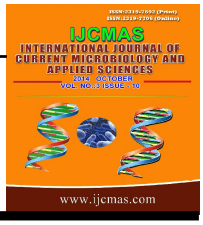
International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 3 Number 10 (2014) pp. 11-22**
http://www.ijcmas.com



Original Research Article

In vitro evaluation of antibacterial activity of infused *Cola nitida* seeds

Jackie K. Obey[1] and T.Anthoney Swamy[2*]

[1]Department of Medical Laboratory Sciences, School of Health Sciences, University of Eastern Africa, Baraton, P. O. Box 2500, Eldoret – 30100, Kenya
[2]Department of Chemistry, School of Science and Technology, University of Eastern Africa, Baraton, P.O. Box 2500, Eldoret, Kenya
**Corresponding author*

Keywords

Cola nitida, Antibacterial activity, Kola, Medicinal plants, Infused

A B S T R A C T

The aim of the current work is to investigate the antibacterial activity of infused *Cola nitida* seeds. Antibacterial screening was done using agar well diffusion method against *Bacillus cereus, Serratia marcescens, Staphyloccus epidermidis, Proteus vulgaris* and *Salmonella typhi*. The extract inhibited the growth of *Bacillus cereus* with a zone of inhibition of 14.33±0.882, *Serratia marcescens* 26.67±1.202 and *Staphylococcus epidermidis* 12.33±0.577. The penicillin control showed large zones of inhibition and DMSO did not show any zones of inhibition. Analysis of variance (ANOVA) performed on the data showed that there were significant differences in the zone of inhibition formed by the extract and antibiotic control ($p<0.0001$). A Tukey's multiple comparison tests also revealed several differences in the zones of inhibition of the extract between organisms and between all the organisms and their controls. Few pairwise comparisons showed that the zones did not differ significantly between organisms. The present study has proved that it is possible to control the growth of some pathogenic microorganisms using the infused extract of *Cola nitida.* The infused extracts from the plant can therefore be considered by pharmaceutical industries in production of cheap, affordable and available drugs for the cure of infections caused by these organisms in situations where expensive conventional drugs are unavailable.

Introduction

This study is the continuation of our previous published work on *Cola nitida* (Obey and Anthoney, 2014). Kola nut is a native stimulant which commonly chewed in many West African cultures. It is often used ceremonially and to honour guest.

Kola nuts were widely used in Central Africa and West Africa long before the arrival of European voyagers (Russell, 1955). Leo Africanus referred to a bitter nut with the name '*goro'* which he encountered during a visit to western Sudan in 1556. This is the name that is used to refer to kola in Nigeria. However, the first definite description of kola nuts was made by

Edouado Lopez who saw seeds with four cotyledons in 1593 (Chevalier *et al*., 1911).

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 species occur in the hot tropical lowland forest with rainfall extending over a period of eight months or more and a temperature of between 23 and 28°C (Ekanade, 1989). Also, it has been cultivated in the transitional zones where the forest gives way to the savanna (Opeke, 1992). It is mainly grown between 6 and 7° north of the equator, but has also been found up to 10º N on the West Coast of Africa. This species requires a hot, humid climate with well-marked wet and dry seasons, and it is capable of withstanding three or more months of dry season (Keay *et al*., 1960).

The nuts of a small number of *Cola* species nuts, including *Cola nitida* and *Cola acuminata,* are good to eat though most species produce seed that is hard and inedible. Some *Cola* species are polycotyledonous. The seed of the edible species is ovoid or ellipsoid, or angular by compression, varying in size up to 3 cm in wide *and 5* cm long. Most of the seed consists of cotyledons to which the minute embryo is attached. In *C. nitida* there are two cotyledons and the seeds readily split into half whilst in *C. acuminata,* where there are three or four cotyledons, sometimes as many as six, the seed splits into a corresponding number of pieces (Irvine, 1956; Keay, 1958 and Russell, 1955).

According to Adiaratou (2008), infused *Cola nitida* has been used to treat Amenorrhoea. Amenorrhoea is a physiological process that can occur in childhood, during pregnancy, during lactation and after the menopause. *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).

