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Nutritional and antimicrobial evaluation of *Saccharum officinarum* consumed in Calabar, Nigeria

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Sugarcane (*Saccharum officinarum*) stem pulp is widely consumed in Nigeria as a snack mainly for sugar content and is believed to possess some remedy against infectious diseases. This study therefore quantitatively evaluated the nutritional and chemical composition of the sugarcane stem pulp with a view to validating this claim. The results of proximate composition indicate relatively high amount of moisture (71.91 ± 0.05%) and a low level of dry matter (28.09%) consisting of carbohydrate (58.55 ± 0.04 g/100 g), fibre (29.88 g/100 g), ash (6.69 g/100 g) and some mineral elements, implying an active role in nutrient supplementation. Generous amounts of phytochemical compounds such as alkaloids (8.07 ± 0.04 µg/100 g), saponins (5.57 ± 0.01 µg/100 g) and flavonoids (1.52 ± 0.02 µg/100 g); and mineral elements including magnesium (1.596 mg/100 g), potassium (0.639 mg/100 g), calcium (0.318 mg/100 g) and others in trace amounts were also obtained. .Antimicrobial results revealed that the sugarcane extract showed the highest growth inhibition against *Staphylococcus aureus* (8.67 - 24.00 mm) among the bacterial isolates and *C. albicans* (6.00 – 14.00 mm) for the fungal isolates studied. Data from the study suggest that sugarcane stem pulp could be suitable for use in rehydration and as a functional food plant. Also, the plant possesses some antimicrobial qualities which could be beneficial to both pharmaceutical and food industries.

Key words: *Saccharum officinarum*, sugarcane, proximate analysis, mineral, phytochemical, antimicrobial.

INTRODUCTION

Diet plays a key role in disease prevention and therapy. Plant foods make up a larger percentage of foods consumed in developing nations including Nigeria because animal foods are relatively expensive hence not easily affordable. It is believed that nutrients in plant foods do more than just prevent deficiency diseases like beriberi or rickets with the most publicized findings indicating notable chemicals like vitamin C, beta-carotene, and polyphenols as powerful antioxidants (Palmer, 2005; Martinez and Martinez, 2007; Mates et al., 2011), which help to prevent molecular damage caused by oxidation. This protection is known to fend off many diseases including cancer, cardiovascular diseases and muscular degeneration (Islam et al., 2002; Mates et al., 2011). This

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has resulted in an increased demand for foods that provide additional health benefits, popularly known as functional foods (Kim et al., 2009).

Sugarcane (*Saccharum* sp.) is one such functional foods, described as one of the world's most efficient living collectors of solar energy, stored in the form of fibre and fermentable sugars (FAO, 1988). It is a giant, thick, perennial grass belonging to the family Poaceae, and constitutes the main crop cultivated in Brazil, India, China, Thailand, Mexico and Pakistan, where it plays a vital role in the economy and provides employment opportunities (OECD, 2011; Chandel et al., 2012). There are six confirmed species of *Saccharum*, two of which are wild while four are cultivated (Bakker, 1999). All modern cultivated varieties of sugarcane are hybrids derived from breeding between these local species (OECD, 2011), and supply about 75% of the world edible sugar (Dillon et al., 2007). Sugarcane and its hybrids are grown for the production of sugar, ethanol and other industrial uses. For instance, Brazil the world leading producer of sugarcane is also the largest exporter of sugar and the second largest exporter of ethanol after the United States (Antunes et al., 2014; FAOSTAT, 2014; Licht, 2015).

Nigeria is among the 106 countries of the world categorized as minor sugarcane producers (Tiamiyu et al., 2013). From the coastal region where it was first introduced by European sailors in the 15th century, the crop has spread to other parts of the country (Busari, 2004). However, much of the sugarcane production in Nigeria is in the northern region (Tiamiyu et al., 2013; Sulaiman et al., 2015). Currently, Nigeria is the second largest producer of sugarcane in West Africa after Ivory Coast and the 19th in Africa (FAOSTAT, 2012; Sulaiman et al., 2015). However, the country depends largely on raw sugar import to meet domestic requirements (Gourichon, 2013; Sulaiman et al., 2015). According to GAIN (2014) report, Nigeria is Africa's largest sugar importer. The yearly average share of imports of raw sugar in the country's domestic supply is about 96% (Gourichon, 2013). Brazil is the largest raw sugar supplier to Nigeria (GAIN, 2015). In 2013/2014, Brazil harvested about 652 million metric tons of sugarcane while Nigeria's domestic sugar production in the same year was 70,000 tons (GAIN, 2014).

