

## **Evaluation of the Antimicrobial Potentials and Adverse Effect of Kolanut (*Kola nitida* Malvaceae) on the Oral Cavity and the Impact on Cariogenic Bacteria: A Socio-Demographic Study**

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### **Authors' contributions**

This work was carried out in collaboration among all authors. Authors AMA and CNF designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors MEAT, CE, ETF and KK managed the analyses of the study, data mining and the literature searches. All authors read and approved the final manuscript.

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### **ABSTRACT**

Kola nut (*Kola nitida*, Malvaceae) is one of the nuts consumed for socio-cultural reasons in West Africa sub-region and it has been used in Western African and Anglo-American herbal medicine as an antidepressant. The aim of this study was to evaluate the beneficial and adverse effect of kola nut consumption on the oral cavity.

This was a cross sectional survey conducted on Kola nuts consumers using a qualitative *in-vitro*

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analytical phyto-chemical screening and microbiological activity testing of the Kola nut samples. One hundred and two volunteers participated in the study that was made up of 66(64.4 %) males and 36(35.3%) of females. Kola nuts consumers had the age of participants ranging between 20 to 60 years and derived from all social classes of the West African community. There was a high prevalence of dental attrition 95(93.3 %), arrested caries 95(93.3 %), and extrinsic stains 91(90 %). The DMFT (Decayed/Missing/Filled Teeth) score was 4.7. The phytochemical screening of Kola nut showed the presence of phenol, tannin, flavonoids, alkaloids, anthocyanin, sterol, and antraquinone. Methanol extracts of *Kola nitida* showed no activity against the isolates of *Candida spp*. But there was less activity against *Streptococcus spp* and an average activity against Actinomycetes and isolate of *Lactobacillus spp* respectively. Kolanut exhibited some degree of remineralization oral hard tissues, causing esthetic problems (dental staining) and non-carious dental tooth wear (attrition). Kola nut inhibited the growth of cariogenic bacteria and did not inhibit the growth of *Candida albicans*. Studies on intra-oral activities needs some further investigations to establish its importance to dental oral care. Results showed that extract of *Kola nitida* had antibacterial effects by inhibiting the growth of cariogenic bacteria of the mouth at certain level of concentration. The poor oral health seeking behaviours of participants were observed as factors contributing to a poor oral hygiene status and consequently the impairment of oral health of the kola nut consumers.

**Keywords:** Bacteriostatic; cariostatic; kola nut; oral cavity.

## 1. INTRODUCTION

Kola nut is the nut of kola tree (*Kola nitida*), a genus of tree native to the tropical rainforest of Africa. Classified in the family of Malvaceae, subfamily of Stercuoideae (or treated in the separate family Sterculiaceae) [1]. Common names include kola nut, cola, kola and bitter kola.

It is chewed in many West African cultures, individually or in a social setting, to restore vitality and ease hunger pangs. Kola nuts are an important part of the traditional spiritual practice of the culture and religion in West Africa. They are used as a religious objects and sacred offerings during prayers, ancestor veneration, and as symbols in significant life events, such as naming, weddings ceremonies, traditional divination and funerals. Therefore they are used in gestures of peace, friendship, hospitality and it is important in various religious and traditional ceremonies in West and Central Africa [2,3].

It is one of the nuts consumed for socio-cultural reasons in West Africa sub-region and have been used in Western African and Anglo-American herbal medicine as an antidepressant [4].

Kola nut is best known for its caffeine containing seeds and are chewed as a stimulant [5]. Those seeds contain many active ingredients that are useful in pharmaceutical industries and in the production of kola type beverages, dyes, wines and confectionery [6]. Kola nut are also reported to suppress sleep, hunger and thirst, have aphrodisiac effect and have been used in

Western and Central Africa for thousands of years to treat headache, sleep migraine, dysentery and diarrhea [5,7]. On the account of their sleep suppressing effect, kola nut chewing is now very popular among students, laborers and long distance drivers in West African countries [4,8].

The oral cavity is a very dynamic environment where there is interaction between saliva, oral bacteria and diet. Dental decay, the irreversible solubilization of tooth mineral by acid produced by bacteria like *Streptococcus mutans* and *Lactobacilli* [9]. Then the elimination of cariogenic bacteria from the oral cavity using antibacterial agents is one of the primary strategies for the prevention of dental caries and a variety of plants with potent activity are known to be used in indigenous community for dental hygiene worldwide [10,11]. A sample kola nut seed is shown in Fig. 1.

