

Studies on Phytochemical Evaluation and Antibacterial Properties of Two Varieties of Kolanut (*Cola nitida*) in Nigeria

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Abstract

Kola nut is a native stimulant which is commonly chewed in many West African cultures, individually or in a group setting. It is often used ceremonially and to honour guest. Phytochemical analysis and antibacterial screening of red and white *Cola nitida* (kola nut) extracts were evaluated using qualitative and quantitative method. Thin layer chromatography (TLC) was used to evaluate the phenolic content. Antibacterial screening was done using agar well diffusion method against odontopathogens; (*Lactobacillus* sp., *Corynebacterium haemolyticum*, *Proteus vulgans*, *Bacillus lentus*, *Streptococcus anginosus* and *Pseudomonas aeruginosa*) obtained from stock cultures. Phytochemical screening revealed the presence of alkaloids, flavonoids, saponin, tannins, glycoside, 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 results of TLC analysis revealed the presence of quercetin and coumarin in red kola and keamferol and coumarin in white kola respectively. The result of antibacterial activity of red *C. nitida* showed a zone of inhibition of 18 mm and 23 mm at 60 mg/ml for aqueous extract on *Proteus vulgans* and *Streptococcus anginosus*. Similarly white kola inhibited the growth of *S. anginosus* at 90 mg/ml with a zone of 18 mm. Methanol extract of red kola inhibited the growth of *P. vulgans* and *S. anginosus* at 60 mg/ml with a zone of 16 mm and 20 mm respectively. Acetone and ethyl acetate extracts of both red and white kola nut did not show any activity against the tested organism at all concentrations. The results obtained indicate that *C. nitida* which posses promising chemotherapeutic and antibacterial potentials respectively that could be useful against odontopathogens. The study may serve as baseline for further studies.

Keywords

Kola Nut. Phenol Zone of Inhibition and Odontopathogens

1. Introduction

Treatment of anaerobic infections is usually empirical but the universality of susceptibility of anaerobes to the

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usual drugs cannot always be assumed as reports of resistant strains have been growing in the literature from all over the world; [1]-[4]. There is therefore a need for continuous search for an urgent alternative to synthetic drugs. In Africa, Asia, North and South America, medicinal plants were used for the treatment of infections before the introduction of antibiotics and other modern drugs [5]-[7].

Traditional chewing sticks are commonly used especially by rural dwellers in maintaining oral hygiene [8] with about 80% - 90% of Nigerians using chewing sticks from a variety of plants [9]. The reasons can be attributed to culture, affordability, accessibility as well as the popular thought misguided belief that natural medicines have no side effects [10]. Studies of many plant materials and standardization of the dosages have being reported [11]-[13]. Medicinal plants such as *Vernonia amygdalina*, *Terminalia glaucescens*, *Nauclea latifolia*, *Serindeia warneckei*, and kola nut are known to inhibit the activities of some anaerobic pathogens [14]. Generally, the mechanical cleansing effect and antimicrobial substances in the seed are seen as major beneficial effects of chewing this nut. The chemical composition of kola nut includes: caffeine (2% - 3.5%), theobromine (1.0% - 2.5%), theophylline, phenolics (phlobaphens, epicatechin, D-catechin, tannic acid), sugar (cellulose), water [15].

Worldwide, approximately 2.43 billion people (36% of population) have dental carries in their permanent teeth. In baby teeth it affects about 620 million (9% of world population) [16]. It is the primary pathological cause of tooth loss in children and about 29% - 59% of adult over the age of 50 experience dental carries [17].

Bacterial dental plaque is involved in the initiation of gingivitis and the progression of periodontitis and plaque control is of great importance in the prevention of these diseases [18]. Adequate plaque control using mechanical methods is difficult to achieve for most individuals. Therefore, a number of chemical dental plaque-control agents have been used to supplement routine oral procedure such as mouth washing or oral irrigation [19]. Antimicrobial agents such as antibiotics, enzymes, chlorhexidine and phenol have shown anti-plaque effect both *in vitro* and *in vivo* [20].

