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Research Article

Antimicrobial Activity of Methanolic Seeds Extract of *Cola nitida* (Kolanut) Against Microorganisms Isolated from the Oral Cavity

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ABSTRACTS

This work examines the antimicrobial activity of methanolic seed extract of *Cola nitida* on microorganism isolated from the oral cavity. The various microorganisms of the oral cavity were obtained by taking mouth swabs from various human donors. The organism isolated and identified using biochemical tests were *Klebsiella pneumonia, staphylococcus aureus, Streptococcus pneumonia, Escherichia coli* and *candida albicans,* occurring in percentages of 5.0%, 75%, 11.7%, 5.0% and 3.3% respectively. When a twenty-four hour old culture of the isolated organisms at a concentration of $1x10^8$ CFU/ml, was exposed to concentration of 100mg/ml, 150mg/ml and 200mg/ml of the methanolic seed extract of *Cola nitida*, by agar diffusion method, *Staphylococcus aureus* showed an inhibition zone diameter (IZD) of 13.00 ± 0.57mm, 14.00 ± 0.28 and 15.00 ± 0.57mm respectively and minimum inhibitory concentration (MIC) OF 12.5mg/ml. when used against *Escherichia coli,* an IZD of 5.00 ± 0.28mm was seen at 200mg/ml concentration, with an MIC value of 200mg/ml. The extract also showed activity against *Klebsiella pneumonia* and *Streptococcus pneumonia* at all concentrations used, with MIC values of 6.25mg/ml and 50mg/ml respectively. The extract had no antimicrobial activity against *Candida albicans* at concentration of 100mg/ml but showed an IZD of 12 ± 1.1mm and 13.00 ± 57mm at concentration of 150mg/ml and 200mg/ml and an MIC of 100mg/ml. The standard antibiotics susceptibility test was also carried out against the test microorganism using the agar disc diffusion method and the result was compared with that of the extract. Statistical analysis is significant with ($P<0.05$). Therefore, the methanolic seeds extract of *Cola nitida* showed varying antimicrobial activity on different test organisms at different concentrations of the extract.

Keywords: *Cola nitida,* methanolic extract, oral cavity, microorganism.

INTRODUCTION

According to the World Health Organization, more than 80% of the world`s population relies on traditional medicine for their health care needs[1]. Plant and plant products have been used as medicine since the start of history and this is the oldest known method for healing. Using higher plants for treatment of diseases had started since man started to live on this planet[2]. Due to limited availability and affordability of pharmaceutical medicines in many tropical countries, the majority of the African populations depend on traditional medical remedies, mainly from plants.

Oral diseases are major health problems with dental caries and periodontal diseases among the most important preventable global infectious disease. Oral health influences the general quality of life and poor oral health is linked to chronic conditions and systemic diseases[3]. The oral cavity, or mouth, includes gingival, tongue, cheek, lip, hard palate and soft palate[4]. The environment present in the human mouth allows the growth of the microorganisms found there, and provides a source of water and nutrients, as well as a moderate temperature. The microbes present in the oral cavity adhere to the teeth and gums in order to resist mechanical flushing from the mouth to stomach, where acid-sensitive microbes are destroyed by hydrochloric acid[5]. The micro-flora in the oral cavity has the capacity to defend and play an important role in healthy oral environment. If the micro-flora in the oral cavity rises then it leads to the development of caries and dental disease[6]

Cola nitida is a species of plant belonging to the family sterculiaceae. It is a tree native to the rainforest of tropical West Africa. The common names include kolanut, cola and kola. It is medium sized (<25m) evergreen forest tree. The bole is usually un-branched reaching to 8-20m in height and sometimes attaining 24m. The trunk may grow to 50cm in diameter width, in old trees, narrow buttresses extending to about 1m. The bark of the tree is grey with longitudinal fissures. The leaves have stalks and are alternate, oblong glabrous, leathery and tough, with un-toothed wavy margins and up to 33cm

Kola nut is a native stimulant which is commonly chewed in many West African countries. It is often used ceremonially to honour guest[7]. It is also used for treatment of morning sickness, migraine headache, and indigestion and cleaning of gum of the teeth[8]. It is also used in controlling vomiting in pregnancy and as a stimulant to keep awake and withstand fatigue by students, drivers and

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Table 1: Percentage of microbial isolates.

