka.

**Sample preparation:** The fresh sweet potato leaves were sorted, destalked and washed thoroughly in water several times until they became clean and free from debris. The leaves were drained, spread on trays and oven dried till the weight became constant. After drying, the dried leaves were ground to powder using a crucible, sieved and then stored in an air tight container ready for use.

**Extraction of antimicrobial components in sweet potato leaves:** The extraction was carried out using three solvents namely; ethanol, peptone and water. The method used for ethanol extraction was the alcoholic extraction method according to Cowan (1999). About 40g of the prepared sweet potato leaves powder was soaked in 100ml of ethanol for extended periods (about 24 hours). The slurry was then filtered and washed with 50ml of ethanol, after which it was dried under reduced pressure and redissolved in the alcohol to a determined concentration.

The water extraction was carried out using cold maceration according to the method applied by Anowi (2012). Then, 40g of the sweet potato leave powder was soaked in 200ml of water and allowed to stand for twenty four (24) hours. It was made into slurry by blending with another 100ml of water and then filtered. The filtrate was concentrated in an oven at 60°C.

Peptone water was prepared by dissolving 15g of peptone in 1000ml of distilled water. The mixture was stirred thoroughly and warmed slightly with frequent agitation to dissolve dispense in the tube. It was then sterilized by autoclaving at 121°C for 15 minutes at 15lbs pressure. 200ml of the peptone water was then used for extraction from 40g of the dried sweet potato leaf and extraction was carried out as was done for the water extraction. The filtrate was then concentrated in an oven at 60°C.
Sample analysis
Proximate Analysis of sweet potato leaves (SPL) powder and its peptone, ethanol and water extract

Determination of moisture content of SPL powder and crude extracts: Moisture content was determined for the prepared sweet potato leaves powder using hot air oven method (Gallenkamp, VWR 1730 England) in triplicates as described by AOAC (2010). Five grams (5g) of the test sample (SPL powder) was weighed using a weighing balance (Electronic Kitchen Scale SF-400 China) in triplicates into already weighed sterilized and cooled petri dishes. The petri dishes were then put into an air oven (Gallenkamp, VWR 1730 England) at 150±2°C for 15 minutes before weighing. The loss in weight was regarded as moisture content. The percentage moisture content was then calculated as thus:

\[
\text{Percentage moisture content} = \frac{W_2 - W_1}{W_1 - W_0} \times 100
\]

Where:
- \(W_1\) = Initial weight of empty crucible
- \(W_2\) = Weight of crucible+sample before drying
- \(W_0\) = Final weight of crucible+sample after drying

Determination of crude protein content of SPL powder and crude extracts: The crude protein content of the sample was determined by the semi-Kjeldahl technique described by AOAC (2010). About two grams (2g) of the sample was put into a Kjeldahl flask and five grams (5g) of anhydrous sodium sulphate was added, followed up with addition of one gram (1g) copper sulphate and one tablet of Kjeldahl catalyst (contains 1g Na:SO₄+0.05g selenium). Twenty five milliliters (25ml) of concentrated sulphuric acid (aids digestion) and five glass beads (prevents bumping during heating) were added into the mixture. The mixture was heated in the fume cupboard. Sample was heated very gently at first and the heat was later increased to above 420°C for about 30min with occasional shackling till solution assumed a green colour. The solution was cooled and black particles showing on the neck and mouth of the flask was washed down with distilled water. The solution was reheated gently until the green colour disappeared and then it was allowed to cool. After cooling, the digest was transferred with several washing into a 250ml volumetric flask and made up to the mark with distilled water. Distillation was carried out using Markham distillation apparatus. Before distillation, the Markham distillation apparatus was steamed through for about 15 minutes. A 100ml conical flask containing 5ml of boric indicator was placed under the condenser such that the condenser tip is under the liquid. 5ml of the digest was pipetted into the body of the apparatus through a small funnel aperture and washed down with distilled water, followed by 5ml of 60% sodium hydroxide (NaOH) solution. Enough ammonium sulphate was collected by steaming through for 7 minutes. The receiving flask was removed and the tip of the condenser was washed down into the flask, then the condensed waster was removed. The solution in the receiving flask was then titrated using 0.01N hydrochloric acid. The nitrogen content was calculated and the protein content of the sample calculated from the obtained result. A blank was run through along with the sample:

\[
\text{Nitrogen (\%) } = \frac{V_a - V_b \times N_{\text{acid}} \times 0.01401 \times 100}{W}
\]