In Nigeria, particularly in the northern part, *Saccharum officinarum,* popularly known as sugarcane is consumed as a snack, by chewing the stem pulp to extract its juice, while the bagasse is thrown away. It is probable that sugarcane may also supply some other vital nutrients alongside its renowned sugar content, since it is eaten in its natural form. Hence, the suggestion that the sugarcane intended for human consumption should be analysed for proximate constituents (OECD, 2011).

Also, it is believed that in the process of chewing sugarcane to extract the juice from the pulp, large amounts of bioactive compounds may not be fully utilized (Deng et al., 2012). According to research reports, these compounds are proven to have important biological and medicinal properties that may make sugarcane a valuable functional food plant (Iacopini et al., 2008). Additionally, the use of *S. officinarum* in traditional medicine in Nigeria and some parts of Asia especially India for the treatment of diseases such as jaundice and liver-related disorders, dyspepsia, haemorrhoids, menorrhagia, dysentery, agalactia, phthisis and general debility (Kadam et al., 2008; Suresh-Kumar et al., 2010), suggest inherent medicinal phytochemicals. Yet scientific information on the chemical composition in relation to its medicinal properties is scanty and largely uncollated. Moreover, edaphic and climatic factors in general are known to influence the chemical and nutrient composition of plants, underscoring the imperative to do a nutrient and chemical screening of the sugarcane consumed in Calabar, Cross River State, Nigeria.

Therefore, the study evaluated the nutritional and phytochemical composition of *S. officinarum* as well as its antimicrobial properties. Data generated from such studies as this, will contribute to the nutrient composition database useful in the assessment of dietary intake of individuals/ population groups, a major prerequisite for solving the problem of malnutrition in developing countries including Nigeria. The study will also serve to provide some baseline data necessary for further investigation into the functional properties of sugarcane.

MATERIALS AND METHODS

Sample collection

Sugarcane (*S. officinarum*) stems were purchased from a vendor in Watt Market in Calabar, who brought them from Kano in Northern Nigeria, and transported to the Department of Botany, University of Calabar where they were properly identified. Thereafter, the stems were taken to the research laboratory in the Department of Biochemistry, where they were washed thoroughly and allowed to drain.

Sample preparation

The outer woody layer of each sugarcane stem was peeled off, while the inner sugary pulp was cut into small cubes using a kitchen knife and further ground to a fine consistency using a homogenizer. From this, 700 g portion was weighed out and used for the study.

Proximate analysis

The moisture, fat, crude protein, ash and fibre contents of the sample were determined by the methods of the Association of Official Analytical Chemists (AOAC, 2005). In brief, moisture content was determined by drying a 20 g portion of the raw homogenized pulp to a constant weight, using a vacuum oven (Astell- Hearson) at 100°C for about 5 h. The moisture content was taken as the difference in weight between the raw and the constantly dried sample. Fat content was determined by exhaustively extracting 20 g of the sample with petroleum ether (B.P. 40 to 60^{o}C) using a Soxhlet apparatus (Corning, England). The crude protein content was determined by the micro-Kjeldahl

digestion apparatus. The method estimated the amount of nitrogen in the sample which was subsequently used to calculate the protein content by multiplying with the factor of 6.25. The crude fibre content was estimated by boiling 20 g of the sample in 1.25% (w/v) sulphuric acid and afterwards with 1.25% (w/v) sodium hydroxide. The residue was then incinerated completely at 550°C. The loss in weight represented the crude fibre content of the sample. Total ash was determined from the residue left after incinerating a 20 g portion of the sample in a muffle furnace at 550°C, whereas carbohydrate content was obtained by difference, that is, by subtracting the protein, fat, ash and moisture contents from the total dry matter and expressed in percentage.

