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PHYTOCHEMICAL AND PROXIMATE CHEMICAL COMPOSITION OF Stigma maydis FROM MAIZE PLANT GROWN IN A CLAY LOAM SOIL

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ABSTRACT

Stigma maydis also known as corn silk is a yellowish thread-like strand of female flower of maize belonging to *Graminae* family. Phytochemical constituents of corn silk of maize plant grown in a clay loam soil constitutes alkaloids, tannins, proteins, flavonoids, cyanogenic glucoside, terpenoid and saponins. The proximate analysis of corn silk showed that it has a moisture content of 86.70%, a protein content of 2.06%, an ash content of 1.20%, a lipid content of 0.20%, a level of carbohydrate of 9.31% and 0.53% of crude fibre. Potential use of corn silk is related to its properties and mechanism of action of its bioactive constituents such as flavonoids and terpenoids. This study indicates that corn silk possesses antihypertensive, antioxidant, anticancer, antidepressant, kaliuretic, neuroprotective properties. However, the phytochemical constituents and proximate chemical composition were suspected to be reduced as a result of high bulk density of the soil and ponding which may have delayed early shoot emergence, root formation, and stunts overall plant performance.

Keywords: Phytochemical, Proximate, corn silk, chemical composition

INTRODUCTION

Most of the medicinal plants species are discovered wildly, while some of them are cultivated in the farm. Some of the regular remedies and aromatic plants are used as a major ingredient in local herbal product namely; aloe vera, citrus, turmeric, cinnamon, jasmine, ginger, black pepper and many more. Studies have been carried out by local research institutes and universities to investigate the biochemical and pharmacological aspects of plant compounds. The utilization of corn silk may help in sustaining our environment by reducing the level of carbon cycle.

Consumers all over the world are becoming increasingly conscious of the nutritional value and safety of foods and their ingredients (Shahidi *et al*. 1992). At the same time, preference for natural foods and food ingredients has been increasing because they are generally believed to be safer, healthier, and less subjected to hazardous elements than the foods containing artificial food additives. Natural antioxidants especially phenolics are safe and bioactive. In recent years, considerable attention has been directed towards the identification of plants with antioxidant ability that may be used for human

consumption. Corn silk comes from the female flowers of the corn crop. At first, the hair color of the corn is usually light green, and then will turn red, yellow, or brown depending on the variety. Corn silk is discarded due to lack of knowledge on the healing properties on renal diseases, urinary problems, diuresis and other applications of corn silk. Corn silk is made up of the stigmas and styles of the maize plant from the grass (Gramineae) family. It is annual grass, growing up to 4 m tall. (Farham, 2003) reported that female inflorescences, the ears developed in leaf axil of the stalk which terminates the male florescence, the tassel. The leaves are arranged in two opposing rows along the stalk. Though the corn plants can grow up to 4 m height, their stems are not woody. It consists of leaves, stems, nodes and ears. Roughly, the leave is 5 – 10 cm wide and 50 – 100 cm long while the stems conventionally erect about 2 – 3 m with many nodes. Each node cast off flag leaves. Normally, under the leaves which were close to the stem, the ears grow. The ears hardly showed themselves until the emergence of the pale yellow silks at the end of the husks.

Corn silk is usually discarded as waste and not used for nutritional purpose. However, it has a great medicinal importance due to the presence of valuable bioactive phytochemical compounds. It has been traditionally used as an eff ective herbal remedy for the treatment of hyperglycemia, diabetes, obesity, hypercholesterolemia, hyperthyroidism, rheumatism, arthritis, gout, tumors, hepatitis, heart problems, jaundice, malaria, inflammation, asthma prostatitis, cystitis, nephritis, kidney stones, bed wetting, renal conditions, and other kidney-related diseases. Corn silk is also known to be urine laxative, antihypertensive, and immune enhancer. Corn silk tea has been used as diuretic for the treatment of urinal irritation. In combination with other herbs, corn silk has been found to be elective against mumps or inflammation of the bladder. It has also been reported to be useful in gonorrhea, acute and chronic cystitis, and bladder irritation due to uric acid and phosphate gravel. Therefore, food or dietary supplement that contained antioxidant properties are important in food daily intake. Fruits and vegetables are good sources of antioxidants. It prevents our bodies from developing diseases like cancer, heart problem, stroke and Alzheimer (Di Matteo and Esposito, 2003).