Where:
- \(V_a\) = Volume (ml) of acid required to titrate sample
- \(V_b\) = Volume (ml) of acid required to titrate the blank
- \(N_{\text{acid}}\) = Normality of acid (0.1N)
- \(W\) = Weight of sample in grams

% crude protein = N \times (conversion factor)
Conversion factor = (100%/ Nitrogen in protein)

Determination of crude fat content of SPL powder and crude extracts: The Soxhlet extraction method of AOAC (2010) was used in determining the fat content of the sample. A 250ml boiling flask was washed, dried in an oven (at 105-110°C) and then cooled in a desiccator. About 2g of the sample (A) was weighed out using an electronic kitchen scale (SF-400China) and put in the extraction thimble and plugged. It was then placed back in the soxhlet apparatus. The cleaned boiling flask (B) was weighed and then filled with about 30ml of petroleum ether of 40-60°C boiling point range. The Soxhlet apparatus was then assembled and allowed to flux for about 6 hours which then completed the extraction. The thimble was carefully removed and the petroleum ether in the top container of the set was drained into a container for reuse. The petroleum ether was recovered by evaporation using water bath (Technicol, SE-20 England). The flask was removed and dried at 80°C for 30 minutes in the oven (Gallenkamp, VWR 1730) and cooled in a desiccator and finally the weight of the empty flask and the flask with oil @ gave the oil content which was calculated as the percentage fat content:

\[
\text{Percentage fat content} = \frac{C - B}{A} \times 100
\]

Where:
- \(A\) = Weight of sample
- \(B\) = Weight of empty flask
- \(C\) = Weight of flask and oil
Determination of crude ash content of SPL powder and crude extracts: The method described by AOAC (2010) was adopted for ash content determination. A silicon dish was heated to 600°C in muffle furnace cooled in a desiccator and weighed using Mettler HAS balance (P-163 England). About 5g of the sample was put into the silicon dish and transferred to the furnace. The temperature was maintained until whitish grey color was obtained indicating that all the organic matter content of the product had been destroyed. The dish was brought out from the furnace and placed in the desiccator, cooled and weighed. The percentage ash content was calculated as:

\[
\text{Percentage ash content} = \frac{C - A}{B - C} \times \frac{100}{1}
\]

Where:

- \( A \) = Weight of empty dish
- \( B \) = Weight of empty dish and sample before ashing
- \( C \) = Weight of dish and ash

Determination of crude fiber content of SPL powder and crude extracts: The crude fiber content of the sample was determined using the method described in AOAC (2010). About 2g (W1) of sample was weighed using Mettler HAS balance (P-163 England) and put in a 250ml beaker, then boiled for 30 minutes with 100ml of 0.12M H2SO4 and filtered through a funnel. The filtrate was washed with boiling water until the washing was no longer acidic. The solution was boiled for another 30 minutes with 100ml of 0.012M NaOH solution; filtered with hot water and methylated spirit three times. The residue was transferred into a crucible and dried in the oven (Gallenkamp, VWR, 1730 England) for 1 hour. The crucible with its content was cooled in a desiccator and then weighed (W3) using Mettler HAS balance (P-163 England). The residue was then taken into a furnace for ashing at 600°C for 1 hour. The ashed sample was removed from the furnace and put into the desiccator to cool and later weighed (W4) using Mettler HAS balance (P-163 England). The percentage crude fiber was calculated thus:

\[
\text{PCFC} = \frac{W_3 - W_4}{W_1} \times \frac{100}{1}
\]

Where:

- PCFC = Percentage crude fiber content
- W1 = Weight of original sample
- W3 = Weight of crude and residue
- W4 = Weight of final ashed sample

Determination of carbohydrate in SPL powder and crude extracts: Carbohydrate content was determined by difference as described by Oyenuga (1968) using the equation below.

\[
\%\text{Carbohydrate} = 100 \%\text{Moisture} + \%\text{Ash} + \%\text{Protein} + \%\text{Crude} + \%\text{Crude fat}
\]

Determination of phytochemicals in SPL powder and crude extracts: Qualitative phytochemical screening of SPL powder and its peptone, ethanol and water extracts. Phytochemical screening procedures carried out were adopted from Oloyed (2005). This analysis determines the biologically active compounds that contribute to the flavor, color and other characteristics of sweet potato 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 proportion 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 Dragendorff's reagent a red precipitate was shown. With Mayer's reagent a creamy white colored precipitate indicated the presence of alkaloid.