Organism isolated	Number of isolates	Percentage of microbial isolate (%)
Staphylococcus aureus	45	75.0
Escherichia coli	3	5.0
Klebsiella pneumonia	3	5.0
Streptococcus pneumonia	7	11.7
Candida albicans	2	3.3

Table 2: antimicrobial activity of the methanolic extract of *Cola nitida* seeds at different concentration against the test organism.

Test organism	Concentration (mg/ml)	Mena inhibitory zone diameter (mm)
Staphylococcus aureus	100	13.00 ± 0.57[a]
	150	14.00 ± 0.28
	200	15.00 ± 0.57
Escherichia coli	100	0.00 ± 0.00[b]
	150	0.00 ± 0.00
	200	5.00 ± 0.28
Klebsiella pneumonia	100	12.00 ± 0.86[a]
	150	14.00 ±0.26
	200	23.00 ± 0.57
Streptococcus pneumonia	100	12.00 ± 0.57[a]
	150	15.00 ± 0.86
	200	17.00 ± 1.15
Candida albicans	100	0.00 ± 0.00b
	150	12.00 ± 1.15
	200	13.00 ± 0.57

a, b: Means with different superscripts are significantly ($p<0.05$) different, ± = SEM

other menial workers[7].

Natural plant products are surfacing as increasingly popular treatments, even for oral health care[9]. The aim of this study is to investigate the antimicrobial activity of *Cola nitida* against the microorganisms isolated from the oral cavity which may be responsible for halitosis and periodontal diseases.

MATERIALS AND METHODS

Materials

Sample collection

The sample used was mouth swabs collected from various human students donors. The mouth swabs were collected using sterile swab sticks

Area of study and population of study

The study was carried out at University of Port Harcourt area, Choba in River state, Nigeria. The population of study was 60 students comprises of 35 male students and 25 female students of the University of Port Harcourt

Plant material

The dried powdered seeds of cola nitida (Sterculiaceae), common name kolanut

Culture Media used;

Nutrient Agar (LifeSave Biotech, san Diego, USA), Batch No, 32256-9, Expiration date, February, 2021,

Mueller-Hinton (Titan Biotech Ltd), batch No, 71865-2, expiration date, November 2019

Sabouraud Dextrose Agar (Titan Biotech Ltd), 61902-3, expiration date, May 2020.

MacConkey Agar (Titan Biotech Ltd) Batch No 71725-4 expiration date, May 2020.

The test organisms isolated were obtained from swabs from the oral cavity of different human donor before they brush their mouth in the morning. These swabs were streaked on various growth media and the organisms found to be growing were isolated and identified using various biochemical tests. The bacteria isolated were maintained on nutrient agar slants, and stored at a temperature of 4^{o}C in a refrigerator. The fungus isolated was stored in Sabouraud Dextrose Agar slant. Both bacteria and fungi were sub-cultured onto fresh media frequently to prevent contamination before storage on Nutrient agar slants. The Organisms used in the research work are; *Staphylococcus aureus, Escherichia coli, Klebsiella pneumonia, Streptococcus pneumonia* and *Candida albicans*

Reagents used are; Crystal violet Kovac`s reagents, Lugol`s iodine, Hydrogen peroxides Safranin red and 95% alcohol.

