

Anti-Microbial and Phyto-Chemical Properties of Crude Extracts of *Garcinia kola* Heckel Stems Used for Oral Health

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Abstract: *Garcinia kola* Heckel is conventionally used for oral cleanliness. Anti-bacterial properties of aqueous extracts from its stem-wood and bark were investigated against *Bacillus subtilis* and *Escherichia coli* (gram +ve and -ve bacteria, respectively) using Agar-diffusion assay and measured in mean±standard deviation. Notable toothpaste (Pepsodent) was the standard/positive control. Its Inhibition Zone (IZ) against each isolate was compared with those of the extracts and sterile distilled water and the Activity Indices (AI) for each extract established. Phyto-chemical composition of the extracts was also determined. The extracts showed considerable inhibitory activities against the isolates. Their inhibition zones (4.50-10.50 mm) compare well with that for the standard against *E. coli* (3.00 mm) and *B. subtilis* (16.50 mm). Inhibition zone (6.00-10.50 mm) and activity index (0.36-0.64) for *B. subtilis* show it is more susceptible than *E. coli* (IZ = 0.00-7.50 mm; AI = 0.00-2.50). Stem-position influenced its anti-bacterial characteristics; the bark was more effective and exhibited greater IZ range (4.50-10.50 mm) with AI (0.64-2.00) than the wood (IZ = 0-7.50 mm; AI = 0.00-2.50). *B. subtilis* was more susceptible and showed less resistance to the extracts (AI = 0.36-0.64) than *E. coli* (AI = 0.00-2.00) which exhibited significant antibacterial activity. Phyto-chemical screening revealed *G. kola* contains saponins, tannins, alkaloids and flavonoids which also indicate its anti-microbial and therapeutic capabilities. This supports folkloric claim that its chewing-sticks are an effective tool for oral cleanliness alongside conventional toothpastes; their purified extracts could be employed in pharmaceutical formulations.

Key words: Agar-well diffusion, activity index, anti-bacteria, *Bacillus subtilis*, dental hygiene, secondary metabolite, wood extract

INTRODUCTION

Global anxiety regarding antibiotic resistance exists (Westh *et al.*, 2004) as there has been recent increase in the incidence of multiple resistance in human pathogenic micro-organisms largely due to the insurgence of antibiotic-resistant microbes and resurgence of previously eradicated diseases (Parekh and Chanda, 2007; Adegboye *et al.*, 2008). Now and then, researchers are exploring new anti-microbial chemicals for several remedies from various organic sources including higher plants. While many herbs have been potential drug sources (Iwu, 2002), screening of extracts and products from natural materials to examine their anti-microbial activities has established that plants generally represent a potential source of novel antibiotic prototypes (Afolayan, 2003). Consequently, a number of studies including those of Basile *et al.* (2000) have identified compounds which are effective antibiotics from higher

plants. As worldwide traditional healing systems have utilized herbal remedies as important sources for the discovery of new antibiotics (Okpekon *et al.*, 2004) efficient against antibiotic-resistant bacterial strains (Kone *et al.*, 2004), Romero *et al.* (2005) advocated that such results urgently call for adequate researches into traditional health systems. Ebana *et al.* (1991) and Manna and Abalaka (2000) also noted that these would facilitate pharmacological studies which could lead to the synthesis of more potent drugs with reduced toxicities.

However, Basile *et al.* (2000) reported that such studies should aim at the discovery and characterization of substances which exhibit activity against infectious micro-organisms yet show no cross-resistance with existing antibiotics. Antwi-Boasiako and Damoah (2010) demonstrated the biocidal effects of water-extracts from higher plants (e.g., *Erythrophleum suaveolens* (Guill and Perr) Breman and *Azadirachta indica* A. Juss) against several microbes and insects. Many of such higher plants

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including lime (*Citrus aurantifolia*) and orange trees (*C. sinensis*) are used as chewing-sticks in West Africa (Fadulu, 1975). Buadu and Boakye-Yiadom (1973) and Adu-Tutu *et al.* (1979) have reported anti-sickling and anti-microbial properties in a few local plant species employed as chewing-sticks for oral hygiene.

The use of senna (*Cassia vinnea*) roots have been predominantly effective in America and those of African laburnum (*C. sieberianba*) in Sierra Leone while *A. indica* stems are widely used in India (Almas, 1993).

