



Original Research Article

Antibacterial activity of Methanolic extracts of *Cola nitida* Seeds on Selected Pathogenic Organisms

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A B S T R A C T

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Cola nitida has been used in folk medicine and is a member of the family *Steculicca*. The aim of the current study was to investigate the antibacterial activity of *Cola nitida*. Antibacterial screening was done using agar well diffusion method against *Bacillus cereus*, *Serratia marcescens*, *Staphylococcus epidermidis*, *Proteus vulgaris* and *Salmonella typhi*. The results of antibacterial activity of red *Cola nitida* showed a zone of inhibition of 23.67 ± 0.882 on *Bacillus cereus*, 22.67 ± 1.452 on *Serratia marcescens*, 24.33 ± 0.667 on *Staphylococcus epidermidis* and 13.00 ± 0.577 on *Proteus vulgaris*. The penicillin control showed large zones of inhibition except for *B. cereus* and DMSO did not show any zones of inhibition. The antibacterial property shown by the plant extracts is an evidence of the ethnomedicinal uses of the plants. Incorporation of active compounds isolated from the methanol extract of *Cola nitida* into conventional drug preparations can also tackle the challenges posed by drug resistant microorganisms.

Introduction

In continuation with our interest in the study on medicinal plants (Anthoney *et al.*, 2013, Anthoney *et al.*, 2014 and Obey *et al.*, 2014), we take up on antibacterial activity of *Cola nitida*.

Kola nut is a native stimulant which commonly chewed in many West African cultures. It is often used ceremonially and to honour guest. *Cola nitida* was originally distributed along the west coast of Africa from Sierra Leone to the Republic of Benin

with the highest frequency and variability occurring in the forest areas of Côte d'Ivoire and Ghana (Opeke, 1992).

Chevalier and Perrott (1911) and Warburg (1902) both quoted in (Opeke, 1992) stated that cultivation of *C. nitida* was carried eastwards through Nigeria towards Cameroon and the Congo around 1900, and spread westwards as far as Senegal (Opeke, 1992). *C. nitida* is planted through Senegal, Guinea, Liberia, Côte d'Ivoire, and Ghana

towards the western part of Nigeria (Voelcker, 1935).

Cola nitida has been used in folk medicine as an aphrodisiac and an appetite suppressant. It is also used to treat morning sickness, migraine headache, and indigestion (Esimone *et al.*, 2007). It has been applied directly to the skin to cure wounds and inflammation (Newall *et al.*, 1996). And also being used to clean the teeth and gums (Esimone *et al.*, 2007).

Cola nitida has been used to control vomiting in pregnant women, and also it is used as a principal stimulant to keep awake and withstand fatigue by students, drivers and other menial workers (Haustein, 1971; Chukwu *et al.*, 2006). *Cola nitida* contain both caffeine and tannin, therefore, it is not advised for individuals with stomach ulcers (Ibu *et al.*, 1986, Newall *et al.*, 1996).

Plant-derived substances have recently become of great interest due to their versatile applications. Medicinal plants are the richest resource of drugs of traditional systems of medicine, modern medicines, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs (Ncube *et al.*, 2008).

For a long period of time, plants have been used as a valuable source of natural products for maintaining human health. The use of medicinal plant for pharmaceutical purposes has gradually increased in Brazil. According to WHO (Santos, 1995), medicinal plants would be the best source to obtain a variety of drugs. These days about 80% of people from developed countries use traditional natural medicine. Therefore, medicinal plants should be investigated for better understanding of their medicinal properties, safety and efficiency (Ellof, 1998).

In the past few years, a number of studies have been conducted in different countries to prove antimicrobial properties of natural medicinal plants (Almagboul, 1985; Artizzu, 1995; Ikram, 1984; Izzo, 1995; Kubo, 1993; Shapoval, 1994 and Sousa, 1991). Many natural plants have been used because of their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant. These products are known by their active substances, for example, the phenolic compounds which are part of the essential oils (Jansen, 1987), as well as in tannin (Saxena, 1994). The antimicrobial properties of many plants have been investigated by a number of researchers worldwide.

Natural products and mainly plants (greater than 80%) are the basis of traditional Chinese medicine. Approximately 5,000 plant species used in traditional Chinese medicine are believed to have therapeutic qualities. About 500 plants are commonly prescribed by doctors of Chinese medicine as Chinese Materia Medica, or traditional drugs, and these can be available in raw and processed or concentrated form. Hundreds of years of practical application and experience have gone into classifying the therapeutic use of 'herbs' and their associated properties. Chinese medicine has over 2,000 years of written history (Lee *et al.*, 2005).