Collection of seeds and extraction

The seeds of *cola nitida* were purchase from Milel market in Port Harcourt local government area in Rivers state, Nigeria. The seeds were air- dried outside away from sunlight for 3 weeks. The dried seeds were pulverized with aid of a hand blender (Corona Nigeria). 120g of the powdered seeds was macerated in 475ml of absolute methanol in a conical flask. It was allowed to stand for 24 hours and filtered. The filtrate was evaporated on a water bath to dryness[10], with slight modification. The dried extract was stored in the refrigerator in sterile universal bottles at 4^{o}C until the time of use

Phytochemical Screening

Phytochemical was screen according to the method by Sofowora, 2008[11], and the phytochemical tested for are alkaloids, tannins, saponins, flavonoids, anthraquinones and cardiac glycosides

Sterility test for seed extract

The sterility test of the seed extract was ascertained by inoculating 1ml of standard seed extract into nutrient agar broth and then poured. The plates were incubated and observed for any visible sign of growth. The absence of growth in the plates indicated sterility of the extracts. The extract was then used for antibacterial and antifungal activities.

Sterilization of materials

Sterilization of glassware was done using an autoclave at 121^{o}C

Isolation of Microorganisms

Table 3: Minimum inhibitory concentration of the methanolic seed extract of *Cola nitida* on the test organisms.

Test organism	Minimum inhibitory concentration (mg/ml)
Staphylococcus aureus	12.5
Escherichia coli	200
Klebsiella pneumonia	6.25
Streptococcus pneumonia	50
Candida albicans	200

The media plates to be used were removed from the refrigerator and dried in the oven. The swab sticks obtained were streaked on the different culture media appropriately. The streaked plates were incubated at temperature of 37ºC for 24 hours and results were observed.

Subculture of colonies

Colonies observed on the streaked plates were sub-cultured into fresh media plates. This was done by using a properly sterilized inoculating loop to pick each colony and streaking this on a new media plate. The isolates were frequently sub-cultured to prevent contamination

Maintenance of isolates

The isolates were maintained by streaking on nutrient agar slants which were stored in the refrigerator.

Biochemical Identification and Characterization of Isolates

Gram staining; a bacterial smear was made on a clean glass slide using distilled water. This smear was air dried and heat fixed by briefly passing through flame. The smear was flooded with 1% crystal violet for 1 minute and then rinsed with excess distilled water. It was flooded again with Lugol`s Iodine for one minute and rinsed again with distilled water. The smear was then flooded with 95% alcohol for 30 seconds rinsed with water and allowed to dry. It was viewed under the microscope at a magnification of x1000, after the addition of immersion oil. The bacteria present were identified due to their different colours and shape when viewed under the microscope.

Catalase test; a drop of hydrogen peroxide is placed on a clean glass slide. This is used to make a bacteria smear. The formation of bubbles in the smear indicates the formation of oxygen by the bacteria cell indicating a catalase positive result.

Indole test; sterile peptone water was aseptically inoculated with a loopful of 24 hour broth culture of the organisms. This was incubated for 24 hours and 0.5ml of Kovac`s reagent was added. The result obtained was observed.

Citrate test; a broth cultured of the organisms isolated was streaked onto the surface of Simmons citrate agar slant and incubated for 24 hours. A positive citrate test is a change in the colour of the agar from green to blue. This test is used to distinguished *Escherichia coli* (citrate negative) from *Klebsiella pneumonia* (citrate positive)

Wet prep test; a wet mount of the swab sticks gotten were soaked in saline solution for about 3days. After this a drop of the saline solution containing the swab sticks was obtained and placed on a clean glass slide. This was then viewed under the microscope to identify the presence of fungi. A confirmatory Germ tube test was carried out on the fungi to confirm that it is *Candida albicans.*

Germ tube test; a wire loop was used to collect the fungal cells and introduced into a tube containing 1ml of plasma followed by incubation for three hour at 37ºC. A smear of this suspension was made on a clean glass slide and a drop of crystal violet was assed and rinsed off after a few seconds and this was viewed under the microscope.

Antimicrobial susceptibility testing

Standardization of the test organisms;

The test organisms to be used where properly standardized by inoculating the test organism using a wire loop into a universal bottle containing nutrient broth. This was incubated for 24 hours and the turbidity was compared to that of 0.5 MacFarland standards. This same procedure was repeated for all the test organisms to be used.