In Cameroon, kola nut is widely consumed and has been observed that many people consuming kola nuts presents with certain peculiarities in the oral environment. Therefore this study was carried out to determine the effects of kolanut on the oral cavity and the impact cariogenic bacteria.

## 2. MATERIALS AND METHODS

This was a cross sectional survey carried out for one month in April 2017 with a convenient sampling method using structured questionnaires and a qualitative *in vitro* laboratory phyto-

chemical and microbiological activity testing of the kola nut samples.

The study was carried out in the Bangwa and Bafoussam area of the West Region of Cameroon. Bangwa is a village made up mainly of an indigenous population, located about 33 km from Bafoussam, the West Regional headquarters known to a busy commercial center made up of a mixed population of people from all over the country with diverse socio-cultural and religious backgrounds.

Regular kola nut consumers of all age groups resident in the study areas who volunteered to participate in this study were recruited.

All laboratory analyses were carried out in the microbiology laboratory of the University of Montagnes –Cameroon.

## 2.1 Data Collection

### 2.1.1 The study was carried out in 3 phases

Collection of sociodemographic information of the participant followed by a clinical dental examination carried out under bright light and finally intra oral (Plaque) samples collected for phytochemical analysis and evaluation of microbial activities. Self-administered questionnaires were used to collect information on the sociodemographic status, the perception of kola nut consumption, the impact of kola nut on the teeth and the oral hygiene practices of participants. Intra-oral examination was carried

out by trained dental personnel in the regional hospital and a designated private dental clinics. Bafoussam to establish dental diseases and abnormalities in the mouth.

## 2.2 Collection and Processing of Samples

Debris and plaque in dental cavities were collected for laboratory analysis by taking the necrotic tooth tissues or plaque swab for the isolation of bacteria. The samples were placed in sterile containers and transported at 4°C to the Laboratory. The fruits of *Kola nitida* were purchased in the localities of Bangoua from local vendors and transported to the laboratory for analysis. The samples were identified by a taxonomist in the University of Dschang, Cameroon.

The fresh seeds of the *Kola nitida* were cut into pieces and dried for three weeks; the dried seeds were ground into powder and 40 g of the powdered seeds of the *Kola 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 with Whatman filter paper 42 and the solvent was evaporated to dryness at a temperature of 40°C using rotary vacuum evaporator. Approximately 2 kg of fresh nuts were cut-up into small pieces, dried at room temperature, grinded and soaked in a methanolic solution within 72 h and filtered extract was brought to dryness using vacuum and pressure pump. The yield was kept at 4°C prior to use.



Fig. 1. Kola nut seed sample for study

## 2.3 Laboratory Analyses

### 2.3.1 Isolation and identification of microorganisms

Dental samples collected from plaque deposits of the tooth using a sterile excavator were inoculated unto Sabouraud Dextrose Agar (SDA), blood agar, De Man Rogosa and Sharpe Agar (MRS) and Potatoes Dextrose Agar (PDA) culture media for the isolation of yeasts; *Streptococcus spp*, *Lactobacillus spp* and *Actinomyces spp*, respectively.

The blood agar, PDA and SDA plates were incubated under aerobic conditions at 37°C for 24 to 48 hours while the MRS plates were incubated in 5% CO<sub>2</sub>. Suspected colonies were sub-cultured and isolates were identified using standard biochemical tests such as Gram staining, catalase, coagulase, oxidase and the germ tube test [12].

### 2.3.2 In vitro evaluation of antimicrobial activity

The stock solution was prepared extract of 4096 mcg / ml in 5% dimethyl sulfoxide (DMSO). Those of Nystatin and Ciprofloxacin (positive controls) were also prepared at a concentration of 32 µg / ml.

Bacterial cultures were diluted in 0.9% NaCl sterile solution to obtain a suspension of  $1.5 \times 10^6$  CFU/ml while the yeast cells were diluted to a final concentration  $1.5 \times 10^3$ .