Medical practice has taught us to understand that ethnopharmacological data is an important source of new drugs. About 140 new drugs have originated directly or indirectly from Chinese medicinal plants by means of modern scientific methods, confirming that these plants are an important resource. Increasing emphasis on the use of medicinal plants in searching for new drugs is undoubtedly a correct strategy (Liu, 2005).

Medicinal plants have been tested extensively and found to have great pharmacological uses such as anti-inflammatory activity, antibacterial activity, anti-diabetic activity, anti-fungal activity, anti-cancer activity, antioxidant activity, hepatoprotective activity, haemolytic activity, larvicidal activity, anthelmintic activity, pain relief activity, central nervous system activity, sexual impotence and erectile dysfunction and hypolipidemic activity (Hosahally, *et al.*, 2012; Anthoney *et al.*, 2013; Farook *et al.*, 2011; Kisangau *et al.*, 2007; Kamatenesi-Mugisha *et al.*, 2005; Adu *et al.*, 2011; Deepa *et al.*, 2007; Joshi *et al.*, 2011; Arivoli *et al.*, 2012; Anthoney *et al.*, 2014).

Antimicrobial potential of different medicinal plants is being extensively studied all over the world but only a few studies have been carried out in a systematic manner (Arora *et al.*, 2009). The aim of this study is to investigate the antibacterial activity of methanol extracts of *Cola nitida*

Materials and Methods

Sample collection and Extraction procedure

The seeds of *Cola nitida* were bought from Monrovia, Liberia in the West Africa region. The samples were identified by a taxonomist in the University of Eastern Africa, Baraton. The fresh seeds of the *Cola nitida* were cut into the pieces and dried for three weeks; the dried seeds were ground into powder. Forty grams (40 g) of the powdered seeds of the *Cola nitida* were mixed with 400 ml of methanol – water (70:30). The mixture was kept for 24 hours on a shaker for effective extraction of the plant components. The extract was filtered and the solvent was evaporated to dryness at a temperature of 40°C using rotary vacuum evaporator. The

extract was brought to dryness using vacuum and pressure pump. The yield was kept at 4°C prior to use.

Bioassay Study

Preparation of the Bacterial Suspension

The turbidity of each of the bacterial suspension was prepared to match to a 0.5 McFarland standard. The McFarland standard was prepared by dissolving 0.5 g of BaCl₂ in 50 ml of water to obtain a 1% solution of Barium chloride (w/v). Sulphuric acid (1%) was prepared in a 100-ml volumetric flask. To prepare the 0.5 McFarland Standard, 0.5 ml of the 1% BaCl₂ solution was mixed with 99.5 ml of H₂SO₄ solution.

Measure the turbidity of the 0.5 McFarland Standards with the aid of a spectrophotometer at a wavelength of 625 nm to read an optical density of between 0.08 to 1.0. At this absorbance, the McFarland Standard represents a bacterial cell density of approximately 1.5×10^8 CFU/ml ($1.0 \times 10^8 - 2.0 \times 10^8$ CFU/ml). It was then transferred to a screw-capped bottle and sealed with parafilm or paraffin to prevent evaporation due to exposure to air. The bacterial suspensions were then tested against the McFarland standards until they reached the absorbance of the McFarland standard and then they were ready for use.

Preparation of the Extract Concentrations and Antibiotic

Stock solutions for the extract were prepared by dissolving 500 mg in 1 ml of dimethylsulfoxide (DMSO). An antibiotic control was made by dissolving 1µg of penicillin in 1 ml of sterile distilled water. DMSO served as a negative control.