Estimation of mineral elements

The mineral elements were determined using a sample digest prepared by digesting completely 5 g of the sample in perchloric and concentrated nitric acids diluted with deionized water in a 50 ml volumetric flask. Sodium (Na), calcium (Ca), magnesium (Mg), potassium (K), phosphorus (P), iron (Fe), copper (Cu) and zinc (Zn) in the digest were measured using the Perkin Elmer Atomic absorption spectrophotometer (Model 306, UK) (AOAC, 1990).

Phytochemical evaluations

The qualitative phytochemical tests were carried out to identify the various constituents using standard procedures described earlier by Harbone (1998), Trease and Evans (2002), and Sofowara (2008). Some of the phytochemical compounds were also quantified using known procedures. Flavonoids, saponins and tannins were determined by the methods of Trease and Evans (1996). The cyanogenic glycosides were assayed by the alkaline picrate calorimeter method (Balagopalan et al., 1988), whereas total alkaloids were determined by the alkaline precipitation gravimetric method (Harbone, 1998).

Antimicrobial studies

Solvent extraction

One hundred (100) g of the sample was extracted with methanol solvent in Soxhlet extractor for 48 h. The solvent extract was concentrated by evaporating to dryness using rotary evaporator. The concentrate obtained was preserved in the refrigerator at - 4°C until further use.

Reconstitution of extract

The stored extract was reconstituted using methanol to obtain a stock solution which was further diluted serially to obtain concentrations of 100, 50, 25, 12.5, 6.25 and 3.13 µg/ml prior to determination of its antimicrobial activity.

Collection and maintenance of test microorganisms

Six clinical microbial isolates namely *Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus, Pseudomonas aeruginosa, Aspergillus fumigatus* and *Candida albicans* collected from the Department of Medical Microbiology, University of Calabar Teaching Hospital were used. The bacteria were maintained on nutrient broth at 37°C, while the fungi were maintained on potato dextrose agar at 28°C.

Determination of antimicrobial activity of the extract

The susceptibility of the test microorganisms to sugarcane extract was determined using the Kirby-Bauer Disk Diffusion method; Ampicillin (10 µg/ml) and Amphotericin B (10 µg/ml) were respectively used as antibacterial and antifungal positive control, while methanol served as negative control. Twenty-four petri-plates previously sterilized in a hot-air oven at 100°C for I h were first labeled accordingly, with the first six set of plates carrying labels of the various concentrations of the sugarcane extract, while the next three set of six plates each carried labels of Ampicillin, Amphotericin B, and methanol control respectively. The labels were placed at specific locations around the petri-plates and their respective clinical isolates were also indicated at the back of the plates. That is, plate 1 for instance carried 100, 50, 25, 12.5, 6.25 and 3.13 µg/ml concentration labels placed at specific locations around the plate. The labeling followed a clock-wise direction. Thereafter, 1 ml each of the clinical isolates was placed in the respective plates and later, 20 ml of Mueller-Hinton agar held at 45°C was added to each plate, which was then swirled to allow for thorough mixing. The plates were kept for 30 min for the agar to solidify.

Afterward, filter paper discs impregnated with various concentrations of the crude extract, Ampicillin, Amphotericin B and methanol control were dried at 60°C for 10 min and the dried discs were later transferred to their petri-plates according to their specific concentrations using sterilized forceps. The plates were then incubated at 37°C for 24 h for the bacteria and at 28°C for 48 h for fungi. All tests were performed in triplicates.

After the incubation period, the sensitivity of the clinical isolates to the various extract concentrations was determined by measuring their zone diameters in millimeters (mm) using a transparent ruler. Antimicrobial activity was expressed as the mean diameter of the clear zone. Extracts producing zones of bacterial growth inhibition 13 to 17 mm and fungal growth inhibition 12 to 15 mm were considered effective (Espinel-Ingroff et al., 2007; CLSI, 2013).

Statistical analysis

Data obtained was expressed as the mean ± SD for the measured variables and further subjected to appropriate statistical analysis such as correlation and one-way analysis of variance (ANOVA) in SPSS statistical package. Statistical significance was accepted at 5% probability level or less.