The antimicrobial activity of minimum inhibitory concentration (MIC) of the kola nut extract was evaluated by the microdilution method as previously described by Newton et al. [13]. For this, in the wells of a 96 well plate, a 100 µm/l volume of MH broth (bacteria) or SD (fungi) was introduced. In the first well of each column were introduced 100 µm/l of sample to test and seven successive 2-fold serial dilutions were made to a final volume of 100 µm/l .. One hundred microlitre inoculum previously prepared was introduced into each well. The plates were then covered with waxed paper and incubated at 35°C for 24 hours for bacteria and 48 hours for yeasts. For each sample, 3 replicates were performed. The culture medium without microorganism, the culture medium containing the microorganism and only the culture medium containing only extract served as a negative control during testing. After the incubation, the bacterial growth was detected using a solution of 2% p-

iodonitrotetrazolium (INT). The colour change was noted and compared to the negative controls. The Minimum Inhibitory Concentrations (MICs) were defined as the smallest concentrations of extract for which there was no growth (absence of the appearance of the pink colour). Regarding yeasts, growth was evaluated after 48 hours with the naked eye. The MIC was defined as the lowest concentration of extract for which we observed no growth.

## 2.4 Data Analysis and Presentation

Data from the survey was analyzed using Epi.info 3.1.3 Microsoft excel 2010. Data was presented in the form of tables and charts. Mean values for the microbial growth parameters was calculated using excel version 7, 2010.

## 3. RESULTS

### 3.1 Socio-demographic Study

A total of 102 participants made up of 66 (64.7%) males and 36 (35.3%) females took part in the study. The 41-50 years age groups 28 (27.5%) were most represented, and 8(37.3%) were business persons, 29(28.4%) were farmers; 63(61.8%) lived in rural areas while 39(38.8%) in urban areas as shown in (Table 1).

### 3.2 Reasons for Kola Nut Consumption

The majority 98(97.1%) of participants consumed Kola nuts principally for pleasure 27(26.6%) for energy resistance 19(18.6%) to prevent nausea/vomiting, 51(52%) to suppress hunger, and 47(48%) to prevent sleep as shown in (Fig. 2).

#### 3.2.1 Duration and frequency of kola nut consumption

Two thirds 62(60.8%) of the participants had been consuming kola nut for more than 13 years and 17 (16.7%) for at least 4-6 years, (Table 2). Some 16 (15.7%) participants consumed 1 kola nut/day A third 33(32.4%) of the respondents consumed an average of 2 kolanuts /day, and 15(14.7%) 3 kolanut/day.

#### 3.2.2 Perception about the effects of kola nut consumption

Thirty (29.4%) participants thought that kola nut had positive effects on the teeth, 16 (53.3%) thought it strengthened the teeth, 6 (20%) were convinced the nuts kills bacteria and 8 (26.7%) that it suppresses halitosis (table 3). More than

80 (83%) thought it caused stains, 16 (16.7%) tooth wear, 8 (7.2%) caries and 5 (7.2%) halitosis as shown in Table 3.

**3.2.3 Oral hygiene practice**

Amongst the respondents 91 (89.1%) who brushed their teeth, 57 (62.6 %) brushed once/daily, 32(35, 2%) brushed twice/daily and 2(2.2%) brushed 3 times/daily (Fig. 3).

**3.2.4 Oral hygiene status**

For oral hygiene status 71 (69.60%) of the respondents examined had a fair oral hygiene, 18(17.60%) have a bad oral hygiene and only 13(12.70%) had a good oral hygiene (Fig. 4).