Kola nut contain caffeine, which may explain their popular use in energy tonics. Native of Africa, the nuts of this towering tree are also a traditional medicine thought to enhance male potency. Kola is listed as having aphrodisiac stimulant, cardiogenic properties in Duke phytochemical database and to maintain alertness. Kolanut is used in peace mission 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".

Considering the use of kola nut by people in Nigeria, who believe that kola nut may prevent dental plaque if proper hygiene is maintained. Also, the antimicrobial activity of phenolic compound, there is the need for thorough biochemical evaluation of kola nut with the view to obtaining the potential which could be vital in providing dental care.

The aim of the study is to evaluate the phytochemical content of kola nut and its antibacterial activity against odontopathogens.

2. Methodology

Two varieties of kola nut (Red and White) *Cola nitida* were collected from major cola nuts market across Nigeria. The plant material was authenticated at the herbarium, Department of Biological Sciences, Usmanu Danfodiyo University Sokoto. A portion of it was shaded dried and pulverized into powder using a mortar and pestle.

2.1. Extraction of Red and White Variety of Kola Nut

A portion of about 250 g of each pulverized kola nut was soaked in 1 liter of water, methanol, acetone and ethyl acetate each respectively in separate conical flask and corked. These were allowed to stand for 24 hrs, filtered and the powered extract was collected separately for red and white variety.

Prepared extract of *Cola nitida* were subjected to standard phytochemical analysis for different constituents such as tannins, alkaloids, flavonoids, anthraquinones, glycosides, Saponins and phenols as described by [21] Jigna *et al.* (2006). The following test were conducted Test for flavonoid Test for tannins, Test for saponin [22]. Test for glycoside and Test for alkaloids [23].

2.2. Antibacterial Assay

Nutrient agar was used for testing of these extracts. Three wells were made on each plate agar using sterilized 14 mm cork borer. Each plate agar was inoculated with different isolates separately. This was done with the use of sterilized wire loop after standardization of each inoculum.

After inoculation, different concentration of the plant extract was mixed with little plain agar and pour into designated well which carry the specific concentration of the extract. The inoculated plates were allowed to stand for 15 minute before incubating then for 24 hours at 37°C. In a good light and colour background the turbidity of the suspension was matched with that of the turbidity standard. The turbidity of the test inoculum was adjusted by either adding more *Candida albican* or more physiological saline until a good match is obtained. Minimum Inhibitory Concentration (MIC) was then conducted on positive inhibited test organisms.

3. Results and Discussion

The results of qualitative phytochemical screening of two varieties of kola nut are represented in **Table 1**. The results revealed the presence of alkaloid, saponin, and glycosides were detected in the aqueous extract of red and white *C. nitida*. However, saponin and steroids were detected in methanol extract while flavonoids, tannins, saponin glycoside, cardiac glycoside, anthraquinone, volatile oil and balsam were little or not detected in both aqueous and methanol extract (**Table 1**).

The results of quantitative phytochemical analysis of both red and white kola nut are presented in **Table 2**. Result shows that there was a significant difference ($p < 0.05$) in the contents of tannins, saponins and phenolic in red variety compared to the white variety. From the result, it was observed that the white variety had the highest percentage of saponin with 1.10% while red had 0.90%. Similarly, white variety had 0.25% phenol while red variety had 0.21%. Moreover, tannins content of white variety was also high 0.12% as compared to red kola with 0.11%.

The involvement of anaerobes in human infections has generated global interest in recent time. Oral environment tends to favour the growth of both aerobic and facultative anaerobic bacteria. Bacteria accumulate on both the hard and soft oral tissue in biofilms [24]. Dental plaque is a biofilm that build up on the teeth and consist of bacteria and their products all in close proximity to the surface of the teeth. The accumulation of food debris on the teeth, might give room for pathogenic bacteria to grow thereby leading to oral infections like dental carries, periodontal infections.

