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Fermenting Infusion of Cola Nitida and Cola Acuminata Husk and Testa

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Abstract: ***The need to ascertain the array of microorganisms associated with fermenting infusion of Cola nitida and Cola acuminata husk and testa as either playing beneficial or benign role is important for its use. This research focused on isolating microorganisms associated with fermenting husk and testa of the mentioned species, its microbial population, microbial occurrences and its antimicrobial potency. The samples were subjected to solid and liquid state fermentation for a period of ten days during which isolation were made. The array of microorganisms consisted of a heterogeneous mixture, with Bacillius and Aspergillus species accounting for a major portion of the total isolates. Antimicrobial potency of infusion generated from liquid state fermentation was determined by testing it against typed pathogenic cultures and some of the isolates, with commercial antibiotics used as the control. The infusion showed antimicrobial activity against some of the typed cultures and isolate. Salmonella typhi (ATCC) 6539 was recorded to have highest susceptibility with a zone of inhibition measuring 19mm and Aspergillus fumigatus was the only fungi recorded to be susceptible from the tested isolates. There is a possibility of harnessing the antimicrobial potency of the infusion for the development of antimicrobial agents to combat resistant pathogenic organisms tested for.***

Keywords: Cola nitida, Cola acuminata, husk, testa, fermentation

I. INTRODUCTION

The environmental nuisance agricultural by-products cause has made researchers device means of converting them into useful forms and so doing have seen the richness and usefulness of agricultural waste or by-products. Kolanut husk and testa is one of such numerous agricultural by-products produced in tonnes yearly and disposed off without a second thought. The genus Cola contains many species numbering up to 50 in west Africa, of which only a few are fruit bearing, while majority are woody species of economic importance. The few fruit bearing ones notably are; *C. nitida* (gbanja or goro), *C. acuminata* (Obi gidi or Obi abata), *C. verticillata* (Obi Olooyo or slimy kola) and *C. millenii* (Mubo *et al.*,2009).

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Kolanut has found usefulness in the food industry as a flavouring ingredient; it is a good source of dye in textiles and thread because of its tannin content (Esther *et al.*, 2010). Tim (2012) reported kolanut to be helpful in increasing mental activity, ease hunger pangs, restrain thirst and reduce sleep. The testa can be used as ingredient in the formulation of fertilizer because of the high potassium content. The root of the tree can be used as chewing stick for cleaning the teeth and can also be used for carving. (Asogwa *et al.*, 2006).

Some plants have the potential of having their waste products be used for a number of economic products (Ubalua, 2007; Adeyi, 2010). Thier use as co-products of cocoa, kola, coffee and cashew for producing animal feed (Hamzat and Adeola, 2011) is another useful form it has been found can be converted to.

Guava leaves and barks for instance have been used in the treatment of malaria, diarrhea, dysentery, sore throats, vomiting, and menstrual complications and some other ailments as reported by Elekwa *et al.* (2009). Mushrooms of different variety have been reported to have antimicrobial potency such as *Russula delica* reported by Aziz *et al.*, (2007) and *Dictyophora indusiata* reported by Oyetayo *et al.*, 2009. The list of medicinal plants and their parts can go on, indicating its importance in combating diseases with little or no resistance as compared to chemically manufactured drugs. The control of microorganisms is critical for the prevention and treatment of diseases but most microorganisms are known to be resistant to a number of antibiotics (Arekemase *et al.*, 2012) leading to huge economic and live losses. Even though pharmaceutical industries are in search of newer antibiotics due to increase in resistance to the drugs used as therapeutic agents (Gislene, 2000). Plant is an alternative to chemically produced drugs which has been found to have antimicrobial potency. These plant parts/plant products cannot be used without first ascertaining the microorganisms associated with it to determine whether they play beneficial or benign role. The objectives of this study is to isolate, characterize and identify microorganisms associated with fermenting infusion generated during fermentation of kolanut husk and testa of *Cola nitida* and *Cola acuminata* as well as check for its antimicrobial potency.

II. MATERIALS AND METHODS

A. Sample collection and preparation

Cola acuminata and *Cola nitida* were harvested from a farm in Owena Osun, Osun State, Nigeria, and authenticated in the Department of Crop Science and production, Federal University of Technology, Akure, Ondo State, Nigeria.

The samples were harvested around March, collected directly from the tree using sickle and transported to Microbiology Department, Federal University of Technology, Akure, Ondo State, Nigeria. Samples were rinsed to remove sand particles and cut open using sterile knife.

