Determination of Phytochemical and Antimicrobial Activities of Corn Starch Extract on *Escherichia Coli* and *Salmonella Typhi*

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Abstract: The phytochemical and antimicrobial effect of cornstarch extracts was investigated. Corn starch of yellow and white corn variety were extracted successively with ethanol, methanol and distilled water. These crude extracts were assessed for antimicrobial activities against *Escherichia coli* and *Salmonella typhi. Escherichia coli* were sensitive to the methanolic and ethanolic extracts of white corn starch with zones of inhibition of 22mm and 20mm respectively. *Escherichia coli* was also sensitive to ethanolic extract of yellow corn starch with a zone of inhibition of 22mm. *Salmonella typhi* was also sensitive to ethanolic and methanolic extracts of white maize starch with zones of inhibition of 20mm and 21mm respectively. The minimum inhibitory concentration of extracts of various corn varieties on *E. coli* and *Salmonella typhi* were investigated. The minimum inhibitory concentration of ethanolic (1.56mg/ml) and methanolic extract (0.78mg/ml) of white maize starch had zones of 17mm and 19mm on *Escherichia coli* respectively. The minimum inhibitory concentration of ethanol and methanol extract of white corn starch on *Salmonella typhi* where found to possess zones of inhibition 17mm and 18mm respectively, while the minimum inhibitory concentration of ethanolic extract (0.78mg/ml) of yellow corn starch on *Escherichia coli* had zones of inhibition of 18mm. Phytochemical screening of both varieties of cornstarch revealed the presence of alkaloid, tannin, saponins and terpenoids. Sensitivity testing of the phytochemicals present revealed that tannins had zone of inhibition on the test organisms *(Salmonella typhi* and *Escherichia coli* (23.20mm and 25mm) respectively, while the other phytochemicals had no zones of inhibition.

Keywords: Antimicrobial, Corn starch, *Escherichia coli,* Phytochemical, *Salmonella typhi.*

Introduction:

Zea mays (Maize or corn) is the most important cereal crop in sub-saharam Africa and with rice and wheat, it is one of the three most important cereal crops in the world (Kim *et al.*,2003). Corn is high yielding, easy to process, readily digested, and cheaper than other cereals. It is also a versatile crop: growing across a range of agro-ecological zones. Every part of the maize plant has economic value. The grain, leaves, stalk tassel and cob can all be used to produce a large variety of food and non-food products. One of the food prepared from maize is "Ogi" fermented cereal porridge made from maize and produced using simple processing methods (Teniolas and Odunfa. 2001).The "ogi" porridge is very smooth in texture and has a poor taste reminiscent of that of yoghurt. Sedimentated ogi has been traditionally found to be of medical importance in th south western part of Nigeria. It is used soaked with bark of the root of some plants to treat not only fever and malaria, but it is popularly used as solvent for herbal extraction, dish stain removal and as insect killers (Chopra *et al.*2003). Maize starch has also been used in the extraction of antimicrobial agents (Zhao *et al.*, 2005). The extracts have also been found to inhibit some gram negative bacteria (Kumar and Jhariya, 2013). Information from indigenes also revealed that maize starch is popularly used in the control of diarrhoea. Series of work has been done on the microbial and therapeutic values of corn starch. Cornstarch has been reported by Falana *et al.*(2012) to contain some microorganisms including *Lactobacillus plantarum.* They also reported that corn starch antimicrobial efficacy against some pathogenic microorganisms including *Escherichia coli* (Falana *et al.*, 2012).

Hence, this research was focused on the Determination of phytochemical and antimicrobial activities of yellow and white corn starch extract on *Escherichia coli* and *Salmonella typhi.*

Materials and Methods Corn starch preparation

A total of 500g of each of the varieties of corn grain was weighed. Thereafter they were washed with sterile water and steeped in a sterile conical flask for 72 hours. The water was decanted and the corn were wet-milled separately using sterile grinder. The resulting pastes were sieved using different sterile muslin cloth, the filtrate collected into different sterile containers to settle for 24hr. After 24h the supernatant was decanted leaving the sediment (starch). The starch was shade-dried for 48h until-completely dried and stored in sterile ziplock bag prior to use.