Preparation of stock solution of extract

A 2 gram of the crude extract was reconstituted into 10ml of distilled water to obtain a stock solution of 200mg/ml. from this stock solution, concentrations of 150mg/ml and 100mg/ml were obtained. The different concentration of the extract was used to carry out the research work and kept in the refrigerator till use

Agar diffusion method

A 0.1ml of standardized inoculums of the different test organism was introduced into 20ml of Mueller-Hinton agar and poured aseptically into a sterile Petri dish. A well was made on the agar using a sterile cork borer 0.1ml of the test extract was inoculated into the wells and this was allowed to stand on the bench for 20 minutes. It was carried out in triplicates and incubated at 37ºC for 24 hours after which the results were observed.

Determination of minimum inhibitory concentration

In determining the minimum inhibitory concentration of the test extracts, the broth dilution method was used. A 2-fold serial dilution was carried out of the stock solution of the extract and the standardized organism was inoculated into the different concentration obtained. This was incubated at 37ºC for 24 hours. The least concentration that was able to inhibit the growth of the microorganisms was taken to be the minimum inhibitory concentration.

RESULTS

The result for the Pytochemical screening showed the presence of alkaloids, saponins, flavonoids and tannins.

Table 1 shows the percentage of the microbial isolates obtained. *Staphylococcus aureus* showed the highest percentage of microbial isolate and *candida albicans* showed the least percentage of microbial isolates

Table 2 shows the mean inhibition zone diameter (IZD) of the methanolic extract of *Cola nitida* seeds at concentration of 100mg/ml, 150mg/ml and 200mg/ml on the organism, *staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumonia*, *Streptococcus pneumonia* and *Candida albcans*. The extract was active against *Staphylococcus aureus* with a mean inhibitory zone diameter of 13.00 ± 0.57mm, 14.00 ± 0.28mm and 15.00 ± 0.57mm respectively at the listed concentration. At these concentrations, it was also effective against *Klebsiella pneumonia* with mean inhibitory zone diameter of 12.00 ±

Table 4: Antimicrobial activity of standard antibiotic on the microorganisms.

Test organisms	Standard antibiotics	Inhibitory zone diameter (mm)	Test organisms	Standard antibiotics	Inhibitory zone diameter (mm)
	Gentamicin	25		Ceftazidime	18
	Ceftazidime	32		Erythromycin	18
	Cefuroxime	Nil		Ceftriaxon	32
	Ciprofloxacin	33		Cefuroxime	Nil
Escherichia coli	Ofloxacin	27		Gentamicin	Nil
	Amoxicillin	Nil	*Staphylococcus*	Cloxacilin	Nil
	Nitrofurantion	Nil	*aureus*	Ofloxacin	12
	Ampicillin	Nil		Augmentin	32
	Ceftazidime	19		Erythromycin	Nil
	Cefuroxime	15		Ceftriaxone	Nil
	Gentamicin	15		Ceftazidime	Nil
Klebsiella	Ciprofloxacin	26	*Streptococcus*	Cefuroxime	Nil
pneumonia	Ofloxacin	22	*pneumonia*	Gentamicin	Nil
	Amoxicillin	10		Cloxacin	Nil
	Nitrofurantion	19		Ofloxacin	Nil
	Ampicillin	Nil		Augmentin	40
Candida albicans	Ketoconazole	20			

0.86mm, 14.00 ± 0.26mm and 23.00 ± 0.57mm respectively and effective against *Streptococcus pneumonia* with inhibitory zone diamteter of 12.00 ± 0.57, 15.00 ± 0.86mm and 17.00 ± 1.15mm. *Escherichia coli* was resistant to the methanolic seed extract at 100mg/ml and 150mg/ml but a mean inhibitory zone diameter of 5.00 ± 0.28mm at 200mg/ml. *Candida albcans* was also resistant at 100mg/ml but however had a mean ingibitory zone diameter of 12.00 ± 1.15 and 13.00 ± 0.57 at 150mg/ml and 200mg/ml respectively.

