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Chemical Evaluation, Free Radical Scavenging Activities and Antimicrobial Evaluation of the Methanolic Extracts of Corn Silk (*Zea mays*)

Stella A. Emmanuel[1], Olutayo Olajide[1], Salisu Abubakar[2], Saheed O. Akiode[2] and Godwin Etuk-Udo[2*]

[1]Chemistry Advanced Research Centre, Sheda Science and Technology Complex (SHESTCO), Abuja, Nigeria.
[2]Biotechnology Advanced Research Centre, Sheda Science and Technology Complex (SHESTCO), Abuja, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. Authors SAE and OO designed the study. Authors SAE, OO and GE collected all data and performed the statistical analysis. Authors SA and SOA performed all microbial screening. Authors SAE, SA, SOA and GEU did the literature search. Authors SAE, OO, SA and SOA wrote the first draft of the manuscript. Author GEU wrote the second draft of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Corn silk is thought to possess medicinal properties according to Nigerian folklore. This study was undertaken to appraise its phytochemical constituents and anti-microbial activities. Data obtained revealed that alkaloids, flavonoids, tannins, saponins, steroids, glycosides and cardiac glycosides were detected in the fresh mature and dried mature samples (FMCSS and DMCSS). The antioxidant activity of the extracts was determined by the DPPH inhibition method. The crude extract of the FMCSS exhibited a stronger free radical scavenging activity than that of the DMCSS (76.14: 73.54, 75.76: 68.23, 68.18: 61.46 and 64.18: 41.58% for the fresh versus the dried samples at 5, 3, 2 and 1 mg/ml concentrations). Results showed that when tested against *Staphylococcus*

**Corresponding author: E-mail: godwin.etukudo@gmail.com;*

aureus, Pseudomonas aeruginosa, Klebsciella pneumonia, Escherichia coli and *Salmonella thypi*, the zones of inhibition obtained ranged from 10 to 16 mm for the fresh samples and between 12 to 20 mm for the dried samples. Mineral analysis revealed the presence of calcium (0.1869: 0.0610 mg/g), iron (0.005: 0 mg/g), magnesium (0.1939: 0.0934 mg/g), copper (0.0073: 0.0094 mg/g), manganese (0.0109: 0.0027 mg/g) and zinc (0.0165: 0.0146 mg/g) in the dried immature/mature silk. The results obtained suggested that the DMCSS sample studied plant possess better anti-bacterial activity with the major activity tailored to the phyto-constituents. GCMS analysis of the hexane extract of the silk showed the presences of straight chain alkanes and poly unsaturated methyl esters.

Keywords: Corn silk; phytochemicals; anti-microbial activity; antioxidants.

1. INTRODUCTION

Plants and herbs constitute a natural reserve of chemotherapeutic agents towards the deterrence or treatment of various diseases [1]. The attention being received in recent times is as a culmination of immune-boosting potential alongside the inhibitory effects of herbal products against several pathogens/microbial agents that plague humans and animals alike [2,3]. Furthermore, with the increased demand for organic materials that serve as food additives, constituents of functional foods, nutraceuticals and prevention of plant diseases, it is therefore conceivable to examine the applicability and benefits of indigenous plant materials to mankind using modern scientific analysis methods [4].

Corn silk is the long style and stigma of flower corn or maize (*Zea mays*). They are fine and soft yellowish to green or purple before pollination and maturity and light to dark brown afterwards. Fresh un-pollinated Corn silk is used in folkloric medicine [5,6]. Corn silk has been used in many parts of the world for the treatment of edema as well as for cystitis, gout, kidney stones nephritis and prostatitis. It is reported to contain proteins, vitamins, carbohydrates, Ca, K, Mg and Na salts, fixed and volatile oils, steroids such as sitosterol and stigmasterol, alkaloids, saponins, tannins, and flavonoids. There have been many reports on the biological activities of Corn silk constituents. Methanol extracts of Corn silk showed an anti-oxidative activity on the level of lipid peroxidation. Volatiles from Corn silk inhibited the growth of *Aspergillus flavus,* indicating that it has an antifungal [6]. Currently products such as tea powder and cosmetic from corn silk are commercially available in China, Korea, Japan and the United Kingdom [7]. From literature review most work done has been focused on the un-pollinated corn silk [5,6,7,8].