**3.2.5 Oral hygiene and pathology**

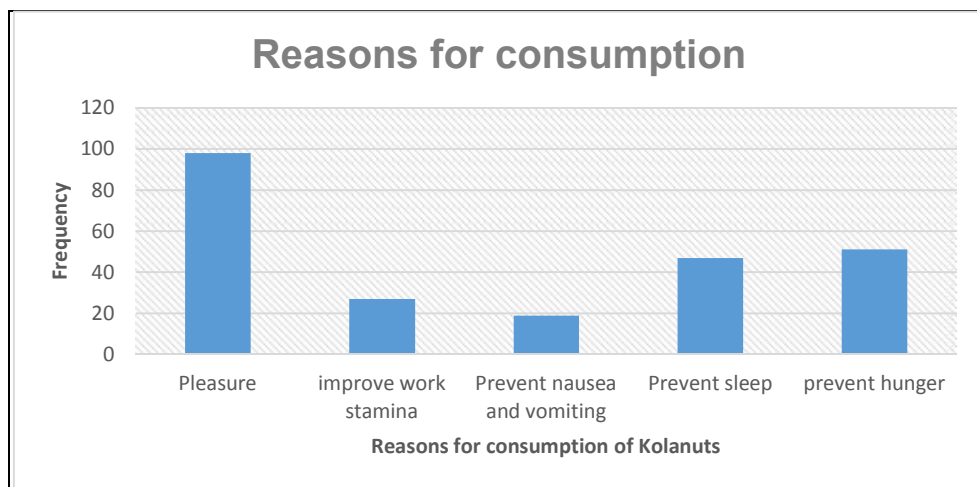
Almost half 48 (47.1%) of the respondents showed the presence of gingivitis, 15 (14.7%) halitosis, 10 (9.9%) retained root, 20 (19.6%) filled teeth, 16 (15.7%) missing teeth, 47 (46.1%) decayed teeth, 72 (70.6%) plaque and almost all the respondents had some level of calculus (Table 4).

**3.2.6 DMFT Score**

More than a third 36 (36.7%) had a DMFT of 0, 48 (49.0%) DMFT 1 to 3, 11 (11.2%) had DMFT 4 to 6 and 2 (2.0%) between DMFT 7 to 9 (Table 5).

**Table 1. Demographic profile of the participants**

Variables	Frequency	Percentages
<b>Gender</b>		
Male	66	64.7%
Female	36	35.3%
<b>Age(years)</b>		
20-30	16	15.7%
31-40	14	13.7%
41-50	28	27.5%
51-60	19	18.6%
>61	25	24.5%
<b>Socioeconomic status</b>		
Business	38	37.3%
Farmers	29	28.4%
House wife	8	7.8%
Retired	14	13.7%
Teacher	13	12.7%
<b>Living area</b>		
Rural	63	61.8%
Urban	39	32.2%



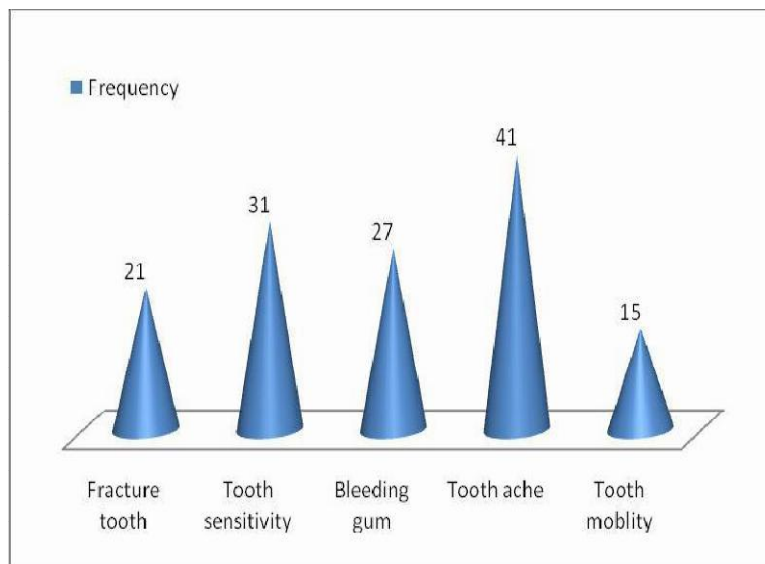
**Fig. 2. Reasons for kola nut consumption**

**Table 2. Duration and frequency of kola nut consumption**

Duration of Kolanut consumption	Frequency	Percentages
1-3yrs	9	8.8
4-6yrs	17	16.7
7-9yrs	3	2.9
10-13 yrs	11	10.8
>13 yrs	62	60.8
Frequency of consumption		
1 kolanut /day	16	15.7
2 kolanuts /day	33	32.4
3 kolanuts /day	15	14.7
4 kolanuts /day	11	10.8
More than 4 kolanuts/day	17	16.7