The research seeks to investigate the proximate chemical composition and content of biologically active ingredients in corn silk (*maydis stigmata*) of maize plant grown in clay loam soil.

According to Duiker (2004) and Hanna and Al-Kaisi (2009), when soil is compacted, the major change that occurs to the soil is in its bulk density, defined as "the mass of oven-dry soil in a standard volume of soil, often given as grams per cubic centimeter (g cm-3)". Generally, with compaction, there is usually a complete alteration of the physical properties of the soil, with notable changes in the soil bulk density, soil strength, porosity and hydraulic properties such as infiltration rate and hydraulic conductivity. Mada et al. (2013) investigated the effect of soil compaction on soils in Southern Adamawa State, Nigeria and observed that soil "compaction due to heavy tractor traffic changes the numerical values of soil bulk density". It increases the soil bulk density and consequently retards or diverts the flow of water in the soil resulting in

ponding or excessive runoff. This is because there is a reduction in the hydraulic properties of the soil, like infiltration and percolation, which are determined as functions of time, in rates. While infiltration is the entering of water from the soil surface into the soil, percolation is the gradual movement of the water downward inside of the soil. The reduction of the soil infiltration rate usually "has a serious consequence to water quality and sediment transport, particularly on sloping soils" (Hanna and Al-Kaisi, 2009).

MATERIALS AND METHODS

The study was conducted at the Plant Anatomy and Physiology Research Laboratory, University of Port Harcourt. The corn samples used were grown on a clay loam soils obtained from Bundu waterside and Eagle Island, Port Harcourt in greenhouse pot experiments at the University of Port Harcourt. The sample used in this study was ±80 – 90 days of age.

Fresh corn silk, as much as 3g, was washed with distilled water, dried in an oven at 60°C for 24hr(31mins) until the final moisture content was 10-11%, ground into powder using a grinder, vacuum packed and stored below -20°^{C} until the analysis was performed.

Soil characteristics determined were: pH, OM, CEC, Texture, Moisture content, and bulk density. pH was determined using electrometric method, Wet oxidation method modified by Eno et al., 2009 was used to determine OM, Soil texture was determined by hydrometer method, and bulk density was determined using the core method modified by Eno et al., 2009. CEC was determine by the EDTA method.

Proximate analysis of corn silk:

The moisture content was determined by drying at 105°C in an oven, until a constant weight was reached. For total ash determination, the corn samples were weighed and converted to dry ash in a muffle furnace at 450 and at 550°C for incineration. The Kjeldhahl method was used for crude protein determination. Crude fat content was determined by extraction with hexane, using a soxhlet apparatus. All these determinations were carried out according to AOAC (1990). Carbohydrate content was determined by calculating the difference between the sum of all the proximate compositions from 100%. Energy values were obtained by multiplying the carbohydrate, protein and fat at water conversion factors of 17, 17 and 37, respectively.

To determine %Ash using furnace method:

1 gram of the dried sample was weighed into the porcelain crucible which was previously preheated and weighed. The crucible was inserted into a muffle furnace and regulated to a temperature of 630°C for three hours and allowed to cool at room temperature and reweighed

$$\%\text{Ash} = \frac{\text{Weight of crucible + Ash sample – weight of crucible} \times 100}{\text{Weight of sample}}$$

Carbohydrate determination by Cleg Anthrone method:

0.1g of sample was weighed into a 25ml volumetric flask, 1ml distilled water and 1.3ml of 62% perchloric acid was added and shaken for a period of 20mins to homogenize completely. The flask was made up to 25ml mark with distilled water and stoppered. The solution formed was filtered through a glass filter paper, allowed to sediment and decanted. 1ml of the filtrate was collected and transferred into a 10ml test tube which was diluted to volume with distilled water. 1ml of working solution was pipetted into a clean

test tube and 5ml Anthrone reagent was added. 1ml distilled water and 5ml Anthrone reagent were mixed. The mixture was read at 630nm wavelength using the 1ml distilled water and the 5ml Anthrone reagent prepared as blank. Glucose solution of 0.1ml was prepared and was treated as the sample with Anthrone reagent. Absorbance of the standard glucose was read and the value of carbohydrate as glucose was calculated using the formula below.