The aim of this study was to evaluate the chemical and biological properties of matured pollinated silk with the view of encouraging its use for possible medicinal and other nutritional purposes and ample opportunity of converting this agricultural waste especially in Nigeria to wealth.

2. MATERIALS AND METHODS

Corn silk was collected from the farmers around the complex. The matured pollinated Corn silk was divided into two groups a portion was air dried and the other half prepared for extraction. Also a little quantity of un-pollinated Corn silk was gotten for elemental analysis. The crude extract of the silk sample was collected [9]. The fresh silk and dried silk were soaked in 600ml methanol in 1 L beaker respectively for three days. It was filtered on the third day and the filtrate was concentrated using a rotary evaporator. This was poured into a 600 cm^3 beaker and allowed to dry further on a water bath. The crude extract was weighed and percentage yield of the extract was calculated.

All reagent employed in this work are of analytical grade obtained from BDH. They were used without further purification except where otherwise stated.

2.1 Phytochemical Analysis

This analysis was carried out to test for the presence of phenols, alkaloids, steroids, terpenes, cardiac glycosides, tannins, flavonoids, anthraquinones, saponins, triterpenoids, glycosides, phlobatannins, resins, balsams and volatile oils [9].

2.2 Elemental Analysis

Two grams of the dry and fresh matured silk was weighed into a beaker. 20 ml of nitric acid was

added to the sample. This was heated on the hot plate at 60ºC for 30 mins. It was then removed from heat and allowed to cool. The solution was diluted with distilled deionized water and made up to 50 ml in a volumetric flask. A blank was prepared in the same manner and poured into a polyproplene bottle. The samples were analysed for metals on Thermo Scientific *iCE* 3000 AA02134104 atomic absorption spectrometer using appropriate working standards.

2.3 GCMC Analysis of Hexane Extract

Hexane extract of the sample was obtained using a soxhlet extractor. This extract was concentrated using rotary evaporator and the extract composition analysis was carried out on Agilent Technology 7890A, 5975C series gas chromatography equipped with FID detector on a splitless (7683B series) injector. A fused silica capillary column (HP5MS) was used with the injector and detector temperature maintained at 230℃ and 280℃, respectively. The oven temperature was programmed at 80℃ for 2 min and finally increased to 280℃ at 6℃/min. The carrier gas was helium at a flow rate of 1.5 mL/min. The area percentages were recorded with a standard Chemstation Data System.

2.4 Antimicrobial Screening

Antibacterial activities of the corn silk extracts was tested on Muller Hinton agar, using agar well diffusion method [10,11]. The bacterial isolates were sub-cultured in peptone water. After 24 hrs, the solutions got turbid and, using spectrophotometer, the turbid solutions were analysed and compared with that of standard 0.5 McFarland solutions. Using sterile cotton board the suspensions were stricken on top of the previously sterilized Petri plates with solidified media and was allowed to dry for 15 minutes. Five wells were punched using sterile cork borer of 6mm size. To test sensitivity properties of the extract, half dilutions of 600, 300 and 150 mg/ml were prepared from stock solution using Dimethylsuphoxide (DMSO) as a diluent. Each well was filled with 0.5 ml of the different dilutions. A standard antibiotic Ciprofloxacin (30 µg/ml) was used as positive Control, while DMSO served as negative Control. This was done to check for sterility and any growth inhibitory potential of the solvent. The plates were kept for 1hour at room temperature to allow diffusion of the extract to take place and then incubated aerobically at 37ºC for 24hours. The zones of inhibition produced by different species were measured in (mm) across the diameter.

