IAA Journal of Applied Sciences 9(1):74-83, 2023. ISSN: 2636-7246

Phytochemical Screening and Antimicrobial Potency of Corn Silk (*Zea mays stigma)* from Corn Planted in Abakaliki Metropolis of Ebonyi State, Nigeria.

Nwafor R. N[1], Nwokonkwo D.C[2] and Oti Wilberforce[3]

Department of Industrial Chemistry Faculty of Science, Ebonyi State University.
Email:nkyrosy@gmail.com

ABSTRACT

Corn silk is the long, thread-like strands of plants material that grow underneath the husk of a fresh ear of corn. It is a shiny, thin fibers that aid the pollination and growth of corn that are used in traditional herbal medicine practices. This research was aimed at exploring the phytochemicals present in corn silk *(Zea mays stigma*) obtained from Abakaliki in Ebonyi State of Nigeria, in addition to the antimicrobial activity of these compounds. Corn silk was collected from seven corn farm fields dried and ground into powder. The powdered corn silk was extracted with organic solvents and the crude extract subjected to phytochemical analyses. The result obtained revealed the presence of the following bioactive compounds flavonoids, saponins, phenols, terpenoids, glycosides, and tannins. THE quantitative analysis of the dried ground sample revealed the concentration of the following bioactive components: alkaloids 0.27%, flavonoids 1.2%, saponins 2.0%, tannins 77.048mg/g, phenols 34.673mg/g and glycosideS 189.44mg/100g. The crude ethanolic extract showed antimicrobial potency against five microorganisms via *E. Coli*, *Staphylococus species*, *Klebisiell*a *species, Salmonella species and Pseudonomas species* at different concentrations with inhibitory zone diameter ranging between 5mm and 16mm. Gentamycin was used as a standard antibiotic and the results obtained significantly revealed the potential effect of corn silk against the pathogens examined.
Keywords: Antimicrobial, corn silk, phytochemicals screening, qualitative, quantitative.

INTRODUCTION

Plants are foods for animals and man. In fact, it has been part of human culture in Africa and most countries of the world to use plants as food and also to treat sicknesses and diseased conditions of both man and animals [1, 2, 3, 4]. This implies that plants have for long had wide acceptance among the human populace [5, 6]. Plants contain chemical compounds produced as a result of their growth metabolic process [7]. These chemical compounds have medicinal values because of the ability of their chemical substances to produce physiological action on the human body. These chemical compounds are referred to as phytochemicals or secondary metabolites [8]. Among the Phytochemicals in plants are alkaloids, tannins, saponins, flavonoids, glycosides, phenols, phlobatannin, coumarins, terpenoids and cardiac glycosides for growth and normal functioning of the body [9].

There are different kinds of plants which are cultivated for food and for health purposes. One of such plants is corn. Corn plant consists of several parts with various functions. The major components of corn are corn fruit or kernel, corn silk, husk, leaves and stem. Corn kernel from corn plant is edible while corn silk, husks, leaves and stem are sometimes used as a feedstock or mainly thrown away. In Nigeria, corn silk is normally discarded after it has been separated from the corn fruits [10]. The elimination of this agricultural by-product is believed to be associated with the lack of knowledge regarding the numerous benefits of corn silk. Corn silk is well-known to give value

to men's health in other countries such as United States of America and China. In addition, corn silk is believed to contain various essential phytonutrients and can significantly heal some illnesses related to kidney, heart and blood pressure. Besides that, corn silk has been used as a functional ingredient in various preparations of foods, cosmetics and pharmaceutical products [11, 12]. Therefore, this paper looked at the qualitative and quantitative phytochemicals present in corn silk *(Zea mays stigma*) obtained from Abakaliki in Ebonyi state of Nigeria and its antimicrobial activity on five different human pathogens.

MATERIALS AND METHODS

Qualitative determination of phytochemicals constituents of corn silk

The phytochemicals present in the corn silk were qualitatively determined in three different solvent extracts namely water, methanol and ethanol. Each of the extracts was exposed to phytochemicals analysis for identification of chemical components using procedures as previously [13, 14, 15, 16, 17].