Table 3 showed the minimum inhibitory concentration of the methanolic extract of *Cola nitida* seeds obtained on the test organisms. It was obtained using the Broth dilution method.

Nil = no activity

Table 4 shows the mean inhibition zone diameter (IZD) of the standard antibiotics on the test organism isolated. The standard antibiotics Ceftazidime, Gentamicin, Ciprofloxacin and Ofloxacin showed inhibitory zone diameter of 32mm, 25mm, 33mm and 27mm respectively, when used against *Escherichia coli*. When used against *Klebsiella pneumonia*, the antibiotics Ceftazidime, Cefuroxime, Gentamicin, Ciprofloxacin, Amoxicillin and Nitrofurantion showed inhibitory zone diameter of 19mm, 15mm, 15mm, 26mm, 22mm, 10mm, and 19mm respectively. For Staphylococcus aureus, inhibitory zone diameter of 18mm, 32mm 18mm, 23mm, 12mm, and 32mm were seen for erythromycin, Ceftriaxone, Ceftazidime, Cefuroxime, Ofloxacin and Augmentin respectively. However for Streptococcus pneumonia, inhibitory zone diameter of 40mm was seen with only Augmentin, while for Candida albicans, an IZD of 20mm was seen with Ketoconazole.

DISCUSSION

The screening of the methanolic extract of *Cola nitida* seeds for phytochemical constituents, showed the presence of saponins, alkaloids, flavonoids and tannins but an absence of anthraquinoies and cardiac glycosides. This conforms to the work done by Adeniyi et al, in 2016[12]; however it differs from the work carried out by Muhammad and Fatima, in 2014[10] which indicated an absence of saponins, tannins, alkaloids and flavonoids from the methanolic extract. The phytochemical constituents detected in the seed extracts of the plant are generally called secondary metabolites. These secondary metabolites exhibit various activities such as antimicrobial activity, anti-inflammatory activity, and anti-fungal activity etc. The antimicrobial activity of the *Cola nitida* seed methanolic extract is as a result of high tannin and saponin content of the seeds[10]

Table 1 showed the percentage of the microbial isolates obtained from the mouth swabs collected. It was observed that *staphylococcus aureus* occurred at a larger percentage of 75.0%, making it the most dominant organism isolated. *Streptococcus pneumonia* occurred at a percentage of 11.67% and was the second most dominant organism isolated. *Escherichia coli* and *Klebsiella pneumonia* followed suit occurring at percentage of 5.0% each and *candida albicans*, with the least percentage occurring at 3.3%.

The antimicrobial activity of the methanolic extract of the *Cola nitida* seeds was assay using the agar diffusion method at concentrations of 100mg/ml, 150mg/ml, and 200mg/ml it was observed that at the used concentrations, the extract had antimicrobial against *Staphylococcus aureus* with inhibition zone diameters (IZD) of 13.00 ± 0.57mm, 14.00 ± 0.28 and 15.00 ± 0.57mm respectively and minimum inhibitory concentration (MIC) of 12.5mg/ml. when used against *Escherichia coli*, it had a mean inhibitory zone diameter of 5.00 ± 0.28mm at

200mg/ml concentration and showed no activity at 100mg/ml and 150mg/ml concentration with a minimum inhibitory concentration of 200mg/ml. It also showed activity against *Klebsiella pneumonia* and *Streptococcus pneumonia* at all concentrations used, and gave inhibition zone diameter of 12.00 ± 0.86mm, 14.00 ± 0.26mm and 23.00 ± 0.57mm for *Klebsiella pneumonia* and 12.00 ± 0.57mm, 15.00 ± 0.86 and 17.00 ± 1.15 for *streptococcus pneumonia* at 100mg/ml, 150mg/ml and 200mg/ml respectively with minimum inhibitory concentration of 6.5mg/ml and 50mg/ml respectively. The extract had no antimicrobial activity against *Candidaa albicans* at concentration of 100mg/ml, but showed a mean inhibition zone diameter of 12.00 ± 1.15mm and 13.00 ± 0.57mm at concentration of 150mg/ml and 200mg/ml and a minimum inhibitory concentration of 200mg/ml. These results were represented in table 2 and 3.