2.5 Scavenging Effect on 2,2-Diphyenyl-1-picrylhydrazyl Radical (DPPH)

This was determined as described by Olajide and co-workers [12]. The radical scavenging activity of the methanol extract against DPPH (Sigma-Aldrich) was determined by UV-Visible Spectrophotometer at 517 nm. Radical scavenging activity (RSA) was calculated as inhibition percentage according to the uncoloured DPPH, with the employ of the equation:

$$\%\, Inhibition = \frac{Ab}{Ab} - \frac{Aa}{Ab} X \frac{100}{1}$$

Where Aa is the absorption of the blank without extract and Ab is the absorption of the extract.

3. RESULTS

Results of the phytochemical screening are shown in Table 1; with the presence of metabolites such as alkaloids, flavonoids, saponins, tannins, phenols, steroids, glycosides, cardiac glycosides, volatile oils and balsams. The presence of phenol and volatile oil were not observed in the Fresh Matured Corn silk sample (FMCSS) but was present in the Dried Matured Corn silk sample (DMCSS) resin was present only in the FMCSS. Tables 2 and 3 show the results of the antimicrobial screening of the extracts; where the activity of the extract against the test organisms showed an increased activity as the concentration of the dried silk increased, with a higher activity compared to the fresh silk sample. Results of the mineral analysis are shown in Table 4; with a significant amount of magnesium in the samples; where the dried immature silk presented the highest quantity. The same trend is observed for the other minerals analysed in the samples. The anti-oxidant activity of the extract (Table 5) showed an increase in scavenging activity as the concentration of the sample extract increased.

Relevant data of the GCMS analysis of the hexane extract are shown in Table 6. The peaks between 16 and 38 minutes.

4. DISCUSSION

The therapeutic value of medicinal plants has been explored towards the treatment of many types of disease conditions particularly in

developing countries. The use of such plants is being boosted by the notion that plant-based drugs are not commonly linked with certain side effects associated with the use of synthetic drugs. Active compounds within plants are now screened to unveil the endless possibilities for their antagonistic nature against a host of microbial agents of disease, thereby positioning them as ideal drug candidates. Furthermore, this serves to validate the claim according to folklore that indigenous plants are being used for traditional African medicinal practices [13]. The therapeutically and dietary needs that specific native plant species meet has been underscored in many developing countries [14].

In this study, the detection of certain phyto-compounds would augment the curative potential of the plant against several pathogens [15,16]. Analysis of the data obtained from table 1 suggests that both extracts contained certain compounds including alkaloids, flavonoids, tannins, saponins, steroids, glycosides and cardiac glycosides. For instance, plant glycosides and cardiac glycosides possess herpato-stimulatory activity thus making them useful for increasing heart muscles contractions as well as in cancer treatment [17,18]. Towards the treatment of extreme upper respiratory tract infections, the administration of aminoglycoside antibiotics; streptomycin and kanamycin, are of importance as these in combination with other similar drugs is used for treating pneumonia and brucellosis [19,20]. The presence therefore of both glycosides and cardiac glycosides in the extracts of the studied plants gives a good indication that these plants may possess important bioactive compounds of different antibiotic class. DMCSS contain all phenolics; flavonoids, phenols and tannins. As a group, these are some of the most abundant phyto-constituents in plants and has also been associated with good mental performance through the consumption certain food and processed items such as berries, chocolate, red wine and tea [21]. Furthermore, the anti-septic nature of phenolics suggests that this plant extract should possess good antimicrobial activity against a selected range of organisms. The success in treating diarrhoea and stomach upsets, attributed to *Staphylococcus aureus* and *Salmonella* spp. infections could be due to the antibacterial effects of alkaloids, polyphenols, saponins and steroids [22].