Test for flavonoids: About 0.5 g of each of the samples were introduced into 10mls of ethyl acetate and heated in boiling water for 1 min. The mixtures were then filtered and the filtrates used for the following test; 4 ml of the filtrates were shaken with 1 ml of 1% aluminum chloride solution and kept. Formation of a yellow color in the presence of 1 ml dilute Ammonia solution indicated the presence of flavonoids.

Test for steroids: Approximately 9ml of ethanol was added into the sample and refluxed for few minutes and then filtered. The filtrate was concentrated to 2.5ml in a boiling bath; then 5mls of hot distilled water was added to the concentrated solution. The mixture was allowed to stand for 1 hour and the waxy matter was filtered off. To 0.5ml of the chloroform extracted in a test tube was added carefully 1ml of concentrated sulphuric acid to form a lower layer. A reddish brown interface showed the presence of Steroids.

Test for saponins: About 0.1 g of each of the samples was boiled with 5ml of distilled water for 5 minutes. The obtained mixtures were filtered while still hot and the filtrates were then used for the following tests. To 1ml of the filtrates, 2 drops of olive oil was added, the mixtures were shaken and observed for the formation of
emulsion. 1ml of each filtrate was diluted with 4ml of distilled water. The mixture was vigorously shaken and then observed on a stand for stable froth.

**Test for tannins:** Into 2 g of each of the samples was added 5ml of 45% ethanol and boiled for 5 min. The mixtures were cooled and filtered. Into 1ml of each filtrate was added 3 drops of lead sub acetate solution. A gelatinous precipitates were observed which indicates the presence of Tannins. Into another 1ml each of the filtrates was added 0.5 ml of bromine water. A pale brown precipitates were observed indicating the presence of Tannins.

**Test for glycosides:** Two grams of each of the samples was mixed with 30ml of distilled water and they was heated for 5 min on a water bath, filtered and used as follows: into 5mls of the filtrates was added to 0.2ml of fehling solution A and fehling solution B until they turn alkaline and heated in a water bath for 2 min. A lightish blue coloration was observed (instead of brick red precipitate) which indicates the absence of glycosides. Test for carbohydrates (Molisch Test). About 0.1g of each of the samples was boiled with 2ml of distilled water and filtered. To the filtrates, few drops of nephthol 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 carbohydrate.

**Quantitative phytochemical analysis of the SPL powder and its extracts:** The quantitative phytochemical analysis was carried out according to the method of Harboune (1973).

**Determination of steroid:** One gram each of the samples were weighed out and macerated with 20ml of ethanol. The mixtures were filtered and 2mls each of the filtrates were pipetted into test tubes. Then 2mls of color reagent was added into the test tubes and they were allowed to stand for 30minutes and the absorbance was measured at 550nm.

**Determination of flavonoid:** About 1g of each of the samples were measured out and macerated with 20ml of ethyl. The mixture was filtered then 5mls of the filtrate was measured into test tubes. To each test tube contain the filtrate, 5mls of dilute ammonia was added and the mixture was shaken. The upper layers were collected and absorbance was measured at 490nm.

**Determination of saponin:** One gram each of the samples were weighed out and macerated with 10ml of petroleum ether. The supernatant was decanted into a beaker and another 10mls of petroleum ether was added. The supernatant was decanted and mixed with the first. The mixture was evaporated to dryness and 6mls of ethanol was added. 2mls of the mixture was pipetted into a test tube. 2mls of color reagent was added and the mixture was allowed to stand for 30minutes, then the absorbance was read at 550nm.

**Alkaloid determination:** About 1g of the samples were weighed out and macerated with 20mls of 20% H2SO4 in ethanol (1:1). The mixture was filtered and 1ml of the filtrate was pipetted into test tubes. 5ml of 60% H2SO4 and 5mls of 0.5% formaldehyde in 80% H2SO4 were added and the content of the test tube was mixed properly. The mixture was allowed to stand for 3h and the absorbance was measure at 588nm.