$$\%\text{CHO as glucose} = \frac{25 \times \text{absorbance of sample}}{\text{Absorbance of standard glucose} \times 1}$$

Crude fibre determination:

2g of sample was extracted with petroleum ether (W1), sample was boiled under reflux for 30minutes with 200ml of dilute hydrochloric acid and filtered. The residue was properly washed with water until it becomes acid free. The residue was transferred into a beaker and boiled for another 30 minutes with 200ml of dilute sodium hydroxide solution and filtered, transferred into ignited crucible. The residue was then washed 3 times with 20ml of ethanol and 2 times with 10mls ether. The residue was dried in an oven and cooled, then weighed (W2). The dried residue was transferred into furnace and ignited, cooled and weighed (W3).

$$\%\text{Crude fibre} = \frac{(W2-W3) \times 100}{W1}$$

Determination of Lipid by Soxhlet Extraction Method:

2g of sample was inserted into a filter paper and was placed into a soxhlet extractor. The extractor was placed into a pre-weighed dried distillation flask. Then the solvent (acetone) was introduced into the distillation flask via the condenser attached to the soxhlet extractor. The setup was held in place with a retort stand clamp. Cooled water jet was allowed to flow into the condenser and heated solvent was refluxed as a result. The liquid solvent chamber was extracted in the process of refluxing. When the lipid was observably extracted completely from the sample under test, the condenser and the extractor were disconnected and the solvent was evaporated to concentrate the lipid. The flask was then dried in the air oven to constant weight and re-weighted to obtain weight of lipid.

$$\%\text{lipid} = \frac{\text{weight of flask and extract} - \text{weight of empty flask}}{\text{Weight of extracted sample}} \times \frac{100}{1}$$

Determination of Protein:

Crude Protein was estimated using micro-kjeldahl method with KELPLUS nitrogen estimation system. 10ml concentrated H_2SO_4, 0.2g corn silk sample and 3g digestion mixture were taken in digestion tubes. Digestion system was switched on and the initial temperature of 100°C was set by pressing the temperature controller keys. The temperature controller was reset to 420°C. Mixture was heated till digested and proper water flow was regulated to ensure absolute removal of acid fumes. After digestion contents were cooled and distilled in classic-DX (VA).

$$\text{Crude Protein (\%)} = \frac{14.01 \times (S-B) \times N \times 100}{W \times 1000}$$

Moisture by Air Oven Method:
1g of the sample was weighed into a clean dried porcelain evaporating dish, this was placed in an oven to maintain a temperature of 105˚C for six hours, and evaporating dish was cooled in desiccators to room temperature, re-weighed and recorded.

$$\%\text{Moisture} = \frac{\text{weighed of fresh sample} - \text{weight of dried sample}}{\text{Weight of sample used}} \times \frac{100}{1}$$

Phytochemical Compositions (screening of the extracts):

Phytochemical analysis of the samples was performed in order to ascertain the presence of bioactive compounds by using described methods (Hussain et al., 2011). The phytochemicals determined included alkaloids, flavonoids, saponins, tannins, glycosides.

Determination of Alkaloids by Harborne,1973 method:

5g sample was weighed into 250ml beaker and 200ml of 10% Acetic acid in methanol was added and covered and allowed to stand for 4hrs. This was filtered and the extracted was concentrated on a water bath to one quarter of the original volume, concentrated ammonium hydroxide was added drop wise to the extract until the precipitate was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide to settle solution and then filtered. The residue which was dried and weighed.

$$\text{Alkaloids (\%)} = \frac{\text{weight of filter paper \& residue-weight of empty filter paper} \times 100}{\text{weight of sample used}}$$

Determination of Flavonoids:
5g of the plant sample was extracted repeatedly with 100ml of 80% aqueous ethanol at room temperature. The whole solution was filtered through a filter paper No 42 (125mm). The filtrate was later transferred into a crude evaporated into dryness over water bath and weighed to a constant weight.

$$\text{Flavonoid (\%)} = \frac{\text{weight of filter paper \& residue - weight of empty filter paper} \times 100}{\text{weight of sample used}}$$