**Table 3. Perceptions about positives and negatives effects**

Effects	Frequency	Percentage
<b>Positive effect</b>		
Kills bacteria in the mouth	6	20%
Make teeth strong Suppress halitosis	16	53.3%
halitosis	8	26.7%
<b>Negative effects</b>		
Causes halitosis	5	7.2%
Caries	8	7.2%
Tooth wear	16	16.7%
stains	80	83%



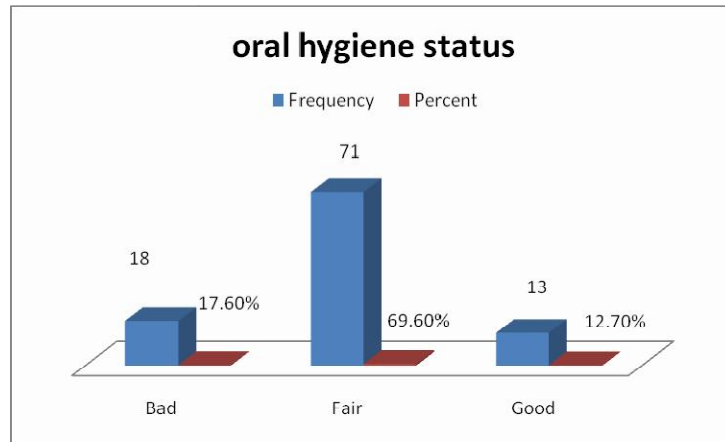
**Fig. 3. Dental problems faced by the respondents**

**3.2.7 Arrested caries**

The prevalence of arrested caries was 93%. The majority 92 (90.2%) had extrinsic stains and 10 (9.8 %) had intrinsic stains (Fig. 5).

**3.2.8 Tooth wear**

Four fifth 85(83.3%) had attrition, while 1 (1%) erosion and 16 (15%) did not have any tooth wear (Fig. 5).



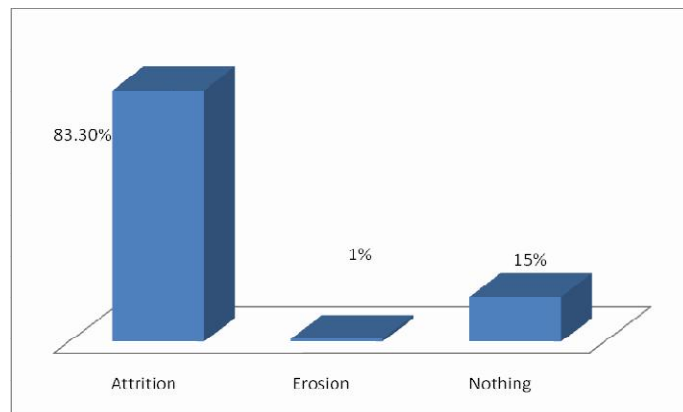
**Fig. 4. Oral hygiene status and dental pathology**

**Table 4. Oral hygiene and pathology**

Oral hygiene status	Frequency	Percentage
Plaque	72	70.6%
Calculus		
+	68	66.7%
++	28	27.5%
+++	6	5.9%
Decayed teeth	47	46.1%
Missing teeth	16	15.7%
Filled teeth	20	19.6%
Retained root	10	9.9%
Halitosis	15	14.7%
Gingivitis	48	47.1%

**Table 5. DMFT assessment**

DMFT score	Frequency	Percentage
0	36	36.7%
1-3	48	49.0%
4-6	11	11.2%
7-9	2	2.0%
TOTAL	98	100%



**Fig. 5. Prevalence of tooth wear**

**Table 6. Phytochemical composition of the extract of Kola nut**

Phenol	Tannin	flavonoids	Alkaloids	Saponin	Anthocyanin	Sterol	Anthraquinone
+	++	+	+	+	+	+	+

**Table 7. Sensitivity test**

<i>Candida spp</i>	MIC	Nystatin
2	> 1024	2
7	> 1024	2
<i>Actinomycetes spp</i>	MIC	Ciprofloxacin
8	256	625
4	128	625
10	> 1024	1
2	> 1024	1.25
3	256	625
<i>Streptococcus spp</i>	MIC	Ciprofloxacin
10	512	625
4	512	625
3	512	312
11	> 1024	1.25
8	256	625
5	512	625
9	256	125
<i>Lactobacillus</i>	MIC	Ciprofloxacin
7	128	312

### 3.2.9 Culture oral swabs

From the 102 respondents examined, 30 samples were collected and from those sample, We obtained 01 isolate of *Lactobacillus acidophilus*, 05 isolates of *Actinomycetes*, 02 isolates of *Candida spp* and 07 isolates of *Streptococcus spp*. It was clear from our tests that 02 out of the 30 patients collected demonstrated positive cultures of 01 *Lactobacillus* infection, 05 *Actinomycete* infections and 7 *Streptococcus spp* infections.