Tests for flavonoids

Alkaline reagent test: Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow solution which becomes colorless on addition of dilute HCl acid, indicates the presence of flavonoids. Lead acetate test: Extracts were treated with few drops of lead acetate solution. Formation of yellow color precipitate indicates the presence of flavonoids. Ferric chloride test: Few drops of 10% w/v ferric chloride solution was added to each of the extracts. The resulting blackish green color designates the existence of flavonoids.

Tests for tannins

Gelatin solution: 5% w/v Gelatin solution and 10% w/v sodium chloride solution were poured into the solution of the extracts. Formation of white precipitates indicates the presence of tannins. Ferric chloride test: 10% w/v Ferric chloride solution was added to the extract solution. The appearance of green or brownish green color indicates the presence of tannins.

Bromine water test: The bromine solution was added to the extract solution. The appearance of yellow precipitates indicates the presence of tannins.

Tests for terpenoids

Liebermann–Burchard test: The extract was treated with acetic anhydride and chloroform followed by concentrated sulfuric acid and shaken well. Appearance of green or green-blue color indicates the presence of steroids and terpenoids.

Salkowski test: Each extract solution was added to chloroform, and then concentrated sulfuric acid was carefully poured into the mixture. The development of reddish brown at the interface confirmed the presence of terpenoids.

Tests for alkaloids

Dragendroff's test: The extract solution was treated with Dragendorff's reagent (bismuth potassium iodide) and the development of orange red precipitates indicates the existence of alkaloids.

Wagner's test: The Wagner's reagent (iodine in potassium iodide) was added to the extract solution. The emergence of reddish brown precipitates indicates the existence of alkaloids.

Mayer's test: Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow colored precipitate indicates the presence of alkaloids.

Tests for phenols

Ferric Chloride test: Extracts were treated with 3-4 drops of 1% ferric chloride solution. Formation of bluish black or intense purple

coloration indicates the presence of phenols.

Test for saponin

Froth test: Extracts were diluted with distilled water of 20ml and this was shaken vigorously in a graduated cylinder for 15minutes. Formation of 1 cm layer of foam that persists for few minutes indicates the presence of saponins. Foam test: 0.5 gm of extract was shaken with 2ml of water. The formation of emulsion on addition of three drops of olive oil indicates the presence of saponin.

Test for glycosides

2ml of the extracts were treated with 3ml of chloroform and 10% ammonia solution and the formation of pink coloration showed a positive test for glycosides.

Test for carbohydrates

Extracts were dissolved individually in 5ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates. Molisch's test: Filtrates were treated with 2 drops of alcoholic α-naphthol solution in a test tube. Formation of the violet ring at the junction indicates the presence of carbohydrate.

Benedict's test: Filtrates were treated with Benedict's reagent and heated and heated gently. Orange red precipitate indicates the presence of reducing sugar.

Quantitative determination of phytochemicals constituents of corn silk

Alkaloids determination

The sample was soaked in a 50ml of 10% acetic acid in ethanol. The mixture was allowed to stand for 4hr at room temperature thereafter the mixture was filtered through a Whatman filter paper. The filtrate was concentrated by evaporation over a steam bath to a quarter of the original volume followed by addition of few drops of concentrated ammonium hydroxide solution until in excess when precipitation reaction is completed. The resulting precipitate was recovered by filtering using filter paper. After filtration, the precipitates were washed with 1% of ammonia solution and dried in an oven at 60°C for 30min, it was cooled in a desiccator and weighed with the aid of weighing balance. The weight of the alkaloids was determined by difference and expressed as the percentage weight of the sample analyzed as shown in equation 1.

$$\text{Percentage alkaloids} = \frac{W2 - W1}{\text{weight of sample}} \times \frac{100}{1} \quad \text{equation 1}$$

Where

W_1 = weight of filter paper

W_2 = weight of filter paper + alkaloid precipitate

Flavonoid determination

This was determined by gravimetric method using the method described by [16, 17, 18, 19, 20, 21]. 5g of the sample was boiled in 50ml of 2M HCl solution for 30 min under reflux. The solution was allowed to cool and filtered through Whatman filter paper. The filtrate was treated with 5ml of ethyl acetate. The resulting solution was filtered using a weighed filter paper recorded as W_1. The residue on the weighed filter paper was placed in an oven to dry at 60°C, then cooled in a dessicator and reweighed until constant was obtained and the weight obtained was recorded as W_2. The resulting weight difference was expressed as the percentage flavonoid in the sample as shown in equation 2.

$$\% \text{ Flavonoid} = \frac{W2 - W1}{\text{weight of sample}} \times \frac{100}{1} \quad \text{equation 2}$$

Where;

W_1= Weight of empty filter paper.