Garcinia sp., chewing-sticks are widely used in several African countries, especially West Africa, the most important source (Agyili *et al.*, 2006). These have been extremely commercialized in the major cities for years to offer natural dental care (Okunji and Iwu, 1991). They are abundant in the South-Western parts of Ghana (i.e., Sefwi-Wiawso, Enchi and Asankragwa forest districts) and the Ghana-Ivory coast border. *Garcinia kola* Heckel (fam: Guttiferae), commonly known as Bitter kola is a medium-sized tree (12-28 m high) with thick and brownish bark with broadly elliptic leaves and yields yellow juice (John *et al.*, 2008).

Interest in *G. kola* is paramount; its stems and twigs are split for dental management as natural toothbrushes for oral cleaning. It is also cultivated for medicinal uses the seeds treat bronchitis, throat infections and have anti-purgative and anti-parasitic properties (Okunji and Iwu, 1991; Igwea *et al.*, 2007). The raw stem bark is also employed as purgative and the powder applied to malignant tumours. Internally, the latex or gum cures gonorrhoea and is externally applied on wounds (Okwu, 2005).

It is ranked third among medicinal plants in Benin where it is incorporated in scores of recipes and has proved as one of the several non-timber forest products of high socio-economic importance (Igwea *et al.*, 2007). Most importantly like other woody plants, *G. kola* can synthesize secondary metabolites including a great variety of phytochemicals which are accumulated in its cells and play various ecological and physiological roles. Flores *et al.* (2001) reported that these chemicals are responsible for the smell, colour, flavour and fragrance of woods and give them the capacity of resisting fungal, bacterial and insect attacks.

They exhibit a wide range of biological effects due to their anti-oxidant and protective properties which could be widely exploited. Moreover, they (particularly flavonoids) inhibit tumour initiation, promotion and progression (Okwu, 2005) besides their anti-allergic and anti-viral properties. Afolayan and Aliero (2006) reported that the number of resistant strains of microbial pathogens is growing as penicillin resistance and the

multi-resistance of pneumococci have caused major human health problems (Meurer-Grimes *et al.*, 1996; Eloff, 1998). This situation, coupled with the undesirable side-effects of certain antibiotics and the emergence of previously uncommon infections is a serious medical problem (Marchese and Shito, 2001; Poole, 2001). This has challenged scientists to search for new anti-microbial substances from various organic sources including medicinal plants. This study, therefore sought to examine the anti-bacterial properties and phyto-chemical composition of the aqueous extracts of *G. kola* traditionally employed for oral hygiene in several local communities.

MATERIALS AND METHODS

Extract preparation: Two *G. kola* stems were collected 1 m from the forest ground from Sefwi-Wiawso in the Western region of Ghana and similarly along the Ghana-Ivory coast border. The bark and stem-wood (4 cm below the bark) were individually sliced into pieces and air-dried to 10-12% moisture content (mc). Each air-dried sample (600 g) was milled with a Wiley mill to fine particle sizes (40-60 mesh). They were kept separately in sterile, dry screw-capped bottles and conditioned (25°C and 65% rh) prior to aqueous extraction.

A warm-water extract was prepared from each powdered sample (200 g) and sterile distilled water (100 mL) kept on water bath (at 70°C) for 72 h and stored in sterile bottles at 25°C for 48 h. Each liquid was decanted, sieved (through 1 mm mesh) and centrifuged (at 2000 × g for 20 min.). The supernatant liquid was decanted from the precipitate, filtered (Whatman paper No. 1) and refrigerated (at 4°C). The pH of each crude extract was then determined with electronic pH meter. The liquid was evaporated to dryness in a desiccator and the dry extracts stored in air tight amber-coloured bottles. The concentration [1 g mL⁻¹ (w/v)] of each dry extract in the liquid was determined and standardized at 3% for each sensitivity test.

Preparation of clinical isolates employed as inocula for anti-microbial susceptibility test: Clinical isolates of *B. subtilis* and *E. coli* were supplied by the Faculty of Bio-Sciences of the College of Sciences, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana. These bacterial cultures were maintained on a sterile nutrient agar medium (Agar (20 g), peptone (5 g), beef extract (3 g), NaCl (3 g) in distilled water), frequently sub-cultured and incubated at 35°C for 72 h. However for assessment of their efficacy, a fresh suspension of each culture was prepared in saline

solution (0.8% NaCl in sterile distilled water) from a freshly grown Agar slant. Each culture was streaked onto a non-inhibitory agar medium using sterile swab sticks and incubated overnight at 35°C to obtain isolated colonies.