### 3.3 Phytochemical Screening of the Extract of Kola Nut

The phytochemical constituents of Kola nut included phenol, Tannin, Flavonoids, alkaloids, anthocyanin, sterol, antraquinone (Table 6).

#### 3.3.1 Test of sensitivity

Kola nut extracts showed no activity against isolates of *Candida spp* while there was little activity against isolates of *Streptococcus* and an average activity against isolates of *Actinomycete* and isolates of *Lactobacillus* (Table 7).

## 4. DISCUSSION

The current study showed that people of all socio-economic groups consume Kola nuts. More males consumed the nuts than females. Apart from its use as a stimulant and also for sociocultural reasons, Kolanut was mostly consumed by males because they used it for several activities involving the male folks. Its caffeine content was the reason why it was popularly used in energy tonics among native of sub-Saharan Africa [4,7]. The nuts are also used in traditional medicine to enhance male potency. Kola is listed as having aphrodisiac stimulant, cardiotoxic properties in Duke's phytochemical database. Kolanut is also used in peace missions or to invite some highly placed personalities to an occasion. There is a popular saying in Nigeria about kolanut; "Yoruba's produce it, Hausa's chew it the most and Igbo ritualize it the most" [12]. This support the reasons why in this study, Kolanuts was widely used by male adults of the 40-50 years age range.

Generally reasons for consumption of Kola included both socio-cultural and stimulating reasons like for pleasure, to suppress hunger, prevent sleep, to prevent nausea/vomiting and for energy resistance. Our study showed that



Kola nut consumption was high and likely to be addictive with the majority of the respondents who have been chewing kolanut for more than 13 years and some of them can chew up to more than 07 kolanuts / day. The current study showed a certain degree of dependence of the consumers. This is similar to studies reported by Burkill [5], Barwick and Van der [7], who confirmed the addictive properties of Kola nut. Some studies attributed these addictive properties to its caffeine contents. Ogutuga [9], reported that the caffeine content of kola nut could be as high as 7% and is often considered to be the agent responsible for the physiological or clinical effect of kola nut in man and other mammals [13,14]. An earlier worker; Somorin reported that Kola nuts contains 0.16-gram caffeine per 100g powder of kola nut [15].

The oral health perceptions of kolanut consumers in this study were diverse with positive effects on the teeth and the oral cavity such as the strengthening of the teeth, killing of bacteria, and the suppression of halitosis. It is because of these that many people believed that kola nut was cariostatic and responsible for the low caries levels [16]. It was also observed in the current study that there were high levels of arrested caries. The possible reasons for the low caries levels may be as a result of the sealing effects of accumulated Kola nut stains which plugged dental fissures and pits resulting to incipient carious lesions. These stain sealants prevented accumulation of food debris in pits and fissures and also allowed leaching of some secondary metabolites that were bacteriocidal and cariostatic thereby arresting already existing carious lesions. Increased saliva secretion during Kola nut chewing and subsequent modification of the oral pH are also contributing factors.

In the current study, negative impacts of kola nut consumption included tooth wear, halitosis, and stains. These were also observed by Tijani and Adetutu in Ibadan [4], Nigeria amongst long distance drivers. Also a very high prevalence of extrinsic stains was observed amongst the respondents and this has been reported as one of the negative esthetic effects of the kolanut on the tooth as confirmed by Castussie et al. [19]. In the current study, the majority of the respondents agreed that kolanut consumption gives an unsightly dull brown colour to the teeth; causes insomnia and therefore general body discomfort (50%). It was discovered that the perceived discomforts associated with kolanut consumption could not deter the subjects from consuming kola nut as 63% of them said kola nut staining of teeth

could be prevented by brushing teeth immediately after consumption or brushing three times daily [4]. This could not be true due to the astringent nature of kolanut which allows precipitates of kolanut to stick to the tooth surface.