W_2 = weight of empty filter paper + flavonoid

Saponin determination

This was determined by double solvent extraction gravimetric method (Harborne, 1973). 5g of the sample was mixed with 100ml of 20% aqueous ethanol solution. The mixture was heated with periodic agitation in a water bath for 4hrs at 55°C. After agitation and heating, the mixture was filtered through a Whatman filter paper no 1 (185mm) and recorded as filtrate 1. The residue obtained was subjected to further extraction with 100ml of 20% ethanol and filtered, the filtrate was recorded as filtrate 2. Then, the two filtrates were pulled together. The combined extract was concentrated to 40ml at 90°C in a water bath and transferred into a separatory funnel where 40ml of diethyl ether was added and shaken vigorously. Separation was done by partition during which the ether layer was discarded and the aqueous layer recovered. This purification exercise was repeated severally until the aqueous layer became very clear in colour. 60ml of n-butanol was added and extracted twice. The combined extract was washed with 5% NaCl solution and evaporated to dryness in a pre-weighed evaporating dish (W_1). This was dried in an electric oven at 60°C and recorded as W_2 after obtaining a constant weight. The saponin content was calculated as a percentage of the original sample using equation 3.

$$\% \text{ Saponins} = \frac{W2 - W1}{\text{weight of sample}} \times \frac{100}{1} \quad \text{equation 3}$$

Where:

W_1= weight of beaker

W_2 = weight of beaker + saponins

Tannin determination

This was determined using Folin Denis Reagent as described by Makkar *et al.,* (1993), In this method, a standard calibration curve was prepared and the absorbance (A) against concentration of tannins at specific wavelength. 5g of the corn silk, 40ml of diethyl ether containing 1% acetic acid v/v were properly mixed to remove the pigment materials. The supernant was discarded after 5min and 20ml of 70% acetone solution was added and the flask was covered very well and shaken with electric shaker for 2hr for extraction. After the extraction period, the solution was filtered and the filtrate used for analysis. 5ml of the filtrate was measured into a tube and the volume of the solution was made up to 10ml with distilled water. 0.5ml of Folin Denis reagent was added to the solution in the tube and mixed properly. Then 2.5ml of 20% Na_2CO_3 solution was added and mixed thereafter, the mixture was allowed to stay for 40mins at room temperature before absorbance was taken at 725nm using spectrophotometer and the concentration estimated from tannin standard curve and also using the equation 4.

$$\text{Tannin (mg/100g)} = \frac{C}{W} \times \frac{Vf}{Va} \times D \quad \text{equation 4}$$

Phenol determination

5g of the sample was put in a beaker containing 50ml petroleum ether and left to stand for 2hr for defatting. The resulting solution was filtered through a Whatman filter paper. The residue obtained was resoaked with diethyl ether, the solution was covered and heated in a water bath for 15 min. 5ml of the extract was pipetted into a 50ml flask, then 10ml of ammonium hydroxide (NH_4OH) was added along with 5ml concentrated Amylalcohol. The resulting solution was made up to the mark and left to react for 30min for colour development. The optical density was measured at 760nm. The

values obtained were used to calculate the phenol content using equation 5.