Determination of Saponins by Soxhlet Extraction Method (Hussain et al., 2011):
2g of sample was inserted into a filter paper and was placed into a soxhlet extractor. The extractor was pre-weighed and dried in a distillation flask. Then the solvent (methanol) was introduced into the distillation flask via the condenser end attached to the soxhlet extractor. The set-up was held in place with a retort stand clamp. Cooled water jet was allowed to flow into the condenser and heated solvent was refluxed as a result. The lipid in the solvent chamber was extracted in the process of continuous refluxing. When the lipid was observably extracted completely from the sample under test, the condenser and the extractor were disconnected and

the solvent was evaporated to concentrate the lipid. The flask was then dried in the air oven to constant weight and re-weighed to obtain the weight of lipid.

$$\%\text{Lipid} = \frac{\text{weight of flask and extract - weight of empty flask}}{\text{weight of sample extracted}} \times \frac{100}{1}$$

Braymer's test for Tannins:
2ml of the crude extract was treated with 10% alcoholic ferric chloride solution. The observation of a blue or greenish colored solution indicates a positive result.

$$\%\text{Tannins} = \frac{\text{C (mg)} \times \text{extract volume(ml)}}{10 \times \text{aliquots (mg)} \times \text{sample weight}}$$

Test for Glycosides:
Excess 20% Potassium hydroxide (KOH) was added to a quantity of the crude extract. Equal amounts of Fehling's solutions A and B was added and the mixture heated on a water bath for 2 minutes. The formation of a brick red precipitate indicates the presence of glycosides

Moisture by Air Oven Method:
1 gram of the sample was weighed into a clean dried porcelain evaporating dish, this was placed in an oven to maintain a temperature of 105˚C for six hours, the evaporating dish was cooled in desiccators to room temperature then it was re-weighed and recorded.

$$\%\text{Moisture} = \frac{\text{weighed of fresh sample – weight of dried sample}}{\text{Weight of sample used}} \times \frac{100}{1}$$

RESULTS AND DISCUSSION

It has been asserted that the changes that occur in the soil physical properties as the soil is compacted have adverse effects on the growth of planted crops, like limiting yields and inhibiting effective site management for crops (in agreement with Passioura, 2002; Rooney et al., 2001; Suddith et al., 2008; and De-Jong-Hughes et al., 2001). Jayan et al. (2006) reported that soil compaction affects the emergence of maize crop. Igoni and Ayotamuno (2016) found that "Maize yield on a sandy loam soil in a humid tropical environment in Nigeria is reduced by compaction induced in the soil. The same applies to clay loam as shown in Table 1 below. The compaction in this study delayed early shoot emergence, root formation, and stunts overall plant performance due to increased bulk density and ponding. Since the corn silk showed evidence of accumulation of phytochemical and proximate composition in this study, it could be that the concentration of the phytochemicals and proximate composition would have been higher if the corn silk was grown in a better textured soil and under a natural condition.

Table 1: Physico-Chemical Parameters of Soil

Soil Parameters	Bundu waterside	Eagle Island	Mean
pH	5.8	6.0	5.9
OM	13.2	12.7	13.0
CEC (cmol kg^{-1})	40.4	38.9	39.7
Moisture (%)	39.0	40.5	39.8
Clay (%)	34.0	35.1	34.6
Sand (%)	48.0	47.9	48.0
Silk (%)	18.0	18.0	18.0
Texture	Clay loam	Clay loam	Clay loam

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Fig. 1 shows that the corn silk tested have different levels of the chemical components. The moisture, ash, protein, fat and carbohydrate content are significantly in corn silk. Plants that have different genetic characteristics will have different adaptations to the environment. This will also lead to differences in the rate and metabolic products created. Therefore, the factors that are suspected to contribute to the differences in the chemical composition of the corn silk are genetic traits, different varieties, growing conditions, antioxidant component, level of maturity at harvest time, storage conditions after harvest and the production process. Variations in composition are influenced by several factors, such as differences in varieties, the climate where it grows, soil fertility, the care of the plant and the treatment method. The chemical composition of corn grain, such as proteins, lipids and starches, is more influenced by genetic traits. Plants that have different genetic characteristics will have different adaptations to the environment.