Five well-isolated colonies were selected with an inoculating needle/loop and transferred into a tube containing sterile saline (i.e., non-selective broth) as well as vortex and cultured. McFarland 0.5 turbidity standard suspension was prepared by adding 1.175% (wt/vol) barium chloride dihydrate ($\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$) solution (0.5 mL) to 1% (vol/vol) sulfuric acid (99.5 mL). For effective comparison, the turbidity standard was liquored into similar test tubes employed to prepare the suspension (i.e., inoculum).

The bacterial suspension was then compared to the 0.5 McFarland standard suspensions (NCCLS, 2000). Each tube with its content was viewed against white background/papers inscribed with sharp black lines and the transparencies of the two suspensions contrasted. When the density of the bacterial suspension differed from that of the McFarland 0.5 suspension, the former's turbidity was reduced by adding sterile saline (or broth) or it could be increased to match that of the standard by adding more bacterial isolates (NCCLS, 2000).

Anti-bacterial activities of *G. kola* crude extracts using Agar-well diffusion method:

The inhibitory activities of the *G. kola* crude extracts were determined by the Agar-well diffusion method (Russell and Furr, 1977). Molten nutrient agar, cooled to 45°C was poured into sterile petri dishes. Standardized inocula of each culture were evenly spread on the surface of the gelled agar plates. Wells (8 mm in diameter) at least 30 mm between adjacent wells and the edges of petri dishes were aseptically punched on the solidified agar using a sterile cork-borer. Fixed volume (0.2 mL of 3% (w/v)) of *G. kola* extract from each stem-position was introduced into the wells of different petri dishes. The plates were incubated at 35°C for 24 h during which microbial activities were evidenced by the presence of clear zones of inhibition surrounding the wells (Fig. 1). There were three replicates. Inhibition Zone (IZ) sizes (mm) indicated by anti-bacterial activities were measured by calculating the difference between cork borer (5 mm) and the zone (diameter) of inhibition and recorded in mean±standard deviation and compared to those of the conventional tooth-paste (i.e., standard/positive control) and sterile distilled water (i.e., negative control).

The Activity Indices (AI) were calculated as the division of inhibition zone of the extract by that of the standard/toothpaste (Tanimowo *et al.*, 2011).



Fig. 1: Agar-well diffusion method illustrating bacterial growth inhibition by *G. kola* aqueous extract; a) = Agar well; b) = Inhibition zone; c) = Inoculated agar and d) = Petri-dish

Phyto-chemical screening test for *G. kola* extracts: The *G. kola* powdered samples from each stem position were also screened for their phyto-chemical components such as alkaloids, tannins, flavonoids, steroids, saponins, reducing sugars and cardiac glycosides based on their anti-microbial properties (Trease and Evans, 1978; Harborne and Harbourne, 1998):

Alkaloids: Each powdered sample (0.5 g) was dissolved in 1% HCl (5 mL) on steam bath. The filtrate formed (1 mL) was treated with 2-5 drops of Dragendorff's reagent. The resultant turbidity (or precipitation) indicated presence of alkaloids.

Tannins: Each extract (1 g) was dissolved in distilled water (20 mL). About 10% FeCl_3 (2-3 drops) was added to the filtrate (2 mL). Blackish-blue, blackish-green colouration or precipitate formation (when the filtrate (2 mL) was added to bromine water (1 mL)) was indicative of presence of tannins.

Flavonoids: Each extract (0.2 g) was dissolved in methanol (2 mL) and heated. A chip of magnesium metal was added to the mixture then 1-4 drops of conc. HCl. Red or orange colouration indicated presence of flavonoids.

Saponins: The powdered samples (1 g) were each dissolved in distilled water (10 mL) in a test tube and shaken vigorously. Distilled water (2 mL) was then added to the filtrate and shaken. Frothing persistence indicated presence of saponins.

Steroids: Salkowski's method described by Adegboye *et al.* (2008) was used to test for steroids in the extracts by dissolving each sample (0.5 g) in chloroform [CHCl₃] (3 mL) and filtered. Conc. H₂SO₄ (3-5 drops) was added to the filtrate to form a lower layer. Reddish-brown colouration was taken as positive for steroid ring.