**Determination of soluble carbohydrate:** One gram of the samples were weighed out and macerated with 50mls of distilled water. The mixture was filtered and 1ml of the filtrate was pipetted into test tubes and 2mls of saturated picric acid was added. The absorbance was then measured at 530nm.

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

**Determination of anti-nutrients in SPL powder and crude extracts**

**Determination of tannin:** The tannin content of the SPL powder and its extracts was determined by the method of Pearson (1976). One gram (1g) of the test sample was weighed out into a conical flask and 10ml of water was added. The mixture was shaken at 5 minutes interval for 30 minutes and filtered to get the extract. About 2.5ml of the supernatant was transferred into a test tube and 2.5ml of standard tannic acid solution was transferred into a 50ml flask. Then 1ml Folin-Denis reagent was added into the flask followed by 2.5ml of saturated Na2CO3 solution. The absorbance was read at 720nm spectrophotometrically after 90 minutes and incubated at room temperature:

\[
\text{Tannin (\%)} = \frac{A_\text{n} \times C \times 100}{A_\text{r} \times W \times V_1 \times V_2}
\]

Where:  
\(A_\text{n}\) = Absorbance of test sample  
\(A_\text{r}\) = Absorbance of standard solution
C = Concentration of standard solution
W = Weight of sample used
V_i = Total volume of extract
V_s = Volume of extract analyzed

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

**Determination of oxalate:** The determination of oxalates was carried out by the titration method of AOAC (2010). Two grams of sample was suspended in a mixture of 190ml of distilled water and 10ml of 6N HCl in a 250ml volumetric flask and digested for one hour at 100°C, cooled and made up to 200ml with distilled water. The digest be filtered through Whatman No. 1 Filter paper using a suction pump. A duplicate proportion of 125ml of the filtrate was measured into 250ml beakers and four (4) drops of methyl red indicator add 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 color (pH 4-4.5). Each portion was heated up to 90°C and 10ml 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 dissolved in 10ml of 20% (v/v) H₂SO₄ solution. At this point, the filtrate resulting from digestion of 2g of the sample was combined and made up to 300ml. Aliquots (125ml) of the filtrate was heated until near-boiling and then titrated against 0.05M standard KMnO₄ solution to a faint pink color. Oxalic acid content was calculated using the formula:

\[
\text{Oxalate \left( \frac{\text{mg}}{100 \text{g}} \right) = \frac{T \times (V_{me}) \times (D_i) \times 10^3}{\text{ME} \times M_i}}
\]

Where:
- \( T \) = Titre of KMnO₄
- \( V_{me} \) = Volume mass equivalent (i.e., 1ml of 0.05M KMnO₄ solution is equivalent to 0.00225g anhydrous oxalic acid)
- \( D_i \) = The dilution factor
- \( \text{ME} \) = The molar equivalent of KMnO₄ in oxalic acid (KMnO₄ redox reaction is 5)
- \( M_i \) = The mass of sample used

**Determination of cyanide:** Cyanide content was determined according to the method of Onwuka (2005). 5g of the sample was weighed into a conical flask and 50ml of distilled water was added to it and the solution was allowed to stand overnight and then filtered. 1ml of the sample filtrate was measured into a test tube and 4ml of alkaline picrate was added and it was allowed to stand for 5min. The absorbance was read at 490nm. The reading was taken with the reagent blank at zero.

**Determination of antimicrobial properties of SPL powder and crude extracts:** The antimicrobial properties of the sweet potato leaves extracts were determined using the agar well diffusion technique described by Wan et al. (1998). About 14g of nutrient agar powder was suspended in 500ml of distilled water. The suspension was then autoclaved at 121°C for 15mins. The medium was allowed to cool after sterilization to about 40-47°C and poured into sterile plates. 0.1ml of overnight broth culture of the respective microorganisms, were diluted in 10ml of sterile water and 0.1ml of the diluent was transferred into the center of a sterile petri dish. Then 20ml of the sterile molten agar was added and the dish was swirled to mix its content. The plates were allowed to stand for 30 minutes so that solidification can take place. Six holes were then bored on each plate using a 6mm diameter cork borer. Each hole was for a concentration of the sweet potato leaf extract. The holes were labeled 1-6 to represent 100, 50, 25, 12, 6.25 and 3.125mg/ml concentrations of the three potato leaf extracts, respectively. Four drops of each concentration was dropped in corresponding holes and the plates were then kept undisturbed for 15 minutes so as to allow the extracts diffuse properly and dry to a considerable level before incubation. The measurements (in millimeters) of the zones of inhibitions of the extracts against the test organisms were taken and recorded.