The systematics of kola species was in a state of "indescribable confusion" by the beginning of the Twentieth Century as a result of profusion of new species, named on the basis of very meagre evidence (Russel, 1955). It was not until the French botanists Chevalier and Perrot's (1911) taxonomic account that clarity was restored. Chevalier created the subgenus Eucola to contain the five species of edible kola nut: *C. nitida, C, C. ballayi, C.verticillata* and *C. sphaerocarpa.* But the latter three species are not known to be cultivated, but their seeds are sometimes use to adulterate the produce of the commercial species when it is scarce.

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 the current study is to investigate the antibacterial activity of infused *Cola nitida* seeds.

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. Fifty grams of the *Cola nitida* was put in conical flask and mixed with distilled water and heated to boiling for 20 minutes. The extract was filtered and the solvent was evaporated to dryness at a temperature of 40^{o}C using rotary vacuum evaporator. The extract was brought to dryness using vacuum and pressure pump. The yield was kept at 4^{o}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 $BaCl_2$ 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% $BaCl_2$ solution was mixed with 99.5 ml of H_2SO_4 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 x 10^8 CFU/ml (1.0 x108 – 2.0 x 108 CFU/ml. It was then transferred to a screw-capped bottle and sealed with parafilm 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, Staphyloccus 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^{0}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 x 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.

Result and Discussion

The main zone of inhibition for *Serratia marcescens* was the highest (26.67±1.202), followed by that of *B. cereus and Staphyloccus epidermidis* (table 1). The extract was not active against *Proteus vulgaris and Salmonella typhi.* The penicillin control inhibited growth at higher zones of inhibition for all the microorganisms. Analysis of variance (ANOVA) showed that there were significant differences in the zones of inhibition among the microorganisms by the extract and the penicillin control ($P < 0.001$). On further comparison with the Tukey's honestly significant difference test, the zone of inhibition for *B. cereus* was not significantly different from *S. epidermidis*, but was significantly smaller than those of *Serratia marcescens*, and larger than *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.* And, *Proteus vulgaris* had significantly lower zone of inhibition than *S. typhi.* The control drug showed zones significantly bigger than all the zones of the extract and the extract had significantly larger zones of inhibition than the negative DMSO control.

The methanolic extract of *Cola nitida* inhibited the growth of *Bacillus cereus* with a zone of inhibition of 23.67±0.882mm, while the infused extract inhibited *Bacillus cereus* with a zone of 14.33±0.822mm. *C. nitida* methanolic extract inhibited *Serratia marcescens with a zone of* 22.67±1.452mm and the infused extract with a zone of 26.67±1.202mm. *Staphyloccus epidermidis* was inhibited with a zone of 24.33±0.667mm for the methanol extract but this was lower for the infused extract (12.33±0.577mm). The methanolic extract was active against *P. vulgaris* with a zone of inhibition of 13.00±0.577 but the infused extract was inactive against the organism.

The methanolic and infused extracts of *Cola nitida* did not show any activity against *Salmonella typhi* (Obey and Anthoney, 2014).

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

According to Russel (1955), *Cola nitida* and *Cola acuminata* were believed to be resistant and biologically robust species and had no important diseases associated with them. *Cola* species are vulnerable to a host of fungal diseases (Emmanuel and Nick Brown) that can attack all parts of the crop. According to Oludemokun (1979), many fungi are capable of infecting kola fruits at an early stage of development, but the disease symptoms will only develop when conditions are favourable.

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 pneumoniae, 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. *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 viridans *Streptococci*. These are heterogenic bacteria with unique pathogenicity than other *streptococci* (Ryan, 2004). Red *kola* also showed the activity against *Proteus vulgaris,* 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 and 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 *Staphyloccus 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 pnuemoniae, 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 (Ajayeioba 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 where 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* Infused Extract Against Selected Pathogenic Microorganisms

Microorganisms	**Mean (mm± S.E.)**	**Penicillin**	**DMSO**
Bacillus cereus	14.33±0.882	42.66±1.201	0.00±0.000
Serratia marcescens	26.67±1.202	40.00±0.577	0.00±0.000
Staphyloccus epidermidis	12.33±0.577	28.67±0.882	0.00±0.000
Proteus vulgaris	0.00±0.000	35.00±0.577	0.00±0.000
Salmonella typhi	0.00±0.000	21.00±0.577	0.00±0.000