RESULTS

Proximate composition

The results of the proximate composition of *S. officinarum* pulp presented in Figure 1 indicates that over two-third (71.91%) of the edible portion of the sugarcane pulp is water, while the dry matter makes up less than one-third (28.09%). Carbohydrate represents the largest constituent (58.55 g/100 g) of the dry matter. The results of the evaluation also reveal that *S. officinarum* contains relatively high amount of crude fibre (29.88 g/100 g) and ash (6.69 g/100 g), but low fat (1.68 g/100 g) and crude protein (3.20 g/100 g) contents.

Elemental composition

Figure 2 shows the levels of mineral elements in 100 g

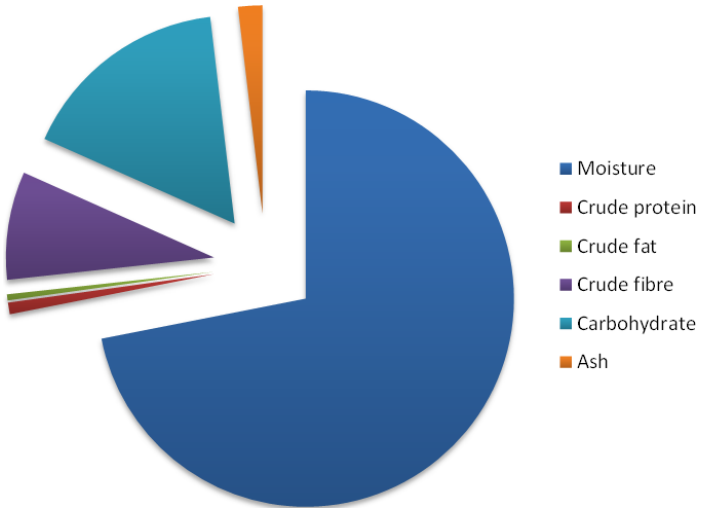

Figure 1. Proximate composition of *S. officinarum*.

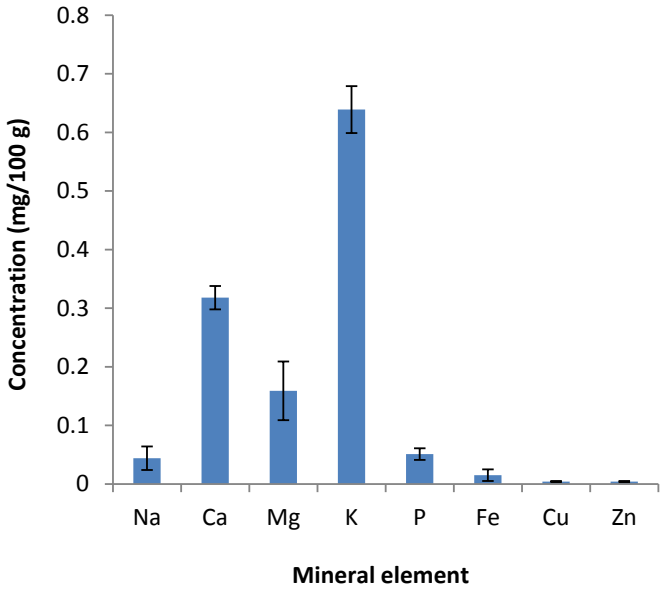

Figure 2. Mineral content of *S. officinarum.*

edible portion of the *S. officinarum* stalk. Potassium had the highest concentration (0.639 ± 0.04 mg/100 g), which was about twice that of calcium (0.318 ± 0.02 mg/ 100 g). Of the trace elements, iron had the highest concentration (0.015 ± 0.01 mg/ 100 g), while copper and zinc had the least concentration (0.004 ± 0.01 mg/ 100 g each) in the sugarcane stalk sample.