In this study, corn silk has a moisture content of 86.70% which could be influenced by the texture of the soil, a protein content of 2.06%, an ash content of 1.20%, a lipid content of 0.20% a level of carbohydrate of 9.31% and has an amount of 0.53% of crude fibre. The differences are due to the accumulation of hydrocarbons at a stage of maturity and this affected the lipid composition. The protein content at the different maturity levels is probably influenced by the function and biosynthesis of amino acids that occurred during the development process. Amino acids are actively metabolized in the immature cob in the early stages of hair emergence to regulate the growth of seedlings. Evidently, the type of soil in which maize crop is cultivated plays a significant role in its yield and growth. Maize is not suited to soils that are acidic, salty or very wet. The soil texture should be intermediate: sandy, sandy-loam to sandy-clay loam (Ministry of Agriculture, Food and Rural Affairs, 2009).

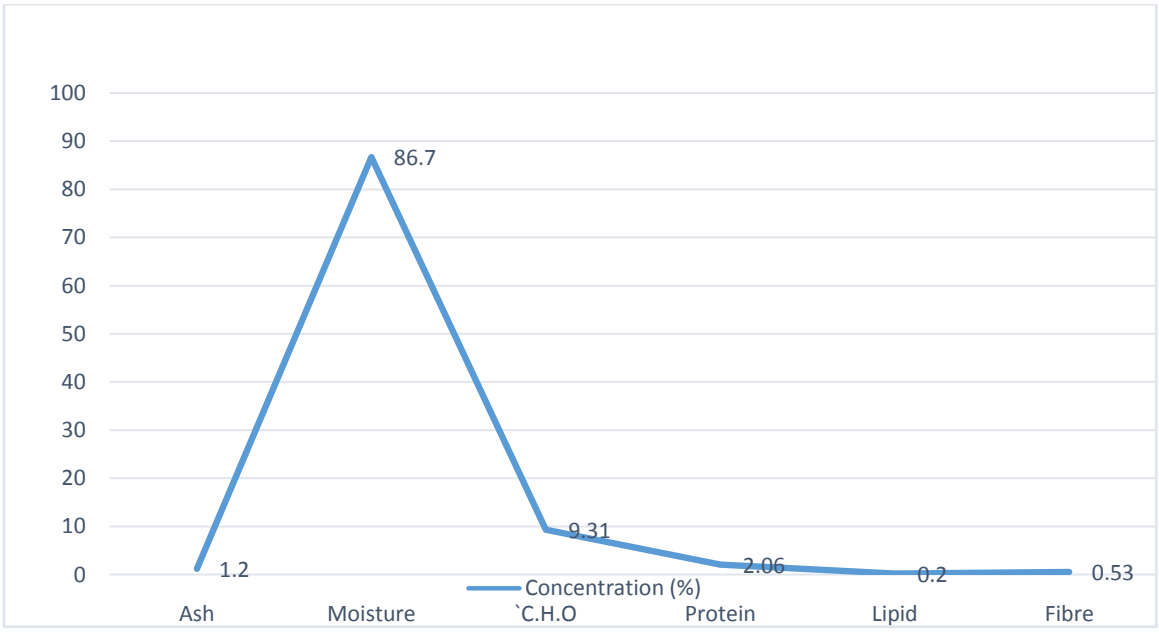

Fig. 1: **Results for the Proximate Analysis of Corn silk**

Prepared corn silk extracts were analyzed for the presence of alkaloids, cyanogenic glycosides, saponins, tannins and flavonoids. The content of flavonoids is recorded to be 0.45%. The difference is believed to be because corn silk contains different levels of flavonoid compounds, depending on agronomic factors, varieties and processes. Flavonoids will be degraded at temperatures above 100°C. Flavonoids are sensitive to heat because of the hydroxyl group and ketones, as well as unsaturated double bonds. The gravimetric analysis for total alkaloid contents in corn silk, *radicata* exhibited that high alkaloid contents were present in the corn silk

sample 2.72%. Only tannin recorded higher amount. The tannins content of corn silk was recorded to have the highest amount (Fig. 2). Among the solvents used the methanol solution was registered high amount of tannin at 19.37%. The saponin content is recorded to be same amount as flavonoid at (0.45%), their biological activity has led to the emergence of saponins as commercially significant compounds with expanding applications in food, cosmetics, and pharmaceutical sectors .The cyanogenic glycoside content was recorded to be the lowest at 0.01%, the toxicity being dependent on the release of hydrogen cyanide. Saponins act as antimicrobial activity and extremely to cold-blooded animals, but toxicity to mammals is low.