Phenol

$$(mg/100g) = \frac{C}{W} \times \frac{Vf}{Va} \times D \qquad \text{equation 5}$$

Where;

C = concentration of standard,

Vf = volume of filtrate,

Va -= volume analyzed

D = Dilution factor

Glycoside determination

5g of the sample was place in 250ml Erlenmeyer flask containing 100ml distilled water. The solution was shaken with mechanical shaker for 3hrs and then filtered through a Whatman filter paper (185mm). 1ml of the filtrate was pipetted into a test tube and 3,5-dinitrosalicylic acid solution was added into the test tube and the resulting solution was boiled in a water bath for15min. The mixture was cooled in cold water. Thereafter, 10ml of distilled water was added into the test tube. The absorbance was ascertained at 540nm with the help of spectrophotometer. The glycoside content was calculated using a standard curve and equation 6.

$$\text{Glycoside (mg/100g)} = \frac{C}{W} \times \frac{Vf}{Va} \times D \qquad \text{equation 6}$$

Where;

C = concentration of standard,

Vf = volume of filtrate,

Va = volume analyzed

D = Dilution factor

Antimicrobial activity screening

40g of the ground powdered corn silk was sent to the Laboratory of Applied Microbiology Department, of Ebonyi State University for antimicrobial activity tests. The 40g was of the sample was macerated in 200ml ethanol for 24hr and the solution was shaken intermittently with an electric shaker to aid extraction. The resulting solution was filtered and the filtrate collected was used for the test. The antimicrobial activity of the ethanolic extract of the corn silk was investigated against 5 pathogen isolates obtained from Clinical Pathology Department of the same University. These include gram negative bacteria, *Eschorichia coli, Pseudomonas species, Klebsiella species* and gram-positive *Staphyloocceus species* and *Salmonella species*. The agar well diffusing method was applied in the investigation, the agar known as Mueller Hinton was used for the culture and was prepared in line with the manufacturer's instruction then sterilized using autoclave 121°C and allowed to cool before pouring into a sterilized petri dish. 6mm corkborer was used to bore the hole on the agar culture plates. Different concentrations of the ethanol extract were placed on the culture alongside with 30µg of gentamycin which was used as a control and incubated at 37°C for 24hr. After the incubation period,

the antimicrobial activities of the extract alongside the control were determined by measuring their inhibitory zone diameter.

RESULTS

Table 1: Qualitative Phytochemicals present in the extracting solvents

Phytochemicals	Water	Methanol	Ethanol
Alkaloid	–	–	–
Flavonoid	–	+	+
Saponin	+	–	–
Tannin	+	+	+
Phenols	+	+	+
Terpenoid	+	+	–
Glycoside	+	+	+

Keynote + = Present

\- = Absent

Table 2: Mean (± SD) of quantitative phytochemicals in corn silk

Phytochemicals	Mean ± SD
Alkaloid (%)	0.4 ± 0.2
Flavonoid (%)	1.2 ± 0.1
Saponin (%)	2.00 ± 0.2
Tannin $_{mg/g}$	77.05 ± 0.19
Phenols $_{mg/g}$	34.67 ± 0.05
Glycoside $_{mg/100g}$	189.44 ± 1.32

n = 3, ± = SD

Table 3: Measurement of Inhibitory Zone diameters (mm) of the Ethanolic Corn silk Extract in Different Concentrations

S/N	Organism names	100	50	25	12.5	Control antibiotics gentamycin(µg)	MIC	MBC
1	*E. coli*	16	10	--	--	22 mm	50	50
2	*Klebsiella Species*	12	7	--	--	22 mm	50	100
3	*Salmonella Species*	13	6	--	--	20 mm	50	100
4	*Staphylococcus Species*	14	8	--	--	18 mm	50	100
5	*Pseudomonas Species*	10	5	--	--	16 mm	50	--

Key: MIC= Minimum Inhibitory Concentrations

MBC= Minimum Bactericidal Concentrations

-- = Nil

mm= Millimetre

DISCUSSION

Phytochemical screening

The medicinal value of plants lies in the phytochemical (bioactive) constituents of the plant which shows various physiological effects on human body. The qualitative phytochemical results revealed the presence of tannin; tannin is one of the major active ingredients found in plant-based medicine [19], carbohydrate which is energy given foods, phenol and glycoside in all the solvents used in extraction, the presence of saponin which responded positively to froth and emulsion tests was found in water (aqueous) extract and terpenoid was found in water and methanol extracts. The result also revealed the presence of flavonoids in ethanol and methanol extracts and absence in water extract while alkaloid was absent in all the solvents used. These results are slightly different when compared to the work done by [20] which revealed the presence of tannin, carbohydrate, flavonoids, phenol, saponin, glycoside and alkaloids in methanol extract of cooked corn silk.