One thousand five hundred and one thousand litres of water was measured into five hundred and three hundred grams of husk and testa respectively in different bowls labeled accordingly. The set up was allowed to ferment for 10 days during which isolation of microorganisms were made on a daily bases. 1ml of the fermenting infusion was taken, serially diluted and plated according to standard microbiological procedures. The antimicrobial potency of the infusion was also tested.

B. Fermentation procedure

Liquid and solid state fermentation were employed. For liquid state fermentation, sterile water of one thousand five hundred and one thousand litres was measured in to bowls containing 500g of husk and 300g of testa respectively and covered immediately with the lids. Ten bowls were used for each sample, labelled accordingly. Microorganisms were isolated from the infusion generated from the liquid state fermentation. Same quantity of sample was weighed for the solid state fermentation but in this case, water was not introduced in to the samples. It was also left to ferment for ten days.

C. Isolation and identification

The bacteria and fungi isolates were identified according to the method of Olutiola *et al*. (2000). Colonial characteristics of the isolated organisms were examined on solidified agar surface after 18-24hr of incubation. The cultural characteristics noted include; colour, shape opacity, translucency, elevation, edges and surface texture. Sugar fermentation and biochemical tests (Gram's reaction, starch hydrolysis, citrate utilization, indole test,
catalase, coagulase, hydrogen sulphide production and motility test) were carried out. Bergey's manual of determinative bacteriology was used to confirm the isolated bacteria.

D. Test organisms

Shigella ATCC 12022 (*Shigella flexneri*), *Salmonella* ATCC 6539 (*Salmonella typhi*), *Entrobacter* ATCC 130A8 (*Enterobacter aerogenes*), *Proteus* ATCC 25933 (*Proteus mirabilis*) and *Citrobacter* ATCC 8090 (*Citrobacter freundii*) were obtained from FIIRO (Federal Institute of Industrial Research Oshodi). The cultures were maintained on Nutrient agar slant, while antibacterial assay was carried out using Mueller Hinton agar.

E. Antimicrobial sensitivity of infusion

Antimicrobial activities of infusion was determined by the agar well diffusion method as described by Esimore *et al.*(1998). A zero-point two millilitre (0.2ml) of 24hour broth culture was aseptically introduced into sterile Petri dishes. Nutrient agar cooled to 45±20C was poured into the sterile Petri dishes. Wells were made on the agar plates using sterile cork borer of 6mm into which varying quantity of the infusion was introduced and incubated at 370C ±2 for 24hours. The plates were observed for zone of inhibition. Sterile water served as the control in the experiment.

F. Antibiotic sensitivity test

The Kirby-Bauer test also known as disc diffusion method was used to determine the effect of commercial antibiotics on the typed cultures obtained from FIIRO (Federal Institute of Industrial Research Oshodi) using the method described by Prescott *et al.*(2005). The antibiotic present on the disc include ofloxacin, cotrimoxazole, nirofurantoin, gentamicin, nalidixic acid, augumentin, tetracycline and amoxicillin. Clear zones around the discs represent zone of inhibition and it was measured in millilitres.

III. RESULTS

The bacterial and fungal counts of fermenting kolanut husk and testa during the ten days fermentation period respectively are shown in figures 1 and 2. Day one, two, five, nine and ten had higher bacterial counts while that of fungi had higher counts between days three to ten. Significant variation was observed in the microbial count between each of the samples during fermentation. The identified bacterial and fungal isolates in the liquid and solid state fermentation mediums are presented in Table 1, indicating a total of nine bacteria and fungi each identified. The array of microorganisms associated with each of the fermentation methods employed is shown on Tables 2 and 3.

Frequency of occurrence of bacterial and fungal isolates from both methods of fermentation is presented on Tables 4 and 5 respectively, thus indicating *Bacillus* species, *Aspergillus* species and *Saccharomyces cerevisiae* to predominate. High occurrences was recorded for *Bacillus subtilis* and *Bacillus laterosporus*, with testa having highest occurrence of 32% and 23% respectively as compared to 16% recorded for the husk samples while *Staphylococcus aureus* occurred the least in both samples. For the fungal isolates, *Saccharomyces cerevisiae* had the highest occurrence of 19% while the least was observed for *Penicillium italicum* (7%) for both husk and testa. It was observed that liquid state fermentation had more bacterial occurrence but lesser of the fungal isolates and vice versa in the case of solid state fermentation.