Cardiac glycosides: Each extract (0.5 g) was dissolved in glacial acetic acid (2 mL) containing 1 drop of 1% FeCl₃. This was under-lid with conc. H₂SO₄. A brown ring at the interface indicated presence of a de-oxy sugar, characteristic of cardiac glycosides. Appearance of a violet ring below the ring while in the acetic acid layer; a greenish ring might form just above the ring and gradually spread throughout this layer to indicate presence of cardiac glycosides.

RESULTS

Microbial growth inhibition by *G. kola* extracts: Anti-bacterial activities exhibited by *G. kola* show that all the crude water-borne extracts generally exhibited varying degrees of inhibitory effects against all the test microbes (Fig. 2; Table 1). However, the bark extracts demonstrated greater degree of anti-microbial activities than the wood extracts (especially against *B. subtilis*).

The greatest mean diameters that is inhibition zones for the extracts (10.50 mm) were recorded by aliquots from both *G. kola* bark from Sefwi and the Ghana/ivory-coast border against *B. subtilis* as compared to the greatest inhibition exhibited by the toothpaste (IZ = 16.50 mm). Consequently, Table 2 shows that the bark extracts recorded the greatest activity index (0.64) against *B. subtilis*. Sterile distilled water exhibited no inhibition

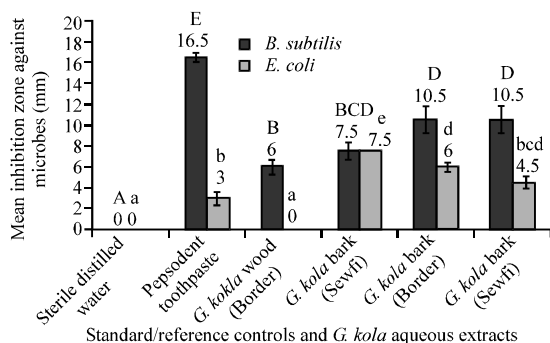


Fig. 2: Ant-bacterial activity of *G. kola* extracts, toothpaste and sterile distilled water against *B. subtilis* (Bars = Standard errors. Means with same letter are not significantly different (p<0.05); LSD = 2.70 (*B. subtilis*) and 1.46 (*E. coli*); n = 3)

zone (i.e., 0 mm) against any of the microbes. However among the extracts, those from *G. kola* wood from the border had the narrowest inhibition diameter (IZ = 6 mm) against *B. subtilis* with activity index of 0.36. Inhibition zones against *E. coli* were less than those for *B. subtilis*. IZ for the extracts and controls against *B. subtilis* ranks as Pepsodent paste>*G. kola* bark (border and Sefwi)>*G. kola* wood (Sefwi)>*G. kola* wood (border)>sterile distilled water (Fig. 2). Except for *G. kola* wood extracts (from the border) which recorded no activity against *E. coli* (IZ = 0 mm), all the extracts exhibited more activity (IZ = 4.50-7.50 mm) than that of the conventional toothpaste (IZ = 3.00 mm). However, *G. kola* wood extract (from Sefwi) inhibited *E. coli* growth most (IZ = 4.50-7.50 mm) as against *B. subtilis* (IZ = 7.50-10.50 mm). In the meantime while *G. kola* wood extract (from the border) recorded the lowest activity (IZ = 6.00 mm) against *B. subtilis*, it completely failed to inhibit the growth of *E. coli* (IZ = 0 mm). Significant differences (p<0.05) exist between the mean diameter zones of inhibition for the various extracts against *B. subtilis* and *E. coli* (Table 2 and 3; Fig. 2). Duncan's Multiple range test also shows significant differences (p<0.05) among the mean diameter inhibition zones for the *G. kola* extracts and the controls. Against a particular micro-organism,

Table 1: Inhibition zones of *G. kola* extracts and standard/reference and activity indices of the extracts against two bacterial isolates

Extract type and control	Bacterial isolates			
	<i>B. subtilis</i>		<i>E. coli</i>	
	Inhibition zone (mm)	Activity index standard of toothpaste*	Inhibition zone (mm)	Activity index standard of toothpaste*
Extracts				
<i>G. kola</i> wood (Border)	06.00±0.67	0.36	0.00±0.00	0.00
<i>G. kola</i> wood (Sefwi)	07.50±0.83	0.45	7.50±0.13	2.50
<i>G. kola</i> bark (Border)	10.50±1.03	0.64	6.00±0.47	2.00
<i>G. kola</i> bark (Sefwi)	10.50±1.27	0.64	4.50±0.60	1.50
Controls				
Standard (Toothpaste)	16.50±0.47	-	3.00±0.67	-
Sterile distilled water	0.00	-	0.00	-