**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 was by Duncan's New Multiple Range Test. While the antimicrobial testing was statistically analyzed using a 3-fatorial or split plot in Completely Randomized Design (CRD).

**RESULTS AND DISCUSSION**

Proximate composition (% of sweet potato leaves powder and its peptone, ethanol and water extracts: Table 1 shows the data on the proximate composition of sweet potato leaves powder and the water, ethanol and peptone extracts. The moisture content of the sweet potato leaves powder was found to be 5.41±0.003 as shown in Table 1. This value is lower than that reported for Amaranthus cruentus (tete) 23.6±4.10, Celusia
argenta (soko) 15.00±1.0 and Corchorus olitorius (ewedu) leaves 30.90±1.30 (Onwordi et al., 2009). The obtained value was also found to be lower than the value of sweet potato leaves reported in Antia et al. (2008) which was as high as 62.21%. The water extract of the sweet potato leaves powder was found to have higher moisture content (31.310±0.008) than the peptone extract (16.921±0.008) and then ethanol extract which had the lowest value (3.603±0.004).

The ash content of the sweet potato leave powder (Table 1) was found to be 5.563±0.004 which is lower than the values of Talinum triangulare (water leaf) 19.4±3.0, Amaranthus cruentus 19.3±5.7 and Telfaria occidentalis (fluted pumpkin) 10.9±8.2 as reported by Fasuyi (2006). It was also lower than the values reported for Celusia argenta (soko) 32.40±1.60 and Corchorus olitorius (ewedu) 21.20±0.80 leaves (Onwordi et al., 2009). The obtained value can be compared to that of early stage Moringa oleifera (5.75±0.21) leaves reported in Barnishaiye et al. (2011). The peptone extract had the highest ash content of 10.414±0.007 followed by the ethanol extract with a value of 8.206±0.004 and the water extract having the lowest value of 7.852±0.003.

The crude fat content of the sweet potato leaves powder was obtained as 1.625±0.004 and was seen to be lower than the reported value of sweet potato leave 4.90±0.02 in Antia et al. (2006). It was higher than the values obtained for Celusia argenta 0.21±0.01, Amaranthus cruentus 0.45±0.03 and Corchorus olitorius 0.32±0.01 (Onwordi et al., 2009). The fat content of the ethanol extract 14.264±0.002 was much higher than the peptone extract (1.625±0.004) and the water extract had a much lower value of 0.208±0.007. The extracts were seen to contain more fat than the sweet potato leave powder.

The crude protein content of the sweet potato leaves powder was found to be 28.183±0.003 as show in Table 1. This value was seen to be much higher than the values obtained for Celusia argenta, Amaranthus cruentus and Corchorus olitorius which were 9.40±0.90, 12.70±1.40 and 11.20±1.30, respectively (Onwordi et al., 2009). Piper guineeses and T. triangulare, had higher values of 28.76 and 31.0%, respectively (Etuk et al., 1998). The value obtained can be compared with that obtained for late stage Moringa oleifera leaf (28.03±2.75) as reported by Barnishaiye et al. (2011). Plant food that provides more than 12% of its caloric value from protein is considered good source of protein. Therefore, sweet potato leaves meet this requirement. The peptone extract had a higher protein content of 21.857±0.002 than the ethanol and water extracts with values of 20.595±0.002 and 16.497±0.002 respectively.

The crude fibre content of the sweet potato leaves powder as shown in Table 1 was found to be 2.764±0.003. The fibre content of Celusia argenta, Amaranthus cruentus and Corchorus olitorius were reported to be 11.70±0.80, 7.80±1.80 and 6.70±1.40, respectively (Onwordi et al., 2009). These values are higher than the obtained value of the sweet potato leaves powder.

The carbohydrate content of the sweet potato leaves powder was found to be 56.472±0.009 as shown in Table 1. The obtained value was seen to be higher than the value reported for Celusia argenta, Amaranthus cruentus and Corchorus olitorius which were 32.80±1.70, 29.40±1.40 and 31.30±1.50, respectively. The obtained value was also higher than the value reported for sweet potato leaves by Antia et al. (2006) which was 51.95±0.05. For the extracts, the ethanol extract had the highest carbohydrate value of 52.947±0.003, followed by the peptone extract with a value of 49.70±0.016 and lastly the water extract with the lowest value of 43.971±0.002.