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

Comparison	**P-value**	**Significance**
Bacillus cereus vs *Serratia marcescens*	0.000	S
Bacillus cereus vs *Staphylococcus epidermidis*	0.505	NS
Bacillus cereus vs *Proteus vulgaris*	0.000	S
Bacillus cereus vs *Salmonella typhi*	0.000	S
Bacillus cereus vs Bacillus cereus control	0.000	S
Serratia marcescens vs *Staphylococcus epidermidis*	0.000	S
Serratia marcescens vs *Proteus vulgaris*	0.000	S
Serratia marcescens vs *Salmonella typhi*	0.000	S
Serratia marcescens vs *Serratia marcescens control*	0.000	S
Staphylococcus epidermidis vs *Proteus vulgaris*	0.000	S
Staphylococcus epidermidis vs *Salmonella typhi*	0.000	S
Staphylococcus epidermidis vs *S. epidermidis control*	0.000	S
Proteus vulgaris vs *Salmonella typhi*	1.000	NS
Proteus vulgaris vs *Proteus vulgaris control*	0.000	S
Salmonella typhi vs Salmonella typhi 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).

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

In conclusion, infused *Cola nitida* extract had effects on the microorganisms such as *Bacillus cereus, Serratia marcescens* and *Staphyloccus epidermidis*. Further studies on structural elucidation of *Cola nitida* using advance spectroscopic procedures like nuclear magnetic resonance, mass spectrometry and UV spectroscopy can be used for structural elucidation of the active ingredients in the seeds of *C. nitida.* This information may offer very useful information on the ethnobotanical use of the plant. It is also recommended that communities can be educated on the correct dose and use of *C. nitida* especially where modern hospital facilities and good healthcare systems are completely lacking.

Acknowledgements

The authors of this paper are very much thankful to the Department of Chemistry and Medical Laboratory Science, University of Eastern Africa, Baraton. Authors also thankful to taxonomist Mr. George Ochieng Ondiek, University of Eastern Africa, Baraton.

References

Adegoke, E., Akinsanya, A., Nagu, A. 1968. Studies of Nigerian Medicinal Plants: A preliminary survey of Plant Alkaloids. *J. West Afr. Sci. Assoc.,* 13: 13–39.

Adeniyi, B.A., Groves, M.J., Gangadharam, P.R.J. 2004. Invitro anti-mycobacterial activities of three species of Cola plant extracts (Sterculiaceae). *Phytother Res.,* 18(5): 414–418.

Adiaratou, T., Ingvild, A., Annette, T., Drissa, D., Berit, S.P. 2008. Ethnopharmacological uses of *Erythrina senegalensis*: a comparison of three areas in Mali, and a link between traditional knowledge and modern biological science. *J. Ethnobiol. Ethnomed.,* 4(6): 1–9.

Adu, F., Gbedema, S.Y., Akanwariwiak, W.G., Annan, K., Boamah, V.E. 2011. The effects of *Acanthospermum hispidum* extract on the antibacterial activity of amoxillin and ciprofloxacin, *J. Drugs. Med.,* 3(1): 58–63.

Agyare, C., Dwobeng, A.S., Agyepong, N., Duah, Y.B., Mensah, K.B., Ayande, P.G., Adarkwa-Yiadom, M. 2013. Antimicrobial, Antioxidant and Wound Healing Properties of Kigelia africana (Lam.) Beneth. and *Strophanthus hispidus* DC., *Adv. Pharm. Sci.,* doi: 10.1155/2013/632927.

Ajaiyeoba, E.O., Fadare, D.A. 2006. Antimicrobial potential of extracts and fractions of the African Walnut - *Tetracarpidium conophorum*. *Afr. J. Biotechnol.,* 5(22): 2322–2325.

Almagboul, A.Z., Bashir, A.K., Farouk, A Salih A.K.M. 1985. Antimicrobial activity of certain Sudanese plants used in folkloric medicine. *Fitoterapia,* 56: 331–337.

Andah, B.W. 1992. Nigeria's Indigenous Technology, (2nd edn). Ibadan University Press, Ibadan. Pp 34–38.

Anthoney, S.T., Jackie, O., Ngule, C.M. 2013. In vitro control of selected pathogenic organism by Vernoia adoensi roots. *Int. J. Pharm. Life Sci.,* 4(8): 285–289.