Similarly, [21] reported a qualitative analysis of baby corn silk in aqueous and ethanol extracts. From their results there were absence of alkaloids in the two solvents used and absence of terpenoids in the aqueous extract while other secondary metabolites were present in both solvents. The differences in these results could be as a result of corn hybrids, maturity stage or the nature of the corn silk used i.e., cooked or raw and baby or matured. The quantitative phytochemical screening as reported on Table 8 revealed alkaloid 0.27%, flavonoid 1.2%, saponin 2.0%, tannins 77.048mg/g, phenols 34.673mg/g and glycoside 189.44mg/100g which suggest that alkaloid is insignificantly present which is in line with its qualitative result. Flavonoid and saponin in trace

amounts which informed their absence in some solvents of extraction. The quantity of tannin obtained is relatively commensurate with the report given by [2] on *Moringa oleifera* wood as 930mg/100g which suggest that corn silk has reasonable amount of tannin and should be exploited for industrial purposes based on the fact that tannin one of the major active ingredients found in medicinal plants. The phenol result of 34.673mg/g as reported showed high concentration when compared with results recorded by Duru (2020) on phenol presence in corn husk evaluated at 12.35mg/g and also of *moringa oleifera* wood at 3.58mg/g by [2]. On another note, glycoside which recorded as 189.44mg/g is lower compared to tannin and phenol which recorded 77.048mg/g and 34.673mg/g respectively. The glycoside concentration is also low when compared with the results of work done by [2] which recorded glycoside to be 800mg/100g. Thus, the phytochemical concentration of glycoside is indeed low in corn silk and may not be a good source for it.

Antimicrobial analysis

The ethanol extract showed reasonable antimicrobial activity against *E. coli, Klebsiella species, Salmonella species*, *Staphylococcus species* and *Pseudomonas species* by demonstrating inhibitory zone 16mm, 12mm, 13mm, 14mm and 10mm at 100mm concentration and 10mm, 7mm, 6mm, 8mm and 5mm at 50mm concentration respectively. The extract showed more sensitivity to *Staphylococcus* species followed by *E coli, Pseudomonas species* and *Salmonella species* and least sensitivity to *Klebsiella species* when compared with the results of gentamycin which is used as control. Zones of observable inhibitory growth of the extracts were compared with that of gentamycin which was used as a criterion for declaring the corn silk extract bioactive. This antimicrobial result of corn silk extract has confirmed its use as antimicrobial agent as reported by Kim *et al.,* (2005). Thus, corn silk could be a potential source of clinical antibiotics in curing diseases caused by the above pathogens especially *E. coli* and *Staphylococcus species*.

CONCLUSION

In conclusion, corn silk has good potentials and also contain various medicinal properties due to the presence of some nutritional composition and phytochemicals. It is safe, non- toxic according to study on the toxicity of corn silk by [12] and should be properly harnessed. Also study of the antimicrobial capability of the corn silk extracts in inhibiting the activities of pathogens showed that corn silk can to a large extent inhibited the activity of some micro-organisms. Therefore, based on the results obtained from the above analyses, corn silk has been found to possess phytochemical parameters and for that it should be properly harnessed and not discarded. The investigative results have shown that corn silk from corn planted in Abakaliki also has the capacity to inhibit the activities of some micro-organisms.

REFERENCES

1. Duru C. E. (2020) Mineral and phytochemical evaluation of zea mays husk. *Sciencetific African*. **7**;224
2. Ezeonu, C. S. and Ejikeme, C. M. (2016) Qualitative and Quantitative Determination of Phytochemical Contents of Indigenous Nigerian Softwoods. *New Journal of Science,* 2016: 1-9
3. Harborne, J.B. (1973). Phytochemical Methods. A Guide to Modern Techniques of Plant Analysis, Ist Edition, New York Chapman and Hall; 206-210.
4. Hasanudin, K., Hashim, P and Mustafa S. (2012). Corn silk (Stigma maydis) in healthcare, a phytochemical and pharmacological review. *Molecules***17**(8):9697-9715.