Phytochemical composition of sweet potato leaves (SPL) powder and its peptone ethanol and water extracts: The phytochemical composition of SPL powder and its peptone, ethanol and water extracts are shown in Tables 2 and 3 below.
Phytochemical analysis is very useful in the evaluation of some active biological components of some vegetables and plants (medicinal). The qualitative and quantitative analyses of phytochemicals were carried out in the SPL powder and its extracts. Alkaloids, flavonoids, saponins, tannins, glycoside, steroid, hydrogen cyanide and soluble carbohydrate were revealed to be present in the SPL powder and its extracts (Table 2 and 3). The qualitative analysis indicates the presence of the phytochemicals and the degree in which they are found in each of the samples.

The quantitative test was used to quantify the phytochemicals that were identified from the qualitative analysis. From Table 2 the alkaloid in the sweet potato powder is 3.794±0.007mg/100g which is much lower than the values reported for some common leafy vegetables consumed in South East Nigeria such as Telfari a occidentalis, Ocimum viride, Gnetum africanum, Crongromena ratfalia, Delissa undulata, Pterocarpus santolinoides, Corchorus olitorius and Vernonia amygdalina (0.81±0.47, 0.99±0.55, 1.28±0.84, 0.82±0.48, 1.19± 0.89, 0.88±0.46, 1.68±1.28 and 1.81±0.34 g/100g, respectively) by Onyeka and Nwambekwe (2007). The alkaloid value of the SPL powder is much lower than the value reported for Talimun triangular (55.56±0.00 mg/100g) by Aja et al. (2010). The ethanol had alkaloid content of 2.964±0.003mg/100g, while the water and peptone extracts have lower values of 2.274±0.004 mg/100g and 3.121±0.579 mg/100g, respectively.

The saponin content was observed (Table 3) to be highest in the SPL powder (0.423±0.002mg/100g), while the peptone extract (0.419±0.001 mg/100g) had the highest value among the three extracts and the water extract had the least value of 0.412±0.00 mg/100g. The SPL powder had a lower value than that reported for dry sample of T. triangular (1.37±0.60 mg/100g) by Aja et al. (2010).

As seen in Table 3 the SPL powder had the highest content of steroid with a value of 0.393±0.003mg/100g. Among the three extract, the peptone extract had the lowest value of 0.393±0.003mg/100g. The water extract had a value of 0.375±0.003mg/100g while the ethanol extract had a value of 0.365±0.005mg/100g. The steroid content of the SPL powder was lower than that reported for common leafy vegetables consumed in South East Nigeria (0.12±0.00-0.27±0.01g/100g) by Onyeka and Nwambekwe (2007).

The SPL powder had flavonoid content of 3.357±0.006mg/100g, this value is lower than that reported for common leafy vegetables consumed in South East Nigeria (0.03±0.01-0.22±0.06g/100g) by Onyeka and Nwambekwe (2007). The water extract had the least flavonoid content of 2.762±0.008mg/100g and the ethanol extract had a value of 3.185±0.003mg/100g. There was no significant difference between the peptone extracts and the SPL powder (3.355±0.004 and 3.357±0.006mg/100g, respectively). The value obtained for the SPL powder and its extract was observed to be much lower than the value reported by Aja et al. (2010) for T. triangular (69.8±4.42mg/100g).

The glycoside content of the SPL powder was observed (Table 3) to be 2.434±0.003mg/100g and this value was higher than that obtained for the peptone, ethanol and water extracts. The peptone extract had the highest value of 2.240±0.004mg/100g, followed by the water extract (1.646±0.002) and the ethanol extract had the least value of 1.445±0.003mg/100g among the three extracts. Soluble carbohydrates content of the SPL powder was 2.353±0.004mg/100g. Water extract had the highest value of (2.343±0.004mg/100g) among the three extracts and the ethanol extract had the least value of 1.723±0.004mg/100g while the peptone extract had a value of 2.053±0.003mg/100g.

Studies have shown that phytochemicals are present in all plant parts and that those present, differ according to the type of extracting solvent used (Tijani et al., 2009). This explains the differences in content of phytochemicals in the peptone, ethanol and water extract extracts. Though there was significant difference between almost all the values obtained, the closeness of the values obtained shows that each extract had the ability to extract the phytochemicals at almost the same capacity, with extent of extraction depending on the individual phytochemicals.