Antimicrobial activity of the infusion is shown on Figure 3 and 4 against typed pathogenic culture and commercial antibiotics respectively. Isolated organisms were also used but only *Aspergillus fumigatus* showed significant susceptibility to the infusion and is presented on Figure 3 alongside with the typed culture. Concentration of the infusion was calculated to be 0.3g/ml and same concentration was prepared for the commercial antibiotics to compare their efficacy. The typed culture used includes; *Shigella* ATCC 12022 (*Shigella flexneri*), *Salmonella* ATCC 6539 (*Salmonella typhi*), *Entrobacter* ATCC 130A8 (*Enterobacter aerogenes*), *Proteus* ATCC 25933 (*Proteus mirabilis*) and *Citrobacter* ATCC 8090 (*Citrobacter freundii*). From the result, *Salmonella typhi* and *Shigella flexneri* showed the highest and lowest susceptibility to infusion from *Cola acuminata* husk and *Cola nitida* testa with a zone of inhibition measuring 19mm and 6mm respectively.

It was observed that the potency of the infusion varied between the days of fermentation with *Cola acuminata* husk of day ten having the highest antimicrobial activity as compared with the other samples. Infusion from *Cola nitida* husk of day four was more effective on *Aspergillus fumigatus* with a zone of inhibition measuring 21mm. Infusion from the husk had more inhibitory effect as compared to the testa on the organism.

In the control experiment, the commercial antibiotics tested against the organisms included; Ofloxacin (OFL), Nalidixin (NAL), augumentin (AUG), tetracycline (TET), amoxicillin (AMX), cotrimoxazole (COT), nitrofurantoin (NIT) and gentamicin (GEN). Ofloxacin was observed to be most effective out of the commercial antibiotics against all the typed pathogenic cultures.

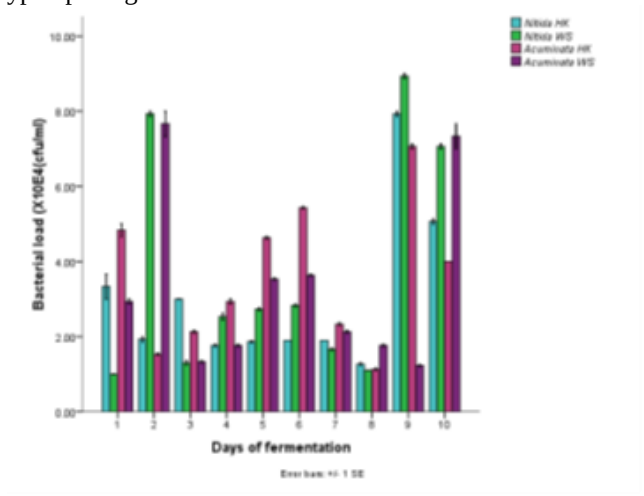

Figure 1: Bacterial count (cfu/ml) of fermenting kolanut husk and testa during LSF

Key: Nitida HK- Cola *nitida* husk Nitida WS- *Cola nitida* testa Acuminata HK- *Cola acuminata* huskAcuminata WS- *Cola acuminata* testaLSF – liquid state fermentation

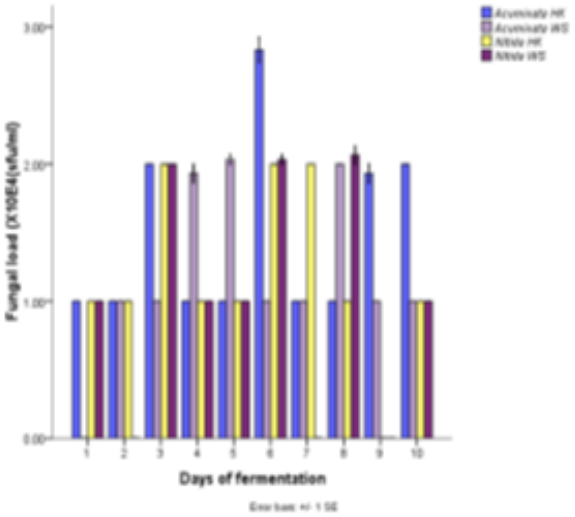

Figure 2: Fungi count (sfu/ml) of fermented husk and testa of kolanut during LSF

Key Nitida HK- Cola *nitida* husk Nitida WS- *Cola nitida* testa Acuminata HK- *Cola acuminata* husk Acuminata. WS-*Cola acuminata* testa. LSF – liquid state fermentation