Screening for the antibacterial potential of the plant extract

The agar well diffusion procedure used in the experiment was similar to that used by Taye *et al.*, (2011) and Jeyachandran and Mahesh (2007). The microorganisms used for this study were laboratory strains of *Bacillus cereus*, *Serratia marcescens*, *Staphylococcus epidermidis*, *Proteus vulgaris* and *Salmonella typhi*. A single colony for each of the organisms was picked from agar plate and dissolved in 5 ml of Mueller Hinton broth. The broth was incubated overnight at 37°C. 5 ml of plain Mueller Hinton broth was incubated alongside the organisms to ensure that the medium was not contaminated. The spectrophotometer was set to 625 nm wavelength and each of the microbial cultures was pipetted into cuvettes to measure the absorbance. A cuvette of plain Mueller Hinton broth was used a blank at 0.000 absorbance. The absorbances of the microorganisms were measured. The bacterial organisms exceeding 0.1 absorbance were adjusted by adding bacterial suspension until the absorbance fell between 0.08 to 0.10, matching the McFarland Standard. The organisms falling below 0.08 absorbance were also adjusted until the McFarland standard absorbance was achieved. All the organisms, therefore, reached a cell density of 1×10^8 cfu/ml (Ngeny *et al.*, 2013). 100 µl of each of the organisms were then inoculated onto agar plates for the bioassay (Agyare *et al.*, 2013). Three 6 mm wells were made into each agar plate using a sterile metal cork borer. 100 µl of the standard drug penicillin was placed in one well, the extract in another well and dimethyl sulfoxide (DMSO) was placed in the third well on each plate. The experiment was run in triplicate for each extract and each organism tested. The plates were incubated for 24 to 48 hours and the zones

of inhibition were measured in millimetres with the aid of a meter rule.

Statistical Analysis

A random sampling procedure was done for the entire test and the experiment was conducted in triplicate assays on Mueller Hinton agar plates (Jeyachandran and Mahesh, 2007). The mean values and standard error were calculated for the zones of inhibition. Analysis of variance was used to determine if there was significant difference among the average zones of inhibition of the bacterial organisms by the extract. The Tukey's honestly significant difference test was used to determine pairwise comparisons between average zones of inhibition among the bacterial organisms by SPSS version 21.0.

Results and Discussion

The main zone of inhibition for *S. epidermidis* was the highest (24.33 ± 0.667), followed by that of *B. cereus*, *S. marcescens* and *P. vulgaris* (table 1). The extract was not active against *Salmonella typhi*. The penicillin control inhibited growth at higher zones of inhibition than all the microorganisms except for *B. cereus*. Analysis of variance (ANOVA) showed that there was significant difference in the zones of inhibition among the microorganisms by the extract and the microorganisms ($P < 0.001$). On further comparison with the Tukey's honestly significant difference test, the zone of inhibition for *B. cereus* was not significantly different from those of *S. marcescens* and *S. epidermidis*, but was significantly larger than those of *P. vulgaris* and *S. typhi* (table 2). *S. marcescens* zone was significantly bigger than those of *P. vulgaris* and *S. Typhi* but was significantly lower than *S. epidermidis*. *S. Epidermidis* was significantly bigger than those of *P.*

vulgaris and *S. Typhi*. Also, *Proteus vulgaris* had significantly higher zone of inhibition than *Salmonella typhi*. The control drug showed zones significantly bigger than the extract for all microorganisms except for *B. cereus* where the extract had a significantly larger zone of inhibition than the control.

Among the organisms such as *Serratia marcescens*, *Bacillus cereus*, *Escherichia coli*, *Proteus vulgaris* and *Salmonella sp* tested with the ethanol extract of *G. kola*, only *B. cereus* with an inhibition zone of 10.17 ± 0.477 and *E. coli* with an inhibition zone of 12.83 ± 0.833 were inhibited. All other organisms were not inhibited (Obey et al., 2014).

According to Mubo et al., (2009), the *in-vitro* antimicrobial evaluation of ethanol extracts of four species of *Cola* Schott & Endl. was done using human isolated strains of *Staphylococcus aureus*, *Staphylococcus albus*, *Bacillus subtilis*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Candida albicans*, *Aspergillus niger* as test organisms. The leaf ethanol extracts of the plants were found to be more effective against the tested fungi than the bacteria at high concentrations. None of the extracts was active against *Staphylococcus aureus*. Plant extract of *C. acuminata* (P. Beauv.) Schott & Endl. and *C. nitida* (Vent) Schott & Endl. showed activity on *S. albus* at concentrations ranging from 10–150 mg/ml having comparable diameters of zone of inhibition of 7.3 ± 0.03 – 16.0 ± 0.0 for *C. acuminata* and 10.0 ± 0.0 – 19.0 ± 0.0 for *C. nitida*. Also, these two species of *Cola* demonstrated activities on *C. albicans* and *A. niger* at concentrations ranging from 90–150 mg/ml with relatively close diameters of zone of inhibition. Only *C. acuminata* inhibited the growth of *K. pneumoniae* at the MIC of 90mg/ml whereas, *C. albicans* was

inhibited by *C. acuminata*, *C. Millenii*, *K. Schum* and *C. gigantea* A.Chev. at the MIC of 120mg/ml. Phytochemical screening of the four species of *Cola* showed the presence of alkaloids, saponins, tannins and cardenolides in all the plants which apart from showing the probable closeness of the species could also be responsible for the observed activities. The antimicrobial property shown by the plant extracts is an evidence of the ethnomedicinal uses of the plants. The similarity observed in the phytochemical constituents and antimicrobial activities demonstrated by *C nitida* (Vent.) Schott & Endl., *C. millenii* and *C.gigantea* A. Chev. and *C. acuminata* suggest a probable closeness among these species.