The activity of saponins would be of immense help to diabetics especially those with diabetic foot ulcers. Herbs which have been used for centuries in treating various illnesses play a major role in forming the basic platform of modern medicines [Wan, 2…010; Nurhana 2010].

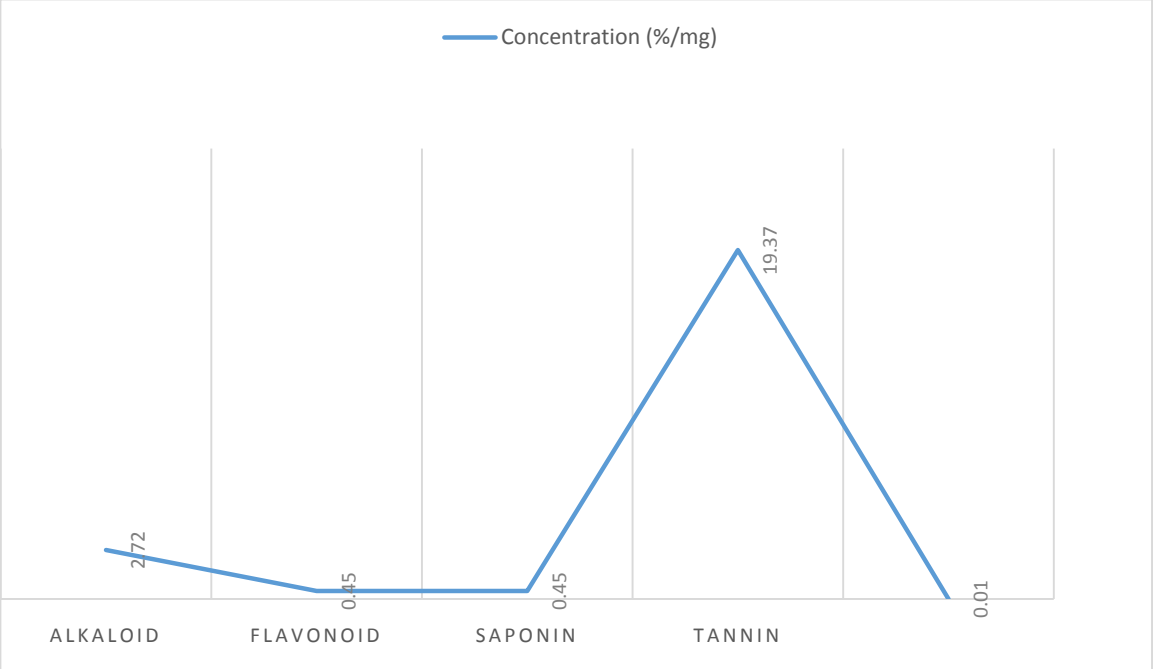

Fig 2: ***Results for the Phytochemical Composition of corn silk***

CONCLUSION

All parts of corn are good sources of phytochemical compounds which possess antioxidant potentials. Corn seed have a valuable role in human nutrition, while corn silk has a great medicinal importance such as Tannins, alkaloids, flavonoids, saponins and cyanogenic glucosides. Due to the presence of a variety of bioactive phytochemical compounds, the present study therefore, confirms that the corn silk has the potential to act as a source of useful drugs because of the presence of various phytochemical constituents. These phytochemical constituents seemed to be the potential to act as a source of useful drugs and also to improve the health status of the consumers as a result of the presence of various compounds that have vital role for good health. The proximate analysis of corn silk showed that it has a moisture content of 86.70%, a protein content of 2.06%, an ash content of 1.20%, a lipid content of 0.20%, a level of carbohydrate of 9.31% and 0.53% of crude fibre. This highlights the potential of corn silk as an herbal drug for healthcare applications. However further studies are necessary for the complete understanding of the antidepressant activity of corn silk. The compaction of soils in this study delayed early shoot emergence, root formation, and stunts overall plant performance due to increased bulk density and ponding (in agreement with Duiker, 2004). Since the corn silk showed evidence of accumulation of phytochemical and proximate composition in this study, it could be that the concentration of the phytochemicals and proximate compositions would have been higher if the corn silk was grown in a better textured soil and under natural condition.

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