Arekemaje, M.O., Aliyu., M.B., Kayode, R.M.O., Ajiboye A.E., Ajijolakewu A.K. 2012. Antimicrobial effects of *Garcinia kola* (Bitter kola) on some selected pathogens from University of

Ilorin Teaching hospital, Ilorin, Nigeria. *J. Asian Sci. Res.,* 2(4): 159–169.

Arivoli, S., Tennyson, S. (2012). Larvicidal efficacy of *Strychnos nuxvomica* Linn.(Loganiaceae) leaf extracts against the filarial vector *Culex quinquefanciatus* Say (Diptera: Culicidae). *World Jm Zool.,* 7(1): 06–11.

Arora, D.S., Kaur, G.J., Kaur, H. 2009. Antibacterial activity of tea and coffee: Their extracts and preparations. *Int. J. Food Prop.,* 12: 286–294.

Artizzu, N., Bonsignore, L., Cottiglia, F., Loy, G. 1995. Studies of the diuretic and antimicrobial activity of *Cynodon dactylon* essencial oil. *Fitoterapia*., 66: 174–175.

Liu, C-X., Yaniv, Z. 2005. Research and development of new drugs originating from Chinese plants. In: Yaniv, Z. & Bachrach, U. (Eds.), Handbook of medicinal plants. Pub.- Food Products Press - Haworth Press, 500 pp.

Chevalier, A., Perrot, A. 1911. Les Vegetaux utiles de l'Afrique tropicale Francaise. Challamell, Paris.

Chukwu, L.U., Odiete, W.O., Briggs, L.S. 2006. Basal metabolic responses and rhythmic activity of mammalian hearts to aqueous kola nut extracts. *Afri. J. Biotechnol.,* 5(5): 484–486.

Dalziel, J.M. 1916. A Hausa Botanical Vocabulary. T. Fischer Unwin Ltd, London. Pp. 67–69

Deepa, N., Rajendran, N.N. 2007. Antibacterial and anti-fungal activities of various extracts of *Acanthospermum hispidum* DC. *J. Nat Remedies,* 7(2): 225–228.

Ebana, R.U.B., Ekpe, B.E., Madunagu, B.E., Otung, I.N. 1991. Microbiological exploitation of cardiac glycosides and alkaloids from *Garcinia kola, Borreria ocymoides, Kola nitida* and *Citrus aurantifolia*. *J. Appl. Bacteriol.,* 71: 398–401.

Ekanade, O. 1989. The effects of productive and non-productive kola, *Cola nitida* Vent. (Schott and Endlicher), on the status of major soil physical and chemical properties in S.W. Nigeria. *Int. Tree Crops J.,* 5: 279–294.

Ellof, J.N. 1998. Which extractant should be used for the screening and isolation of antimicrobial components from plants? *J. Ethnopharmacol*. 60: 1–6.

Emmanuel, T., Nick, B. A State of Knowledge Report undertaken for the Central African Regional Program for the Environment. Biodiversity Support Program**,** Washington, DC.

Esimone, C.O., Adikwu, M.U., Nworu, C.S., Okoye, F.B.C. Odimegwu, D.C. 2007. Adaptogenic potentials of Camellia sinensis leaves, *Garcinia kola* and *Kola nitida* seeds. *Sci. Res. Essay.,* 2(7): 232–237.

Ezeanya, C.C., Daniel, E.O. 2013. Antibacterial activity of *Garcinia kola* seed and leaf extracts on some selected clinical isolates. *Science J. Microbiol.,* Pp. 1–9.

Farook, S.M., Atlee, W.C. 2011. Antidiabetic and hypolipidemic potential of *Tragia involucrata* Linn. In streptozotocin-nicotinamide induced type II diabetic rats. *Int. J. Pharm. Pharm. Sci.,* 3(4): 103–109.

Haustein, A. 1971. Cola nut: Custom and rituals of some of ethais rating Ivore. *Anthropos,* 69: 457–493 (in French).

Hosahally, R.V., Sero, G., Sutar, P.S., Joshi, V.G., Sutar, K.P. and Karigar, A.A. 2012. Phytochemical and pharmacological evaluation of *Tragia cannabina* for anti-inflammatory activity. *Int. Curr. Pharm. J.,* 1(8): 213–216.