Distorting the enzyme activity within bacteria is usually observed in the presence of phyto-tannins [23]. The anti-diarrheal effectiveness of alkaloids is reportedly potent against intestinal infections associated with AIDS, thereby linking the screening of plants with a high presence of alkaloids to the treatment of HIV infections [24]. Other phyto-constituents observed included saponins which alongside alkaloids are known to be very effective against both gram positive and gram bacteria such as *Salmonella* spp. and *Streptococcus* spp [25] used in this study. In this report, the respective fresh and dried extracts of corn silk were tested against different microorganisms in an attempt to gauge their activity spectrum. The varied antimicrobial activity (Tables 2 and 3) is indicative of the antibacterial compounds present, thereby supporting the data obtained from the phytochemical studies.

Table 1. Result of phytochemical screen of extract of FMCSS and DMCSS

Test	FMCSS	DMCSS
Tannins	+	+
Saponins	+	+
Steroids	+	+
Tritepenoids	-	-
Alkaloids	+	+
Flavonoids	+	+
Phenols	-	+
Terpenoids	-	-
Glycosides	+	+
Cardic glycosides	+	+
Volatile oils	-	+
Phlolbatannins	-	-
Cardenolides	-	-
Balsams	+	+
Resins	+	-

Reports indicate that corn silk have been employed therapeutically towards the amelioration of cystitis, edema, kidney stones, diuretic, prostate disorder, and urinary infections as well as bedwetting and obesity [26]. Also, it is known to exhibit for strong antioxidant capacity [27].

It has been determined that phenolic compounds; particularly the flavonoids and anthocyanins are the most commonly found phytochemicals present in corn silk thus suggesting that the plant possesses an inherent range of bioactive abilities [28,29].

In a study by Solihah and co-workers [5], phenols, flavonoids, tannins, phlobatannins, alkaloids, saponins and cardiac glycosides were

detected in both aqueous and methanolic extracts of corn silk in which the methanolic extracts alone contained terpenoids. The data from that report is similar to that obtained in this study.

Disease causing pathogens like *Pseudomonas aeruginosa* and *Staphylococcus aureus* are two of the most opportunistic infections commonly associated with respiratory diseases, urinary tract and gastrointestinal infections, etc [30,31] that have gained a lot of attention owing to its raising prevalence among community acquired infections. Another contender for significant opportunistic infections is *Escherichia coli*, which has been identified among the array of causative agents responsible for acute diarrhoea and other urinary tract infections globally [32].

Table 2. Antimicrobial activity of methanolic extract of FMCSS

Microorganism	Inhibition zone (mm) and extract concentrations (mg/ml)				
	(600mg/ml)	(300mg/ml)	(150mg/ml)	Positive Control Ciprofloxacin (30µg/ml)	Negative Control DMSO
Pseudomonas aeruginosa	14.00	11.00	08.00	23.00	NA
Salmonella typhi	10.00	07.00	-	23.00	NA
Escherichia coli	14.00	10.00	07.00	23.00	NA
Klebsiella pneumonia	13.00	11.00	08.00	23.00	NA
Staphylococcus aureus	16.00	13.00	09.00	25.00	NA

Table 3. Antimicrobial activity of methanolic extract of DMCSS

Microorganism	Inhibition zone (mm) and extract concentrations (mg/ml)				
	(600mg/ml)	(300mg/ml)	(150mg/ml)	Positive control ciprofloxacin (30µg/ml)	Negative control DMSO
Pseudomonas aeruginosa	16.00	13.00	10.00	23.00	NA
Salmonella typhi	12.00	09.00	07.00	23.00	NA
Escherichia coli	15.00	12.00	09.00	23.00	NA
Klebsiella pneumonia	16.00	14.00	11.00	23.00	NA
Staphylococcus aureus	20.00	16.00	12.00	25.00	NA

Table 4. Elemental analysis

Element mg/g	Fresh matured silk	Dried immature silk	Dried matured silk
Ca	0.1465	0.1869	0.0610
Mg	0.1602	0.1939	0.0934
Cu	0.0072	0.0073	0.0094
Fe	0.0198	0.005	B.D.L
Mn	0.0187	0.0109	0.0027
Zn	0.0136	0.0165	0.0146