Oral hygiene practices were moderate though it was noted that the oral health seeking behavior was very poor. This might be due to the low level of oral health education, poor infrastructure and inadequate oral health manpower. This confirmed the fact that the prevalence of toothache was high amongst the respondents. Tooth wear and dental discoloration (tinting) are the primary cited side effects of Kolanut because of its aesthetic concerns. Tooth wear can be attributed to persistent frictional contacts due to persisted mastication a phenomenon that is similar to bruxism.

The findings from the mouth revealed a high prevalence of plaque, decayed teeth and calculus. This was probably due to poor oral hygiene as a result of food impaction and debris collection. Poor oral hygiene practice and the absence of patient motivation had been described as the reasons for the high prevalence of periodontal diseases [17,24]. This contradicts the perception that dental caries is very low amongst Kola nut consumers as a result of increase saliva secretion and modification of the oral pH [18]. It was observed that there is always a rise in pH after a sugar meal challenge drives the pH below the critical level and this is attributed to the low caries level amongst kola nut consumers thereby stimulating remineralization [18]. Remineralization might be also reasons for painless retained roots and high prevalence of arrested caries observed in the current study. Ferrazzano et al. [11], reported that remineralization can be attributed to the potential activity of polyphenoid compound of kola nut. Actually the prevalence of dental caries amongst kolanut eaters is high because of the high prevalence of arrested carries but the prevalence of active caries is low which is the reason the D index of the DMFT score of the respondents was very high.

*In vitro* phytochemical analysis using methanol extracts showed that kola nut was composed of secondary metabolites such as Phenol, Tannin, Flavonoids, Alkaloids, Saponin, Anthocyanin, Sterol and Anthraquinone. Though not detailed, the phytochemical constituents was similar to a previous study carried on oral micro-organisms by Muhammad and Fatima [12] using methanol

and aqueous extracts where phytochemical screening revealed the presence of alkaloids, flavonoids, saponin, tannins, steroids, volatile oil and balsams. Higher content of alkaloids, saponin and glycoside was obtained in the aqueous extract than the methanol for both red and white kola nut. The presence of alkaloids and saponins might also explain the therapeutic properties of this plant, since alkaloid-containing plant has been used by humans since ancient times for therapeutic and recreational purposes [19].

The antibacterial effects on the bacteria implicated in dental caries and periodontal diseases showed that kola nut extracts had no activity against isolates of *Candida spp*, but little activity against isolates of *Streptococcus* was observed and an average activity against isolate of *lactobacillus*. This is in contrast with a study carried out in the laboratory of oro-facial infectious diseases of faculty of dental surgery of University of Toulouse [20]. Polyphenols extracts of Kola nuts have been reported to exhibit antibacterial activities with distinguished characteristics in their reactivity with protein related polyamide polymer [21].

In the current study aqueous and methanol extracts 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* [22]. Though inhibition was not observed on other odonto-pathogens used in this study that is (*Lactobacillus sp*, *Corynebacterium haemolyticum*, *Bacillus lentus* and *Pseudomonas aeruginosa*). *C. nitida* inactivity exhibited against these organisms and also that of acetone and ethyl acetate extract of both red and white variety, may be due to the absence of inhibitory alkaloids against the tested organisms. This is because alkaloids have been attributed to be responsible for the antimicrobial effects [23].

## 5. CONCLUSION

This study showed that kola nut consumption have both positive and negative effects on the teeth.

Apart from its cariostatic effects and low tooth sensitivity, a high rate of attrition, extrinsic stains, gingivitis, plaque deposit and calculus associated with poor oral hygiene are some of the perceived and clinical negatives factors that impair oral health. Others factors like inadequate poor

brushing habits, low dental clinic attendance are some of the factors that negatively influenced dental health.

However, *in vitro* study showed that extract of *Kola nitida* had antibacterial effects by inhibiting the growth of cariogenic bacteria of the mouth at certain level of concentration.

## CONSENT

As per international standard or university standard written participant consent has been collected and preserved by the authors.

## ETHICAL APPROVAL

It is not applicable.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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