According to Muhammad (2014), aqueous and methanol extracts of red and white variety of *kola* nut showed antibacterial activity against *Streptococcus anginosus*, gram positive bacteria which, is a member of the viridian *Streptococci*. These are heterogenic bacteria with unique pathogenicity than other *Streptococci* (Ryan, 2004). Red *kola* also showed the activity against *Proteus vulgans*, gram negative bacteria at 60 mg/ml, which gave a zone of inhibition of 18 mm and 16 mm at 60 mg/ml for methanol and aqueous extracts. Similar report was observed in the work of (Saravana kumar et al., 2009) on *kola* extract against *Proteus mirabilis*, which gave an inhibition zone of 16 mm at 1000 µg/ml.

According to Indabawa (2011), methanol and water soluble fractions of *Garcinia kola* and *Cola nitida* possess antibacterial activity, *G. kola* was more active against some members of Enterobacteriaceae, namely, *Escherichia coli* and *Salmonella typhi*, whereas, methanol extracts of *Cola nitida* showed grater activity on *S. aureus*. Thus, the plants possess potentials for the

manufacture of potent drugs for the treatment of infections caused by the test organisms, such as typhoid fever, gastroenteritis, urogenital tract infections and boils.

In Nigeria, various concoctions are made from roots, seeds and leaves obtained from a variety of the *kola* tree. And they are administered orally as purgatives, as direct cures or preventions of all sorts of diseases (Andah,1992; Dalziel, 1948).

Cola nitida increases gastric acid secretion in wistar rats. The indication of gastric acid secretion of *cola nitida* could be entirely due to the presence of xanthine or it may involve gastric secretagogues in cola yet to be discovered (Tende *et al.*, 2011).

Antibacterial activities shown by the four *Cola* species are in line with previous antimicrobial works on the species of *Cola* (Reid *et al.*, 2005; Adeniyi *et al.*, 2004; Ebana *et al.*, 1991) where *Cola* extracts were found to exhibit important inhibitory activities against the growth of certain bacteria and fungi. The crude ethanolic extract of *C. acuminata*, *C. nitida* and *C. gigantea* showed important activity against *Staphylococcus albus*. The diameters of the zones of inhibition of these extracts were found to be remarkably close to that of the control drug: erythromycin. The MICs were 10 mg/ml, 10 mg/ml and 60 mg/ml respectively. However, the leaf ethanol extract of *C. millenii* was inactive against this organism. *C. acuminata* showed the most important activity against *Staphylococcus albus*, *Klebsiella pneumoniae*, *Aspergillus niger* and *Candida albicans*. This is probably due to the strong presence of alkaloids in *C. acuminata* as reported by Adegoke *et al.*, (1968). *C. gigantea* also had a high antimicrobial activity against *Staphylococcus albus*,

Bacillus subtilis, and on *Aspergillus niger* and *Candida albicans* whereas, *C. nitida* and *C. millenii* had weak inhibitory effects on the growth of all the microorganisms. There is a need for further study to ascertain if the yield in these species would be increased by using stronger fractionating solvents such as ethyl acetone or methyl acetone. These solvents have been reported to be more vigorous than other solvents used in crude extraction of plants (Ajayejoba and Fadare, 2006).

In an agar well diffusion assay, *G. kola* was tested against several microorganisms and showed the ethanol extracts exhibited zones of inhibition ranging from 17 to 23 mm while the aqueous hot water extract showed zones ranging from 20 to 27 mm. Antifungal activity was shown against *Staphylococcus aureus* at 0.008 mg/ml. Phytochemical compounds such as flavonoids, tannins saponins, steroids, cardiac glycosides and reducing sugar were found to be present (Arekemaje *et al.*, 2012).