Table 5. Result of anti-oxidant analysis

Concentration mg/ml	% inhibition for Vit C	% inhibition for FMCSS	% inhibition for DMCSS
5	85.00	76.14	73.54
3	90.15	75.76	68.23
2	90.41	68.18	61.46
1	91.50	64.18	41.58
0.5	91.32	45.89	37.66
0.1	90.47	29.68	28.54
0.05	90.19	31.77	26.14

Table 6. Relevant GCMS analysis chromatogram data

Peak No	Retention time (RT)	Peak height	% of total
1.Hexadecanoicacid ME	16.770	2694303	0.752
2. 9,12,octadecadienoic acid ME	18.491	3989276	1.020
3. 11-octadecenoic acid ME	18.546	2721710	0.853
4. Octadecanoic acid ME	18.778	1640635	0.370
5. 1,2- Benzenedicarboxylic acid, mono(2-ethylhexyl)ester	23.732	11394452	11.089
6. Heptacosane	26.473	3689788	2.688
7. Tetracosanoic acid, ME	27.209	1082364	0.797
8. Octacosane	29.065	992794	0.808
9. Nonacosane	32.963	3694808	9.958
	33.065	434105	

Key: ME= methyl ester

The antimicrobial activity is indicative of the antibacterial compounds present, thereby supporting the data obtained from the phytochemical studies. The obtained results indicate that the polar fraction of the extract possess reasonable activity against gram positive and gram negative bacteria, thereby demonstrating the potential of the plant to be used in curing diseases caused by these organisms. The data revealed that the extracts were general effective against *Staphylococcus aureus, Pseudomonas aeruginosa, Klebsciella pneumonia, Escherichia coli* and *Salmonella typhi* with zones of inhibition ranging from 10-16±0.50mm for the fresh samples and 12-20 ±2.50mm for the dried samples respectively tested against Ciprofloxacin. Both FMCSS and DMCSS were most effective against *Staphylococcus aureus*. The mineral analysis result indicated the presence of calcium (Ca), iron (Fe), magnesium (Mg), copper (Cu), manganese (Mn), and zinc (Zn) which were all higher in the dried immature sample than either of the two other prepped samples tested. The concentrations of the essential elements appear to be within the approved permissible limit for minerals consumed therapeutically as well as for general human health [33]. From the data, the plant is useful towards bone formation, energy, normal functioning of metabolic and metallo-enzymes. The presence of zinc and other trace minerals further boosts the plants' application as an anti-microbial agent.

Analysis of its antioxidant potential suggests that presence of certain phyto-compounds particularly flavonoids in concert with other compounds, triggers its free radical scavenging (FRSA) activities. The FRSA of the samples were determined as 76.14: 73.54, 75.76: 68.23, 68.18: 61.46 and 64.18: 41.58% for the fresh versus the dried samples at 5, 3, 2 and 1 mg/ml concentrations. This result is high for plants in comparison to the synthetic standards of vitamin C which gave 85.00%, 90.15%, 90.41% and 91.50% respectively at the same concentrations. The presence of key enzymes and catalytic agents in plant samples aids in quelling the lethal effects of reactive oxygen species to the body. Their ability to suppress free radicals before they can attack the cells and biological targets, prevents impairment to carbohydrates, DNA, enzymes, lipids and proteins [34].

5. CONCLUSIONS

This study serves to validate the local beliefs as well as bolster the collective knowledge of plant materials, identified as a fountain of pharmaceutically useful compounds. The data obtained in this study proffer scientific support for the folklore notion that the studied plant possesses anti–bacterial activity, specifically the dried extracts of matured corn silk, when compared to using the standard drug (Ciprofolxacin). The phytochemical analysis of the plant shows that the extracts contain alkaloids, flavonoids, tannins, saponins, steroids, glycosides and cardiac glycosides. Hence the range of pharmacologic activities could be deduced from the above phyto-constituents.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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