Ibu, J.O., Iyama, A.C., Ijije, C.T., Ishmael, D., Ibeshim, M., Nwokediuko, S. 1986. Effect of *Cola acuminate* and nitida on gastric acid secretion. *Scand. J. Gastroenterol.,* 124 (Suppl.): 39–45.

Ikram, M., Inamul, H., 1984. Screening of medicinal plants for antimicrobial activities. *Fitoterapia.,* 55: 62–64.

Indabawa, I.I., Arzai, A.H. 2011. Antibacterial activity of *Garcinia kola* and *Cola nitida* seed extract. *Bayero J. Pure Appl. Sci.,* 4(1): 52–55.

Irvine, F.R. 1956. Plants of the Gold Coast. Oxford University Press, Oxford.

Izzo, A.A., Di Carlo, G., Biscardi, D., Fusco, R., Mascolo, N., Borreli, F., Capasso, F., Fasulo, M.P., Autore, G. 1995. Biological screening of Italian medicinal plants for antibacterial activity. *Phytother. Res.,* 9: 281–286,1995.

Jansen, A.M., Cheffer, J.J.C., Svendsen, A.B. 1987. Antimicrobial activity of essential oils: a 1976-1986 literature review. Aspects of test methods. *Planta Med.,* 40: 395–398.

Jeyachandran, R., Mahesh, A. 2007. Antimicrobial Evaluation of Kigelia Africana (Lam). *Res. J. Microbiol.,* 2(8): 645–649.

Joshi, C.G., Gopal, M., Byregowda, S.M. 2011. Cytotoxic activity of *Tragia involucrata* Linn. extracts. *American-Eurasian J. Toxicol. Sci.,* 3(2): 67–69.

Kamatenesi-Mugisha, M., Oryem-Origa, H. 2005. Traditional herbal remedies used in the management of sexual impotence and erectile dysfunction in western Uganda. *Afr. Health Sci.,* 5(1): 40–49.

Keay, R.W.J. 1958. Trees of Nigeria. Oxford University Press, New York.

Keay, R.W.J., Onochie, C.F.A., Stanfield, D.P. 1960. Nigerian trees, Federal Department of Forest Research, Ibadan, Nigeria.

Kisangau, D.P., Hosea, K.M., Joseph, C.C. and Lyaruu, H.V.M. 2007. *In vitro* antimicrobial assay of plants used in traditional medicine in Bukoba rural district, Tanzania. *Afr. J. Traditional, Complement. Altern. Med.,* 4(4): 510–523.

Kubo, L., Muroi, H., Himejima, M. 1993. Structure-antibacterial activity relationships of anacardic acids. *J. Agri. Food Chem*., 41: 1016–1019, 1993.

Kurz, C.L., Chauvet S., Andres E., Aurouze M., Vallet I., Michel G.P.F., Uh M., Celli J., Filloux A., de Bentzmann S., Steinmetz I., Hoffmann J.A., Finlay B.B., Gorvel J., Ferrandon D., Ewbank J. 2003. Virulence Factors of the Human Opportunistic Pathogen Serratia marcescens Identified by *In Vivo* Screening. *The EMBO J.,* 22(7): 1451–1460.

Lee, H-H., Itokawa, H., Kozuka, M. 2005. Asian Herbal Products: The basis for development of high-quality dietary supplements and new medicines, Pp. 21–72. In: Shi, J., Ho, C.T. & F. Shahidi (Eds.) Asian Functional Foods. CRC Press, Taylor & Francis Group, 647 Pp.

Michelim, L., Lahude, M., Araujo, P.R., Giovanaz, H., Muller, G., Delamare, A.P.L., Olavo, S., da Costa, P., Echeverrigaray, S. 2005. Pathogenic factors and antimicrobial resistance of *Staphylococcus epidermidis* associated with nosocomial infections occurring in intensive care units. *Braz. J. Microbiol.,* 36(1).

Mubo, A.S., Micheal, O.S, Oyedokun, O.E., Oluwadayo. O.S. 2009. Phytochemical and Antimicrobial Studies of Four Species of *Cola* Schott & Endl. (Sterculiaceae). *Afr. J. Tradit. Complement. Altern. Med*., 6(4): 518–525.