The acetic acid extract of *Garcinia Kola* has also shown zones of inhibition as high as 17.5 mm for *S. aureus*, 18.5 mm for *E.coli*, 35.0 mm for *Streptococcus pyogenes* and 37.0 mm for *Salmonella typhi*, (Ezeanya *et al.*, 2013). Between 5 mg/ml to 25 mg/ml of *Garcinia kola* and *Cola nitida* showed zones of inhibition against *E.coli*, *S. aureus*, *S. typhi* and *K. pneumoniae*. The methanol soluble extract of *G. kola* showed zones of inhibition of 20 mm, 25 mm, 24 mm, 9 mm and 20 mm against *E. coli*, *S. aureus*, *S. typhi* and *K. pneumonia* respectively. That of *Cola nitida* showed 23mm, 9 mm, 18.5 mm, 12.5 mm against *E. coli*, *S. aureus*, *S.typhi*, *K pneumonia* respectively. This study showed that *Cola nitida* is a better antimicrobial against *E. coli* while *G. kola* is good for *S. aureus*, *S. typhi* and *K. pneumonia*, (Indabawa *et al.*, 2011).

Table.1 Antibacterial activity of *Cola nitida* methanol extract against selected pathogenic microorganisms

MICROORGANISMS	Mean (mm± S.E.)	Penicillin	DMSO
<i>Bacillus cereus</i>	23.67±0.882	20.33±0.333	0.00±0.000
<i>Serratia marcescens</i>	22.67±1.452	43.66±0.882	0.00±0.000
<i>Staphylococcus epidermidis</i>	24.33±0.667	44.67±0.882	0.00±0.000
<i>Proteus vulgaris</i>	13.00±0.577	26.00±0.577	0.00±0.000
<i>Salmonella typhi</i>	0.00±0.000	26.00±0.577	0.00±0.000

Table.2 Tukey's honestly significant difference test for zones of inhibition of *Cola nitida* methanol extract against selected pathogenic microorganisms

Comparison	P-value	Significance
<i>Bacillus cereus</i> vs <i>Serratia marcescens</i>	0.994	NS
<i>Bacillus cereus</i> vs <i>Staphylococcus epidermidis</i>	1.000	NS
<i>Bacillus cereus</i> vs <i>Proteus vulgaris</i>	0.000	S
<i>Bacillus cereus</i> vs <i>Salmonella typhi</i>	0.000	S
<i>Bacillus cereus</i> vs <i>Bacillus cereus</i> control	0.131	NS
<i>Serratia marcescens</i> vs <i>Staphylococcus epidermidis</i>	0.868	NS
<i>Serratia marcescens</i> vs <i>Proteus vulgaris</i>	0.000	S
<i>Serratia marcescens</i> vs <i>Salmonella typhi</i>	0.000	S
<i>Serratia marcescens</i> vs <i>Serratia marcescens</i> control	0.000	S
<i>Staphylococcus epidermidis</i> vs <i>Proteus vulgaris</i>	0.000	S
<i>Staphylococcus epidermidis</i> vs <i>Salmonella typhi</i>	0.000	S
<i>Staphylococcus epidermidis</i> vs <i>S. epidermidis</i> control	0.000	S
<i>Proteus vulgaris</i> vs <i>Salmonella typhi</i>	0.000	S
<i>Proteus vulgaris</i> vs <i>Proteus vulgaris</i> control	0.000	S

Key: NS= Not Significant; S=Significant

The search for alternative treatment for diseases caused by certain human pathogens have given rise to interest in research on plant extract against microorganisms. *Bacillus cereus* is associated with food poisoning, gastrointestinal and non-gastrointestinal infections due to virulence factors that include enterotoxins, beta-lactamase, proteases and collagenases (Didelot *et al.*, 2000). *Serratia*

marcescens, is a human opportunistic pathogen associated with nosocomial infections and is resistant to multiple antibiotics (Kurz *et al.*, 2003). *Staphylococcus epidermidis* inhabit the skin of both healthy and ill individuals, hence making it an opportunistic and nosocomial pathogen and has been associated with infections in intensive care units of hospitals (Michelim *et*

al., 2005). *Proteus vulgaris* and other members of the genera are usually found in soil, water and sewage but have been associated with hospital-acquired infections due to the presence of virulence factors like fimbriae, flagella and membrane proteins, lipopolysaccharide, capsule and other associated molecules (Rozalski *et al.*, 1997). *Salmonella typhi* is the causative agent of typhoid fever, diarrhea and enteritis, diseases which are associated with several complications (Santos *et al.*, 2003).

From the data obtained in this study it is therefore worthy to mention that the plant can be used to treat against all the infections caused by *Bacillus cereus*, *Serratia marcescens*, *Staphylococcus epidermidis* and *Proteus vulgaris*.

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