Preparation of Corn Starch Extract

Aqueous Extract: Total of 50g of cornstarch was weighed out using mechanical weighing balance and placed into sterile conical flask. Then 500ml of sterile water was measured out and dispensed into the flask containing the corn starch and covered with sterile foil paper. The mixture was agitated intermittently and left to soak for 48h at 25°C. Thereafter, the mixture was

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filtered through sterile Whatman No.1 filter paper into sterile beaker. It was concentrated using hot water bath at 30°C for 6 hours and then allowed to cool and stored in the refrigerator until when needed.

Ethanolic Extract: A total of 50g of corn-starch was weighed using mechanical weighing balance and placed into a sterile conical flask. Then 500ml of ethanol was measured out and dispensed into the flask with the corn-starch. Then it was covered with sterile foil paper. The mixture was agitated intermittently and soaked for 48 hours. Thereafter, the mixture was filtered using sterile Whatman No.1 filter paper into sterile beaker and covered with sterile perforated foil paper to allow for natural evaporation of the extract. After evaporation it was stored in the refrigerator until when needed.

Methanolic Extract: Total of 50g of corn starch was weighed out using mechanical weighing balance and placed into sterile conical flask. Then 500ml of methanol was measured out and dispensed into the flask with the corn starch. Then it was covered with sterile foil paper. The mixture was agitated intermittently and left to soak for 48 hours. Thereafter, the mixture was filtered through Whatman No.1 filter paper into sterile beaker and covered with sterile perforated foil paper to allow for natural evaporation of extract. After evaporation it was stored in the refrigerator until when needed.

Antimicrobial Disc preparation: A perforator was used to perforate about 6mm of Whatman No.1 filter paper, the disc were wrapped in foil paper and placed in a beaker covered with foil then sterilized using hot air oven at 80°C for 1 hour and the sterile discs were stored in the refrigerator until when needed.

Sensitivity Testing: The antimicrobial assay of corn starch was carried out using two methods; Agar well and Disc diffusion methods.

Agar-Well Method: Serially diluted broth culture (10^{-4}) of test organisms were inoculated into sterile nutrient agar and spread using a sterile swab stick. Sterile cork borer was used to make three wells on each plate. Thereafter, 0.1ml of each extract was carefully pipette into the wells. The preparations were incubated for 24h at 37°C. Then the preparation was observed for zones of inhibition and zones were measured and recorded.

Disc Diffusion Method: Serially diluted broth culture (10^{-4}) of test organism were inoculated into already prepared nutrient agar plates respectively, spread out using sterile swab stick. The already prepared sensitivity discs were placed at different location on the plates and incubated for 24-48h at 37°C. Then zones of inhibition were observed, measured and recorded.

Determination of Minimum Inhibitory Concentration: The minimum inhibitory concentration was determined using agar well technique. Serial dilution of the various extracts which showed zones of inhibition on the test organisms was made with their respective solvents. Total of 0.1ml of the diluted extracted were incorporated at varying concentrations into the agar plate containing the test organisms (*Salmonella typhi* and *Escherichia coli*). The plates were incubated at 37°C for 24h. The lowest concentration of extracts that had zones of inhibition within the incubation period was taken to be the minimum inhibitory concentration. The diameter of zones of inhibition were measured and recorded accordingly.

Phytocemical Testing: The methods of Ostlund *et al.*, (2002) was adopted.

Test for Alkaloids: A quantity (0.4ml) of extracts was stirred with 8ml of 1% HCL and the mixture was warmed and filtered. The filtrate (2ml) was treated with potassium bismuth (Dranendroff's reagent). Turbidity or precipitation with this reagent was taken as evidence for existence of alkaloids.