Muhammad, S., Fatima, A. 2014. Studies on phytochemical evaluation and antibacterial properties of two varieties of Kolanut (*Cola nitida*) in Nigeria. *J. Biosci. Med.,* 2: 37–42.

Ncube, N.S., Afolayan, A.J., Okoh, A.I. 2008. Assessment techniques of antimicrobial properties of natural compounds of plant origin: current methods and future trends. *Afr. J. Biotechnol.,* 7(12): 1797–1806.

Newall, C., Anderson, L.A., Phillipson, J.D. 1996. Herbal Medicines: A Guide for

Health-Care Professionals. Pharmaceutical Press, London, England. 84 Pp.

Ngeny, L.C., Magiri, E., Mutai, C., Mwikwabe N., Bii C. 2013. Antimicrobial properties and toxicity of hagenia abyssinica (Bruce) J. F. Gmel, Fuerstia africana, T. C. E. Fries, Asparagus racemosus (Willd.) and Ekebergia capensis Sparrm. *Afr. J. Pharmacol. Theurap.,* 2(3): 76–82.

Obey, J.K., Anthoney Swamy, T. 2014. Antibacterial activity of methanolic extracts of *Cola nitida* seeds on selected pathogenic organisms. *Int. J. Curr. Microbiol. Appl. Sci.*, 3(8): 999–1009.

Obey, J.K., Anthoney Swamy, T., Ngule, C.M. 2014. *In Vitro* control of selected pathogenic organisms by ethanolic extract of *Garcinia Kola Seeds*. *Int. J. Curr. Microbiol. App. Sci.,* 3(4): 183–196.

Oludemokun, A.A. 1979. A review of cola diseases. *Pans* 25: 265–269.

Opeke, L.K. 1992. Tropical tree crops. Spectrum Books Ltd., Ibadan.

Reid, K.A., Jager, A.K., Light, M.E., Mulholland, D.A., Staden, J.V. 2005. Phytochemical and pharmacological screening of Sterculiaceae species and isolation of antibacterial compounds. *J. Ethnophamacol.*, 97(2): 285–291.

Russell, T.A. 1955. The kola of Nigeria and the Cameroons. *Trop. Agri. (Trinidad),* 32: 210–240.

Ryan, K.J., Ray, C.G. 2004. Sherris Medical Microbiology, 4th ed. McGraw Hill. Pp. 293–294.

Santos, P.R.V., Oliveira, A.C.X., Tomassini, T.C.B. 1995. Controle microbiógicode produtos fitoterápicos. *Rev. Farm. Bioquím.*, 31: 35–38.

Saravana kumar, A., Venkateshwarank, Vanitha, J., Vasudevan, M., Sivakumar, T. 2009. An evaluation of antibacterial activity of phenol and flavonoid content of *Thespesia populnea* flower extract. *Pakistan J. Pharmacol. Sci.,* 22: 282–286.

Saxena, G., McCutcheon, A.R., Farmer, S., Towers, G.H.N., Hancock, R.E.W. 1994. Antimicrobial constituents of *Rhus glabra*. *J. Ethnopharmacol.,* 42: 95–99.

Shapoval, E.E.S., Silveira, S.M., Miranda, M.L., Alice, C.B., Henriques, A.T. 1994. Evaluation of some pharmacological activities of *Eugenia uniflora*. *J. Ethnopharmacol.,* 44: 136–142.

Sousa, M., Pinheiro, C., Matos, M.E.O., Matos, F.J., Lacerda, M.I., Craveiro, A.A. 1991. Constituintes Químicos de Plantas Medicinais Brasileiras. Universidade Federal do Ceará, Fortaleza, Pp. 385–388.

Taye, B., Giday M., Animut A., Seid J., 2011. Antimicrobial activities of selected medicinal plants in traditional treatment of human wounds in Ethiopia. *Asian Pac. J. Trop. Biomed.,* 1(5): 370–375.

Tende, J.A., Ezekiel, I., Dare, S.S., Okpanachi, A.O., Kemuma, S.O., Goji, A.D.T. 2011. Study of the effect of aqueous extract of Kolanut (*Cola nitida*) on gastric acid secretion and ulcer in white wistar rats. *Brit. J. Pharmacol. Toxicol.,* 2(3): 132–134.

Voelcker, O.J. 1935. Cotyledon colour in kola. *Trop. Agri.,* 12: 231–234.