Antinutrient content of sweet potato leaves powder and its extracts: Table 4 shows the antinutrient content of the sweet potato leaves powder and the water, ethanol and peptone extracts. The results for antinutrient composition (Table 4) revealed low values of cyanide (0.774±0.005mg/100g), phytic acid
Table 4: Anti-nutrient Content of Sweet Potato Leaves (SPL) Powder and its Extracts

<table>
<thead>
<tr>
<th>Antinutrients</th>
<th>Peptone extract</th>
<th>Ethanol extract</th>
<th>Water extract</th>
<th>SPL powder</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phytate (mg/100g)</td>
<td>3.89±0.001</td>
<td>3.89±0.003</td>
<td>5.84±0.002</td>
<td>5.93±0.003</td>
</tr>
<tr>
<td>Oxalate (%)</td>
<td>1.86±0.004</td>
<td>3.89±0.109</td>
<td>5.11±0.005</td>
<td>6.25±0.004</td>
</tr>
<tr>
<td>Tannin (mg/100g)</td>
<td>5.88±0.002</td>
<td>5.52±0.002</td>
<td>8.34±0.002</td>
<td>9.01±0.002</td>
</tr>
<tr>
<td>Cyanide (mg/100g)</td>
<td>0.77±0.003</td>
<td>0.35±0.003</td>
<td>1.67±0.003</td>
<td>1.44±0.004</td>
</tr>
</tbody>
</table>

Values are mean ± standard deviation of triplicate readings. Superscripts a, b, c and d show that samples are significantly different.

Table 5: Antimicrobial activity of sweet potato leaf extracts (peptone, ethanol and water extracts) using the Agar well diffusion technique

<table>
<thead>
<tr>
<th>Zone of Inhibition of organisms (mm)</th>
<th>Conc. (mg/ml)</th>
<th>S. aureus</th>
<th>E. coli</th>
<th>K. pneu</th>
<th>S. typhi</th>
<th>P. aero</th>
<th>A. niger</th>
<th>Penicil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>100</td>
<td>15.7±0.6</td>
<td>NI</td>
<td>12.0±0.0</td>
<td>18.0±0.0</td>
<td>13.7±0.6</td>
<td>26.3±0.6</td>
<td>NI</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>12.7±0.6</td>
<td>NI</td>
<td>8.3±0.8</td>
<td>14.7±0.6</td>
<td>11.0±0.0</td>
<td>21.7±0.6</td>
<td>NI</td>
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<tr>
<td></td>
<td>25</td>
<td>9.0±0.0</td>
<td>NI</td>
<td>11.3±0.6</td>
<td>8.7±0.6</td>
<td>16.3±0.6</td>
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<tr>
<td></td>
<td>12.5</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>9.3±0.6</td>
<td>NI</td>
<td>12.0±0.0</td>
<td>NI</td>
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<td></td>
<td>6.25</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>8.7±0.6</td>
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<td></td>
<td>3.125</td>
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<tr>
<td>Peptone</td>
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<td>NI</td>
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<td>12.3±1.2</td>
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<tr>
<td>Ethanol</td>
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<td>NI</td>
<td>NI</td>
<td>12.0±1.0</td>
<td>NI</td>
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</table>

Values are means ± standard deviation of triplicate readings. Superscripts a, b, c and d show that samples are significantly different. Key: S. aureus = Staphylococcus aureus; E. coli = Escherichia coli; K. pneu = Klebsiella pneuonia; S. typhi = Salmonella typhi; P. aero = Pseudomonas aeroginosa; A. niger = Aspergillus niger; Penicil = Penicillium spp.; NI= No inhibition.

(3.897±0.001mg/100g) and tannins (5.933±0.002mg/100g) but exceptionally high value of oxalate (1.684±0.004%).

The phytate content of the sweet potato leaves was 5.933±0.003mg/100g as shown in Table 4. This value was seen to be higher than that reported for sweet potato leaves (1.44±0.01) by Antia et al. (2006) and very much lower than that reported for Telferia occidentalis (48.8mg/100g) as obtained by Areghore (2012). There was no significant difference (p = 0.05) between the phytate content of the peptone extract (3.897±0.001mg/100g) and that of the ethanol extract (3.897±0.003). The SPL powder had the highest value of 5.933±0.003mg/100g followed by the water extract with a value of 5.847±0.002mg/100g.