Table 4 showed the antimicrobial activity of the standard antibiotics on the test organism, and it was observed that ciprofloxacin had the best activity at a mean inhibition zone diameter of 33mm, followed by Ceftazidime, Ofloxacin and Gentamicin with inhibition zone diameter of 32mm, 27mm, and 25mm respectively when used against *Escherichia coli*. In comparison with the Clinical Laboratory Institute standard, it could be said that *Escherichia coli* was sensitive to the listed antibiotics above but was however resistant to Amoxicillin, Nitrofurantion and Ampicillin. For, *Klebsiella pneumonia*, the organism was seen to be sensitive to Gentamicin, Nitrofurantion, amoxicillin, ofloxacin and ciprofloxacin with inhibition Zone Diameter of 19mm, 15mm, 19mm, 10mm, 22mm, and 26mm respectively, but had intermediate activity to cefuroxime and ceftazidime with inhibition zone diameter of 15mm and 19mm, it was however resistant to Ampicillin with no IZD seen. For *Staphylococcus aurenus*, the organism was sensitive to cftriaxone and Augmentin with IZD value of 32mm, and had intermediate activity to ceftazidime, Erythromycin and cefuroxime with IZD of 18mm and 23mm but was resistant to Ofloxacin and Augmentin. For *Candida albicans*, an IZD of 20mm was seen with Ketoconazole and for *Streptococcus pneumonia*, an IZD of 40mm was seen with only Augmentin and all other antibiotics were resistant.

In comparison to previous works carried out, *Candida albicans* showed antimicrobial activity at concentration of 150mg/ml and 200mg/ml, similar report was observed in the work of Obey and Anthony in 2014[7], which reported that the antifungal activity of the methanolic extract was seen on *Staphylococcus aureus* at concentrations of 100mg/ml and 200mg/ml. This falls in line with the work done by Mubo et al, in 2009[13], reported that *Staphylococcus aureus* had a high susceptibility to the methanolic extract of *Cola nitida* seeds. For *Escherichia coli*, no inhibition zone diameter was seen at concentrations of 100mg/ml and 150mg/ml. However at 200mg/ml, inhibition zone diameter of 5.00 ± 0.28mm was seen. This shows a weak inhibitory activity by the extract on the organism. For *Klebsiella pneumonia*, average inhibition zone diameters of 12.00 ± 0.86mm, 14.00 ± 0.26 and 23.00 ± 0.57mm were seen at the concentration used respectively. This however contradicts the report given by Mubo et al, in 2009[13] which reported that the methanolic extract of the seeds had very low inhibitory activity against this organism.

Antimicrobial activity was seen for the different concentration (100mg/ml, 150mg/ml and 200mg/ml) of the methanolic extract on *Streptococcus pneumonia*. This supports the report given by Muhammad and Fatima in 2014[10] and Obey and Anthony in 2014[7]. Statistical analysis of the result obtained was carried out using the SPSS Software, version 16. P values obtained were less than 0.05 indicating that the results obtained at different concentration, were significant.

CONCLUSION

From the result, it can be deduced that the seeds of *Cola nitida* contain Phytochemical constituents such as: Tannins, flavonoids, alkaloids and saponins. The methanolic extracts of the seeds have antimicrobial activity against *Klebsiella pneumonia*, *Staphylococcus aureus* and *Streptococcus pneumonia* at a lower concentration of 100mg/ml but had activity at a high concentration of 200mg/ml for *Candida albicans* and *Escherichia coli.* Therefore, the methanolic extract of the*Cola nitida* seeds has antimicrobial properties and as such has prospects for incorporation into oral hygiene products such as mouthwashes and toothpastes.

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