Test for Saponins: The ability of saponins to produce emulsion with oil was used for the screening test. The extract (20ml) was boiled in 20ml of distilled water in a water bath for five minutes and filtered. Thereafter, 10ml of the filtrate was mixed with 5ml of distilled water and shaken vigorously for froth formation. Then, 3 drops of olive oil were mixed with froth, shaken vigorously and observed for emulsion development.,

Test for Terpenoids: The extract (5ml) were mixed with 3ml of chloroform, followed by 3ml of conc. H_2SO_4. A reddish brown colouration at the interface confirmed the presence of terpenoids.

Test for Steriods: Concentrated H_2SO_4 (5drops) was added to 1ml of the extract in a test tube. A red colouration indicates the presence of steroid.

Test for Flavonoids: A quantity (50ml) of extract was suspended in 100ml of distilled water to get the filtrate. A 5ml of diluted ammonia solution was added to 10ml of filtrate followed by few drops of concentrated H_2SO_4. Presence of flavonoids was confirmed by yellow colouration.

Test for Tannins: The extract (50ml) was boiled in 20ml of distilled H_2O and filtered. A few drops of 0.1%. $FeCl_3$ was added in filtrate and observed for colour change; brownish green or a blue-black coloration was taken as evidence for the presence of tannins.

Test for Resin: A quantity (0.2g) of the powdered material was extracted with 15ml of 96% ethanol. The alcoholic extract was then poured into 20ml distilled water in a beaker. A formation of precipitate indicates the presence of resins.

Test for Glycosides: Few drops of ferric chloride and concentrated sulphuric acid were added into the solution of the extract in glacial acetic acid and observed for a reddish brown coloration at the junction of two layers and the bluish green colour in the upper layer.

Quantitative Phytochemical Screening
Determination of Tannins: The extract was poured into a column containing sephadex LH20 (15X45cm) that had been equilibrated in 95% ethanol. The column. was eluted with 95% ethanol until uv absorbance (280nm) indicated that no material was eluting. The ethanol fraction that contained other polyphenolics was found to have no enzyme inhibiting ability. The column was then eluted with 50% aqueous acetone until absorbance at 420nm. The eluted material was exposed to warm air current to remove the acetone, after which the remaining solution was freeze dried. The resulting fluffy brown powder was weighed and kept until when needed.

Determination of Alkaliods: Total of 5g of the sample was weighed into a 250ml conical flask and 200ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 hours. This was filtered and the extract was concentrated on water bath to one – quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete.
The whole solution was allowed to settle and the precipitated was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried, weighed and kept until when needed.

Determination of total saponins: The samples were ground and 20g of each were put into a 250ml conical flask and 100ml of 20% aqueous ethanol were added. The samples were heated over a hot water bath for 4 hours wit continuous stirring at 55^{0}C. The mixture was filtered and the residue re-extracted with another 200ml of 20% ethanol. The combined extracts were reduced to 40ml over water bath at about 90^{0}C. The concentrate was transferred into a 250ml separating funnel and 20ml of diethyl ether was added and shaken vigorously, the aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. A total of 60ml of n-butanol was added, the n-butanol extracts were washed twice with 10ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath after evaporation. The samples were dried in an oven to a constant weight. The saponin content was calculated and kept until when needed.

Determination of Terpenoid: The extract was concentrated under a nitrogen stream without drying the samples. Then the concentrated sample was loaded into a small silica column. The sample was allowed to be absorbed into the column, then about 1-3ml of hexane was added and the column eluate was collected. The eluate was dried under a nitrogen stream, weighed and stored until when needed.

Sensitivity Testing of Phytochemicals : The assay was conducted using agar well technique. Serially diluted broth culture (10^{-4}) of test microorganism was inoculated into sterile nutrient agar and spread using a sterile swab stick. Sterile cork borer was used to make three wells on each plate. Thereafter 0.1ml of phytochemicals were carefully pipetted into the wells. The dissolution of phytochemicals was aided by 1% (v/v) DMSO. The preparations were incubated for 24h at 37^{0}C. Then antibacterial activities were evaluated by measuring zones and recording the zones of inhibition.