5. Kim K. A., Shin H.H., Choi S. Kand Choi H. S. (2005). Corn silk induced cyclooxygenase-2 in murine macrophages. *Bioscience, Biotechnology and Biochemistry*. **69**; 1848-1853.
6. Lawal, H. O, Etatuvie, S.O. and Fawehinmi, A. B. (2012). Pharmacological Properties of *Morinda Lucida.Journal of Nigeria Natural Medicine Development, Agency*, Victoria Island, Lagos, Nigeria, **5**: 25-41.
7. Limmatvapirat C. and Limmatvapirat S. (2020). Phytochemical analysis of baby corn silk extracts, *Journal of Ajureda and intergrative medicine*:**11**(3); 344- 351
8. Makkar, H.P.S., Blummel, M., Borowy, N. K. and Becker, K. (1993). Gravimetric determination tannins and their correlations with chemical. *Journal of the Science of Food and Agriculture* 61(2): 161-165.
9. Okwu, D. E (2004). Phytochemicals and vitamin content of indigenous spices of South Eastern Nigeria. *Journal of Sustainable Agriculture and the Environment*. **6**;30-34
10. Okwu. D. E (2005) Phytochemical composition and nutritional quality of *Glycine max* and *Vign*a *unguiculata (L) walp. American Journal of Food Technology*. **2**(6) 512-520
11. Umar M. S. (2016); Anti-diabetic potential of methanol extract of cooked corn silk (*Stigma maydis*) on alloxan induced diabetes in albino mice, *The Pharmaceutical and Chemical Journal,* 3(4); 68-72.
12. Wang C., Zhang T., Liu J., Lu S, Zhang C and Wang E (2011). Subchronic toxicity study of corn silk with rat. *Journal of Ethnopharmacology*. **137**; 36-43.
13. Ugwu Okechukwu PC, FC Nwodo Okwesili, E Joshua Parker, Bawa Abubakar, C Ossai Emmanuel, E Odo Christian (2013). Phytochemical And Acute Toxicity Studies Of Moringa Oleifera Ethanol Leaf Extract. International Journal Of Life Science Biotechnology And Pharma Research 2(2): 66-71.
14. Adonu Cyril C, OPC Ugwu, Ossai Esimone Co, A Bawa, AC Nwaka, CU Okorie (2013). Phytochemical Analyses Of The Menthanol, Hot Water And N-Hexane Extracts Of The Aerial Parts Of Cassytha Filiformis (Linn) And Leaves Of Cleistopholis Patens. Research Journal Of Pharmaceutical, Biological And Chemical Sciences 4:1143-1149.
15. Offor CE, PC Ugwu, PM Okechukwu, IO Igwenyi (2015). Proximate and phytochemical analyses of Terminalia catappa leaves. European Journal of Applied Sciences 7 (1), 09-11.
16. Igwenyi IO, OE Isiguzo, PM Aja, PC Ugwu Okechukwu, NN Ezeani, AJ Uraku Proximate composition, mineral content and phytochemical analysis of the African oil bean (Pentaclethra macrophylla) seed. American-Eurasian J Agric Environ Sci 15, 1873-1875.
17. Aja PM, PCU Okechukwu, K Kennedy, JB Ibere, EU Ekpono (2017). Phytochemical analysis of Senna occidentalis leaves. IDOSR J Appl Sci 2(1): 75-91.
18. Chukwuemeka IM, IP Udeozo, C Mathew, EE Oraekwute, RC Onyeze, OPC Ugwu (2013). Phytochemical analysis of crude ethanolic leaf extract of Morinda lucida, Int. J. Res. Rev. Pharm. Appl. Sci 3(4): 470-475.
19. Ali Ikechukwu A., UA Ibiam, PC Ugwu Okechukwu, OR Inya-Agha, U Orji Obasi, David Obasi Chukwu (2015). Phytochemistry and acute toxicity study of Bridelia ferruginea extracts. World J. Med. Sci,12(4): 397-402.
20. Phytochemical composition of Datura stramonium Ethanol leaf and seed extracts: A Comparative Study.
21. Esther Ugo Alum, Mathias C. Diana, Okechukwu P. C. Ugwu, Patrick M. Aja, Emmanuel I. Obeagu 2023). IAA

Journal of Biological Sciences 10 (1), 118-125. IAA Journal of Biological Sciences.10(1); 118-125.