Table 1: Identified bacterial and fungal isolates from husk and testa of kolanut subjected to solid and liquid state fermentation

s/n	Bacterial isolates	Fungal isolates
1	Bacillus subtilis	Trichoderma viridiae
2	Bacillus sphaericus	Articulospora inflata
3	Bacillus cereus	Aspergillus fumigatus
4	Bacillus laterosporus	Aspergillus flavus
5	Bacillus licheniformis	Aspergillus niger
6	Bacillus firmus	Saccharomyces cerevisiae
7	Micrococcus luteus	Geotricum albidum
8	Lactobacillus fermentum	Penicillium italicum
9	Staphylococcus aureus	Mucor mucedo

Table 2: Bacteria isolates associated with husk and testa of kolanut subjected to liquid and solid state fermentation

Isolates	**Samples**			
Liquid state fermentation	AB	AW	GB	GW
Bacillus subtilis	+	+	+	+
Micrococcus luteus	+	-	+	-
Staphylococcus aureus	+	+	+	+
Bacillus laterosporus	+	+	-	+
Lactobacillus fermentum	+	-	+	-
Bacillus firmus	+	-	+	-
Bacillus sphaericus	+	+	-	-
Solid state fermentation				
Bacillus subtilis	+	+	+	+
Bacillus laterosporus	+	+	-	+
Bacillus licheniformis	+	-	+	+
Bacillus cereus	+	+	+	+

Key: AB- *Cola acuminata* husk , AW- *Cola acuminata* testa**,** GB- Cola *nitida* husk, GW- *Cola nitida* test

Table 3: Fungi isolates associated with husk and testa of kolanut subjected to liquid and solid state fermentation

Isolates	**Samples**			
Liquid state fermentation	AB	AW	GB	GW
Trichoderma viridiae	+	+	+	+
Articulospora inflata	+	+	+	+
Aspergillus fumigatus	+	+	+	+
Aspergillus flavus	+	+	-	+
Aspergillus niger	+	-	+	-

Saccharomyces cerevisiae	+	+	+	+
Solid state fermentation				
Trichoderma viridiae	+	+	+	+
Articulospora inflata	+	-	+	-
Aspergillus fumigatus	+	-	+	+
Aspergillus flavus	-	-	+	+
Aspergillus niger	+	+	+	+
Aspergillus rapens	-	+	-	-
Geotricum albidum	+	+	-	+
Penicillium italicum	+	-	-	-
Mucor mucedo	+	-	+	-

Key AB- *Cola acuminata* husk AW- *Cola acuminata* testa**,** GB- Cola *nitida* husk. GW- *Cola nitida* testa

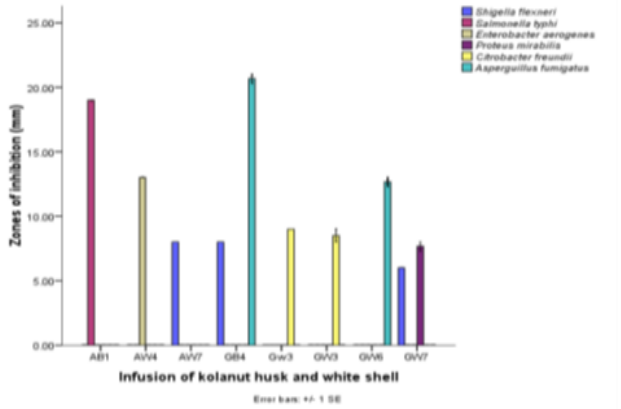

Figure 3: Zones of inhibition of infusion of kolanut husk and testa against typed pathogenic culture and isolated organism

Key *Shigella* ATCC 12022 (*Shigella flexneri*), *Salmonella* ATCC 6539 (*Salmonella typhi*), *Entrobacter* ATCC 130A8 (*Enterobacter aerogenes*),*Proteus* ATCC 25933 (*Proteus mirabilis*) and *Citrobacter* ATCC 8090 (*Citrobacter freundii*).**AB**-*Cola acuminata* husk. **AW**- *Cola acuminata* testa **GB**- *Cola nitida* husk **GW**- *Cola nitida* testa ****Numbers represents days of fermentation**

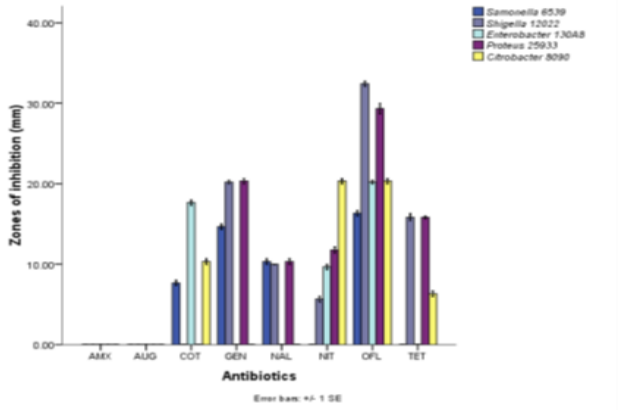

Figure 4: Antibiotic sensitivity pattern of typed pathogenic organisms against commercial antibiotics