Results and Discussion: Table 1 and 2 shows the antimicrobial sensitivity of different concentration of the methanolic, ethanolic and aqueous extracts of white corn starch on *Escherichia coli and Salmonella typhi.* The methanolic extract and ethanolic extracts of the white corn starch have antimicrobial properties. The methanolic extract was the most effective on *Escherichia coli* at 100mg/ml concentration with zone of inhibition of diameter 22mm using Agar well method and 20mm using Disc diffusion method. The ethanolic extract at 100mg/ml concentration had a zone of inhibition of diameter 20mm using the Agar-well diffusion method, 19mm using the Disc diffusion method while the aqueous extract did not show any zone of inhibition in the Agar-well method. It was only the ethanolic extract of yellow corn starch that was effective on *Escherichia coli* with a zone of inhibition of 22mm and 23mm using both the disc diffusion and agar well methods. The aqueous and methanol extracts showed no zone of inhibition in both disc diffusion and agar well methods.

The result of antimicrobial sensitivity testing of methanolic, ethanolic and aqueous extracts of corn starch on *Salmonella typhi.*. The methanolic extract of white corn starch was most effective with zone of inhibition of 23mm and 20mm using the agar well method and disc diffusion methods respectively . The aqueous extract was not effective against *Salmonella typhi.* None of the extracts of yellow maize starch was effective on *Salmonella typhi.*

The result of minimum inhibitory concentration of corn starch on *Escherichia coli* and *Salmonella typhi* as stated in table 3and 4. The ethanolic extract of white corn starch had a minimum inhibitory concentration of 1.56mg/ml while the

methanolic extract recorded 0.78mg/ml. The ethanolic extract of yellow maize starch on *Escherichia coli* recorded 1.56mg/ml.

The minimum inhibitory concentration of ethanolic and methanolic extracts of white maize starch on *Salmonella typhi.* The ethanolic extract of white maize starch had a minimum inhibitory concentration of 0.78mg/ml while the methanolic extract had 0.78mg/ml.

Table 5 also shows the result of the qualitative phytochemical screening of white corn starch and yellow corn starch. The result revealed the presence of tanning, alkaloids and terpenoids and saponins.

The quantitative phytochemical screening of white and yellow corn starch as stipulated in table 6 shows that yellow corn starch had higher percentage Alkanoids of 1.67% to compare to white corn starch. It also shows that saponins is absence in white corn starch but Tannin is presence in both corn starch samples.

Table 7 shows result of sensitivity testing of phytochemicals on the *Escherichia coli* and *Salmonella typhi.* The result indicated that the sensitivity testing of phytochemicals (Saponins, Alkanoids and Terpenoids) had no zone of inhibition on *Escherichia coli* and *Salmonella typhi.* While Tannin had zone of inhibition of 25.00mm and 23.00 on *Escherichia coli* and *Salmonella typhi* respectively.

Conclusion

On the basis of present investigation, it is concluded that there exists a great potential in the search of new and more potent antimicrobial substances from the natural sources. The potential for developing antimicrobials from plants appear rewarding as it will lead to the development of phytomedicines to act against microbes. Plant based antimicrobials have enormous therapeutical potential as they can serve the purpose without any side effects that are often associated with synthetic antimicrobials. From this study corn starch from both corn varieties have potential therapeutic properties.

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Table 1: Antimicrobial Activities of Corn Starch on *Escherichia coli* and *Salmonella typhi* Using Disc Diffusion Method

Corn starch	Reagents	Zone of inhibition(mm)		Remarks	
		E. coli	*S.typhi*	*E. coli*	*S. typhi*
WCS	Ethanol	19.0 0	20.00	Sensitive	Sensitive
	Methanol	20.00	21.00	Sensitive	Sensitive
	Aqueous	-	-	Resistance	Resistance
YCS	Ethanol	20.00	-	Sensitive	Resistance
	Methanol	-	-	Resistance	Resistance
	Aqueous	-	-	Resistance	Resistance

Key:-= No zone of inhibition, *E. coli* = *Escherichia coli*, *S. typhi*= *Salmonella typhi*, WCS=White corn starch, YCS=Yellow corn starch.