Phytochemical constituents

The results of the qualitative and quantitative assay of phytochemicals in the *S. officinarum* sample are presented in Table 1. There was a high correlation (r = 0.92) between the two methods of analysis. Alkaloids were the predominant (8.07 ± 0.04 µg/100 g) phytochemicals, followed by saponins (5.57 ± 0.01 µg/100 g). Flavonoids and cyanogenic glycosides were almost equal in concentration (1.52 ± 0.02 and 1.20 ± 0.01 µg/100 g, respectively), while tannins showed the least concentration (0.25 ± 0.01 µg/100 g) in the sample.

Antimicrobial activity

Table 2 presents the results of the antimicrobial activity of

Table 1. Concentration of phytochemicals in 100 g of *S. officinarum* stem.

Constituents	Qualitative	Quantitative (µg)
Flavonoids	++	1.52 ± 0.02
Saponins	+++	5.57 ± 0.01
Tannins	+	0.25 ± 0.01
Alkaloids	++++	8.07 ± 0.04
Cardiac glycosides	–	ND
Steroids	–	ND
Hydrogen cyanide	++	1.20 ± 0.01
Polyphenols	–	ND

Values are presented as mean of 3 determinations ± SD; ND = not detected; + = present in trace amount; ++ = present in low conc.; +++ = present in medium conc.; present in high conc. r = 0.92; p < 0.05.

Table 2. Antimicrobial activity of methanol extract of *S. officinarum* stem pulp.

Test organism	100	50	25	12.5	6.25	3.12	Methanol control	**Positive control
E. coli	15.33±0.67*	14.00±0.58*	13.33±0.33*	11.00±0.58*	10.33±0.33*	8.67±0.33*	N	19.33±0.88
K. pneumoniae	23.00±0.58	21.00±0.58	20.67±0.33	20.33±0.33	14.33±0.88*	12.33±0.33*	N	19.33±1.45
S. aureus	24.00±0.58*	18.33±0.33*	12.33±0.33	12.00±0.58	10.00±0.58	8.67±0.88*	N	14.33±0.88
P. aeruginosa	14.33±0.33	14.67±0.33	9.33±0.33*	8.33±0.33*	8.00±0.58*	6.00±0.33*	N	14.38±0.87
A. fumigatus	12.00±0.58*	11.33±0.88*	10.33±0.33*	8.00±1.15	6.33±0.33	6.00±0.00	N	7.00±0.58
C. albicans	14.00±0.58*	12.33±0.88*	9.33±0.33	8.33±0.33	6.33±0.88*	6.00±0.58*	N	9.67±0.88

Values are expressed as mean ±SEM of 3 determinants; N = no inhibition; * = significantly different from positive control; p<0.05; ** = Ampicillin was positive control for bacteria; Amphotericin B for fungi.

the *S. officinarum* stem pulp extract against the test microorganisms: *E. coli*, *K. pneumoniae*, *S. aureus*, *P. aeruginosa, A. fumigatus* and *C. albicans*. The extract showed growth inhibition against the various isolates at varying degrees. Among the bacteria, inhibition was highest against *S. aureus* (8.67-24.00 mm) which was even above that of the control antibiotic (Ampicillin, 14.33 mm), while inhibition against *P. aeruginosa* (6.00-14.38mm) was the least. Susceptibility appeared to be concentration-dependent as the mean zones of growth inhibition were generally highest at the 100 µg/ml extract concentration and lowest at the 3.12 µg/ml. For the fungal isolates, the extract showed a stronger growth inhibition against *C. albicans* (6.00-14 mm) than *A. fumigatus* (6.00-12.00 mm). Also, the antimicrobial activity of the extract appeared to be broad spectrum as it was independent of Gram reaction. Methanol which served as negative control showed no activity against any of the test organisms.