Kola nut is regularly chewed and by Nigerian, posses polyphenols which have been reported to exhibit anti-bacterial activities with distinguished characteristics in their reactivity with protein related polyamide polymer [25]. In this study, red and white variety of kola nut aqueous and methanol extracts showed antibacterial

Table 1. Results of qualitative phytochemical content of aqueous and methanol extract of red and white *C. nitida*.

Component	Aqueous extract		Methanol extract	
	Red	White	Red	White
Alkaloids	+++	+++	ND	ND
Flavonoids	+	Trace	ND	ND
Saponins	++	++	+++	+
Tannins	+	+	ND	ND
Glycosides	++	+++	+	+
Saponin glycoside	Trace	Trace	ND	ND
Cardiac glycoside	ND	ND	ND	ND
Steroids	+	+	+++	+
Anthraquinone	ND	ND	ND	ND
Volatile oil	Trace	Trace	+	+
Balsams	+	+	+	+

Key: +++ = Present in high concentration; ++ = Moderately present; + = Slightly present; ND = not detected.

Table 2. Results of quantitative analysis of red and white *Cola nitida* Kola nut variety % constituent (mg/ml).

	Tannins	Saponins	Phenols
White variety	0.12	1.10	0.21
Red variety	0.11	0.90	0.25

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* [26]. However, red variety also showed 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 seen in the work of [27] on kola extract against *Proteus mirabilis*, which gave an inhibition zone of 16 mm at 1000 µg/ml.

The inhibition of micro-organisms by kola nut which contain phenolic compounds may be due to iron deprivation or hydrogen bonding with vital proteins such as micro-enzyme. Coumarine, quercetin and kaferol which might be present in kola nut as detected from the TLC ran on both red and white varieties, are belief to posses inhibitory characteristics against gram positive and gram negative bacteria [28]. Though inhibition was not observed on other odontopathogens 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 chained to be responsible for antimicrobial effect [29] [30]. However, the inactivity may not be justified that no other bioactive agent present in the extract. This is similar to the report of [31], who showed that no active substance exhibited its maximum activity under laboratory experimental condition. Therefore, if there is increase in concentration these may enhance activity.

Preliminary qualitative test is useful in the detection of bioactive principles and subsequently may lead to drug discoveries and development [32] [33] conducted phytochemical screening of leaves of twenty eight woody species from different plants families in Nigeria and discovered the presence of tannins, alkaloids and flavonoids in all samples, thus these secondary metabolites seem to be cosmopolitan in plants but, in varying degrees and type.

In this study, the presence of alkaloids, saponins, and glycoside were present in aqueous extract of both red and white kola nut. However, saponin and steroid were denser in methanol extract of red kola nut. Anthraquinone and cardiac glycoside were not detected in both extracts of red and white *C. nitida*. This is similar to the finding of [34] on some Niger Delta plants who observed the presence of flavonoid, tannins, alkaloids and saponin in large quantity. 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 [35].

4. Conclusions

Phytochemical investigation of *Cola nitida* traditionally used in Nigeria revealed the presence of medicinal naturals. kaemferol, quercetin and coumarin were identified by using simple TLC method. The qualitalitative analysis also revealed the presence of alkaloids, flavonoids, saponin, tannins, steroids, volatile and balsam. These result showed varying degrees of antibacterial activities on some odontopathogens.

The red kola aqueous and methanol extract impaired the growth of *Proteus vulgans* and *Streptococcus anginosus*. These organisms help in plague formation therein, initiates different dental problems.

The *C. nitida* extract showed its potential in the impairment of growth of test isolates and can serve as an alternative remedy for oral infections. This is a step forward for further evaluation of the plant for bio-remedies.

Recommendation

Based on the result of the study, the following recommendations are provided:

- 1) Considering the antibacterial activity of red *Cola nitida* further studies should be carried out on odontopathogens and how best it can be recommended for use against dental infections.
- 2) The phenolic compounds present in *C. nitida* should be isolated, purified and tested to obtain their maximum therapeutic potentials.
- 3) Vigorous research work should be geared towards the investigating the antimicrobial potential of *Cola nitida*, for harnessing the numerous volatile compounds from the plant for industrial uses to better the lots of humanity.

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