Table 2: Antimicrobial Activities of Corn Starch on *Escherichia coli* and *Salmonella typhi* Using Agar-Method

Corn starch	Reagents	Zone of inhibition(mm)		Remarks	
		E. coli	*S. typhi*	*E. coli*	*S. typhi*
WCS	Ethanol	20.0 0	20.00	Sensitive	Sensitive
	Methanol	22.00	23.00	Sensitive	Sensitive
	Aqueous	-	-	Resistance	Resistance
YCS	Ethanol	22.00	-	Sensitive	Resistance
	Methanol	-	-	Resistance	Resistance
	Aqueous	-	-	Resistance	Resistance

Key:-= No zone of inhibition, *E. coli* = *Escherichia coli*, *S. typhi*= *Salmonella typhi*, WCS=White corn starch, YCS=Yellow corn starch.

Table3: Minimum Inhibitory Concentration of Methanol Extract of Corn Starch on *Escherichia coli* and *Salmonella typhi*

Concentration(%)	Zone of Inhibition(mm)		Remarks
	E. coli	*S. typhi*	
100	28.00	28.00	Sensitive
50	26.00	27.00	Sensitive
25	25.00	25.00	Sensitive
12.25	23.00	24.00	Sensitive
6.25	22.00	23.00	Sensitive
3.13	21.00	21.00	Sensitive
1.56	20.00	19.00	Sensitive
0.78	19.00	18.00	Sensitive

Key: *E. coli = Escherichia coli, S. typhi= Salmonella typhi*

Table4: Minimum Inhibitory Concentration of Ethanol Extract of Corn Starch on *Escherichia coli* and *Salmonella typhi*

Concentration (%)	Zone of Inhibition(mm)		Remarks
	E. coli	*S. typhi*	
100	25.00	26.00	Sensitive
50	23.00	25.00	Sensitive
25	22.00	24.00	Sensitive
12.25	21.00	23.00	Sensitive
6.25	19.00	22.00	Sensitive
3.13	18.00	20.00	Sensitive
1.56	17.00	18.00	Sensitive
0.78	15.00	17.00	Sensitive

Key: *E. coli = Escherichia coli, S. typhi= Salmonella typhi*

Table 5: Qualitative Phytochemical Screening of Corn Starch

Phytochemical Screening	White Corn Starch	Yellow Corn Starch
Saponins	-	+
Tanin	+++	+
Flavonoids	-	-
Glycosides	-	-
Alkaloids	+	+
Steroids	-	-
Terpenoids	+++	+++
Resin	-	-

Key: - =Absent, + =Present in trace concentration, +++ =Present in very high concentration

Table 6: Sensitivity Testing of Phytochemicals on *Escherichia coli* and *Salmonella typhi*

Phytochemical	Weight of Samples	WCS	YCS
% Alkanoids	5.00	1.54	1.67
% Saponins	20.00	-	196.56
conc of Tannin(mg/ml)	500.00	1.03	1.01

Key:-%= percent, WCS=White corn starch, YCS= Yellow corn starch

Table 7: Sensitivity Testing of Phytochemicals on *Escherichia coli* and *Salmonella typhi*

Phytochemical	Zone of Inhibition(mm)		Remarks
	E. coli	*S. typhi*	
Saponins	-	-	Resistant
Alkanoids	-	-	Resistant
Tannin	25.00	23.00	Sensitive
Terpenoids	-	-	Resistant

Key: *E. coli* = *Escherichia coli*, *S. typhi*= *Salmonella typhi*

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