DISCUSSION

In Nigeria and other parts of the world, the stem pulp of *S. officinarum* (sugarcane) is consumed as

a snack due largely to its sweet taste occasioned by high sucrose content. In this study, the dry matter was found to be less than one-third of the sugarcane stalk, while over two-third represented the water content, indicating its usefulness as a natural and perhaps a safer source for rehydration compared to carbonated beverages. The moisture content obtained in this study (71.91%) was also found to compare favourably with the range reported in a recent study for four sugarcane varieties (65.72 to 67.29%) cultivated in India (Singh and Singh, 2012). The nutrient with the highest value in the dry matter was carbohydrate (58.55 g/100 g). However, this partly contrasts with the report of Madan et al. (1998) who showed a much higher carbohydrate content (74 to 96%) than that obtained in this study. The observed variation may be attributed to differences in study design. Whereas, carbohydrate evaluation in the present study was based on total dry matter, including the crude fibre content, Madan et al. (1998) based theirs on the total soluble solids (TSS) only, of the cane juice. The variation in study design could also account for differences in other proximal components observed between this study and others (Madan et al., 1998; Singh and Singh, 2012) such as crude protein, fat and ash contents.

Moreover, other factors such as the sugarcane cultivar, degree of maturity, soil type, and other cultivation factors, are also among the many sources of variation in the nutrient content of plant foods (Osagie and Onigbinde, 1998). Changes in the nutritional composition of plant foods caused by age, varietal and climatic/-environmental factors have long been recognized and documented (Bhatnager et al., 2004; Eppendorfer et al., 2006; Marques da Silva and Silva, 2006). Therefore some of the minor data variations observed may be location bound, since this study submits for the first time nutrients and chemical composition of sugarcane consumed in Calabar, Nigeria. Studies on the influence of intrinsic and environmental factors on sugarcane nutrient composition are highly warranted as these could offer more insight into the observed variations.

The present investigation further reveals appreciable quantities of individual elements in the sugarcane stem. It has been reported that the mineral composition of the sugarcane stem is highly dependent on its age and decreases as the internode grows older (Bakker, 1999). Studies by Bakker (1999) and van Dillewijn(1952) showed that the mineral composition of the stem is affected by translocation of some elements especially potassium and nitrogen from the maturing internodes towards the younger ones, while others like magnesium and phosphorus increase towards the bottom of the stalk. However, in this study, whole mature sugarcane stems as commercially available for consumption were used and included both the top and bottom parts. Thus, the mineral content recorded can be said to represent the proximate mineral composition of the sugarcane stem consumed in Calabar.

The study data also indicated the relative presence of alkaloids, saponins, flavonoids, hydrogen cyanide and tannins in the sugarcane. Quantitative data showed a relatively high percentage of alkaloids (8.07 ± 0.04 µg/100 g) and saponins (5.57 ± 0.01 µg/100g), suggesting a validation of its involvement in some pharmacological activities. Alkaloids have been reported to be the active components of numerous medicinal plants and plant-derived drugs with numerous physiological activities (Atangwho et al., 2009). It is plausible that *S. officinarum* owes its reported medicinal properties at least in part to the presence of these alkaloids. The presence of flavonoids in this study agrees with the findings from other studies (Vila et al., 2008; Colombo et al., 2009; Silvia et al., 2009) that have reported the occurrence of several flavonoids in sugarcane. Flavonoids are commonly known for their strong antioxidant activity and have been referred to as “nature’s biological response modifiers” because of strong experimental evidence of their inherent ability to modify the body’s reaction to allergens, viruses, carcinogens, and inflammatory agents (D’Mello et al., 2010; Tiwari and Rao, 2002).

The antimicrobial data obtained indicate that the microorganisms used in this study were susceptible to the *S. officinarum* extract but at varying degrees. Studies have shown for the most part that biological and medicinal properties of plant extracts, including the antimicrobial properties, conferment of disease resistance and reduction in the risk of major degenerative diseases (Liu, 2004) depend largely on their phytochemical composition (Atangwho et al., 2009). It is most likely that the observed antimicrobial activity of *S. officinarum* extract against the select organisms derives from the phyto-compounds (alkaloids, saponins, flavonoids, and tannins) found in the extract studied.

Conclusion

Taken together, data from the present investigation suggest that besides being the major source of sucrose, sugarcane available in Calabar can be exploited as a natural source for rehydration, nutrient supplementation and medicinal or pharmaceutical agents. The study further reveals that apart from eating sugarcane to obtain energy and nutrients, the crop can also be used to prevent or treat intestinal problems of microbial origin.

Conflict of interests

The authors have not declared any conflict of interest.

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