The oxalate content of the sweet potato leaves powder was 6.25±0.004mg/100g. This value is very high compared to the value obtained for the phytate, cyanide and tannin contents of the sweet potato leaves. The value was found to be higher than that reported for sweet potato leaves (30.00±1.04mg/100g) by Antia et al. (2006) and Moringa oleifera (0.45±0.01%) as reported by Ogbe (2012). The oxalate content of the SPL powder was higher than that in the extracts with the values of 1.66±0.004, 3.66±0.100 and 5.11±0.005% for the peptone, ethanol and water extracts respectively.

The tannin content of the sweet potato leaves powder was 9.01±0.002mg/100g. This value is lower than that reported for Telferia occidentalis (40.6mg/100g) by Areghore (2012) and higher than that reported for Vernonio amygdalina (0.5%) by Obob (2008). The tannin content of the water extract was highest for the three extracts with a value of 6.34±0.002, followed by the peptone extract (5.86±0.002mg/100g) and finally, the ethanol extract with a value of 5.52±0.002.

The cyanide content of the sweet potato leaves powder was 1.44±0.004mg/100g. As reported by Antia et al. (2006), the cyanide content of sweet potato leaves was 30.24±0.02mg/100g which was higher than the value obtained for the sweet potato powder in Table 4. The cyanide content of the extracts were 1.67±0.003, 0.77±0.003 and 0.35±0.003mg/100g for water, peptone and ethanol extract in order of decreasing content.

**Antimicrobial properties of sweet potato leaf extracts:**

The antimicrobial properties of sweet potato leaves extracts (peptone, water and ethanol extracts) is shown...
in Table 5. It was observed that *Escherichia coli* and *Penicillium* spp. were resistant to the three (3) extracts at the different concentrations used for the antimicrobial testing. *Staphylococcus aureus*, *Klebsiella pneumonia*, *Pseudomonas aerogenosa* and *Aspergillus niger* were inhibited by the water extract at concentrations of 25mg/ml and above for *Staphylococcus aureus*, 50mg/ml and above for *Klebsiella pneumonia*, 12.25 mg/ml and above for *Salmonella typhi*, 25mg/ml and above for *Pseudomonas aerogenosa* but 6.25mg/ml and above for *Aspergillus niger*. The micro-organisms were observed to be resistant at a concentration of 3.125 of the water extract and to both the ethanol and peptone extracts at the various concentrations used. *Salmonella typhi* was inhibited by the three extracts at varying concentrations. It was inhibited by the water extract at concentrations above 12.5mg/ml and at concentrations above 50mg/ml for the peptone and ethanol extracts. The data represented in Table 6 revealed that the water extract of sweet potato leave powder exhibited significant
well diffusion tests. The antimicrobial activity of sweet potato leaves may depend on their antioxidative compound and/or pectin-like materials. Thus, the practical use of sweet potato leaves is expected to prevent food poisoning caused by bacteria (Islam et al., 2004). According to Islam et al. (2004), sweet potato leaves strongly suppressed the growth of both gram-positive and gram-negative bacteria: Escherichia coli 0157:H7, Bacillus cereus and Staphylococcus aureus.

Conclusion: This research shows that sweet potato leaves' extracts possess antimicrobial activity and this is attributed to both the polysaccharide and phytochemical constituent of the leaves. The water extract was seen as the most potential extract of the three extracts used. This antimicrobial property of sweet potato leaves merits special consideration since sweet potato production and consumption is immensely increasing worldwide. This research justifies the use of sweet potato leaves for the treatment of minor ailments in some parts of the world. It also indicates the potential for medicinal vegetables in the production of food preservatives and delivery of primary health care to the poor people who are facing the harsh economic crisis. Meanwhile, the use of natural preservatives and medicines such as sweet potato leaves extract should be advocated since their use is considered safer than the use of synthetic or artificial substances which have adverse effect and may lead to one or more complications in humans. Also, the benefit or effectiveness of sweet potato leaves extract as an antimicrobial agent is not limited to the microorganisms mentioned this work. Therefore, more research should
be embarked upon to investigate on other organisms that the extract could be effective against. Also, novel methods of producing extracts from sweet potato leaves could be researched upon.

REFERENCES