Key *Shigella* ATCC 12022 (*Shigella flexneri*), *Salmonella* ATCC 6539 (*Salmonella typhi*), *Entrobacter* ATCC 130A8 (*Enterobacter aerogenes*), *Proteus* ATCC 25933 (*Proteus mirabilis*) and *Citrobacter* ATCC 8090 (*Citrobacter freundii*)AMX: Amoxycillin, AUG: Augumentin, COT: Cotrimoxazole, GEN: Gentamicin, NAL: Nalidixin, NIT: Nitrofurantoin, OFL: Ofloxacin, TET: Tetracycline

IV. DISCUSSION

The possibility of consuming fermenting infusion of kolanut husk and testa prompted the need to determine the microbial load and frequency of occurrence of microorganisms associated with the samples under study.

Low microbial count observed during the initial days of fermentation could be due to the organisms adjusting to new environment, but decreased towards the end of fermentation, possibly due to the utilization of the available nutrient by the organisms (Prescott *et al*., 2005). Gradual increase and decrease in microbial count might be due to nutrient depletion by microorganisms involved in fermentation of the samples due to competition for food and survival and presence of essential minerals and nutrients thus promoting microbial growth and enzyme activity (Oboh and Akindahunsi, 2003a). Sugar bioconversion generates energy for cell metabolism there by enhancing microbial growth (Rainbult and Tewe, 2001). Decrease in fungal counts could be due to the presence of saponins, which have been reported to possess antifungal activity (Bader *et al.,* 2000**).** Depletion of available nutrient and increase in acidity of the fermenting medium are likely reasons why decrease in fungal count was observed (Prescott *et al.*, 2005).

Bacillus, *Saccharomyces cerevisiae* and *Aspergillus* were the predominate organisms isolated. *Bacillus* have been reported to be heterogeneous in nature and versatile in their adaptability to the environment (Gislene, 2000; Bandow, 2002). *Bacillus* species are also known to have the ability to initiate fermentation of both nitrogenous and carbohydrate products thus, giving them edge over other bacteria resulting in their high occurrence in this study. Some species of *Bacillus* have been found be good sources of microbial enzymes. Microbial enzymes are more useful than enzymes derived from animal or plant because of variety of catalytic activities, possibility of higher yield, regular supply due to the absence of seasonal fluctuations and rapid growth of microorganisms and ability to manipulate the genetic makeup of the organisms (Iftikhar, 2010). High occurrence of *Aspergillus* might be traced to its ability to utilize an enormous variety of substances from food due to their enzyme producing capacity (Oyeleke, 2002). Isolation of *Saccharomyces cerevisiae* might be due to its tolerance to moisture (Aboloma and Onifade, 2011) and also to their involvement in fermentation in which case help break down complex substances to simpler useable forms. The presence of *Aspergillus* species suggests that, the agricultural by-product under study could be a threat to the health of either animal or man consuming it, if found out to be the aflatoxin producing strains. The study did not carry out test on the *Aspergillus* species isolated to determine if they are the aflatoxin producing types. There is therefore a need to test the *Aspergillus* isolates for presence of this toxin.

Infusion from *Cola nitida* and *Cola acuminata* testa was observed to be effective against the typed pathogenic organisms in different degrees especially those of *Cola acuminata* testa and husk. *Cola acuminata* was more effective than *Cola nitida* as observed from the study. Ogundare and Akinyemi (2013) reported that antimicrobial properties of plants are due to the presence of phytochemicals. Zone of inhibition recorded by some of the commercial antibiotics.

when compared with the infusion might be due to high standard of purity employed in the preparation of such antibiotics (Doughari *et al.*, 2007). Mailard, 2002 also reported that the effectiveness of antibiotics may be due to their molecular size which aids in their solubility in diluents.

V. CONCLUSION

This study has revealed the array of microorganisms associated with fermenting husk and testa of both species of kolanut. It has also revealed its antimicrobial potency, with infusion from *Cola acuminata* husk and testa showing highest potency. It was most effective against *Salmonella typhi.* These antimicrobial properties can therefore be harnessed in the development of novel antimicrobial drugs against resistant pathogenic microorganisms when the active components are determined and adequately purified.

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