*Activity index = Inhibition zone of test sample (i.e., *G. kola* extract)/Inhibition zone of standard (i.e., toothpaste)

Table 2: ANOVA for the anti-bacterial activity of *G. kola* extracts against *B. subtilis* using Agar-well diffusion method

Source of variation	Sum of squares	DF	Mean sum of squares	F-ratio	p-value	F-crit.
Extracts	134.67	05	26.93	20.2	0.001	4.39
Error	008.00	06	01.33			
Total	142.67	11				

*Significant: F = 20.2 ≥ F-crit.(0.05, 5, 6) = 4.39

Table 3: ANOVA for anti-bacterial activity of *G. kola* extracts against *E. coli* using Agar-well diffusion method

Source of variation	Sum of squares	DF	Mean sum of squares	F-ratio	p-value	F-crit.
Extracts	42.67	05	8.53	25.6	0.00056	4.39
Error	02.00	06	0.33			
Total	44.67	11				

*Significant: $F = 25.6 \geq F_{\text{crit.}(0.05, 5, 6)} = 4.39$

Table 4: Phyto-chemical analysis of *G. kola* chewing-stick crude extracts

Extract (Source)	Bio-active agents					
	Saponins	Cardiac glycosides	Tannins	Steroids	Alkaloid	Flavonoids
<i>G. kola</i> wood (border)	+	-	+	+	-	+
<i>G. kola</i> wood (Sefwi)	+	-	+	+	+	-
<i>G. kola</i> bark (border)	+	-	+	+	+	+
<i>G. kola</i> bark (Sefwi)	+	-	+	+	-	+

+ = Present; - = Absent

the greater the activity index for the plant extract, the greater is its virulence and the more susceptible is the microbe. In general, *B. subtilis* (AI = 0.36-0.64) was more susceptible to the *G. kola* extracts and the tooth-paste than *E. coli* (AI = 0.00-2.00). The corresponding activity indices for the *G. kola* extracts.

Phyto-chemical screening: Crude phyto-chemical composition of *G. kola* exhibited in Table 4 shows the absence of cardiac glycosides but substantial amounts of saponins, tannins and steroids in all the extracts from the two radial *G. kola* stem positions under investigation. Alkaloids were absent from the stem-wood of the plant (from the Ghana/ivory-coast border) and the bark (from Sefwi-Wiawso) while flavonoids were absent only in *G. kola* wood from Sefwi.

DISCUSSION

Anti-microbial analysis: Generally, *G. kola* chewing-stick extracts, especially from the bark, exhibited stronger anti-microbial activity than those from the wood against *B. subtilis* (IZ = 6; AI = 0.36) and *E. coli* (IZ = 0; AI = 0). However, no Inhibition Zone (IZ = 0) does not mean the absence of bio-active compounds in the crude extracts as they may be present in insufficient quantities to exhibit any anti-microbial characteristics.

Taylor *et al.* (2001) and Adegboye *et al.* (2008) explained that the dose level could be so low to be effective against the test microbe. Similar screening made by Omar *et al.* (2000) for stem-wood and bark extracts of 14 Eastern North American hardwoods, traditionally employed as medicine against 8 bacterial and 6 fungal strains showed that the bark extracts were more active

than from the wood. The bark extracts were active (about 86%) against methicillin-sensitive *Staphylococcus aureus*; 71% against *Bacillus subtilis* and 79% against *Mycobacterium phlei*. The bark extract of *Juglans cinerea* was active against *Pseudomonas aeruginosa* 187, *Salmonella typhimurium* and *Klebsiella pneumoniae*. However, the wood extracts were less active; they were 72% active against *S. aureus*, 36% against *B. subtilis* and 43% against *M. phlei*. Their anti-fungal tests also indicated that 36% of the extracts were active against at least one strain with the bark extracts being similarly more active than the wood's. *Juglans cinerea* bark extract had the broadest spectrum of activities against *Candida albicans*, *Saccharomyces cerevisiae*, *Cryptococcus neoformans*, *Trichophyton mentagrophytes*, *Microsporum gypseum* and *Aspergillus fumigatus*. The significant differences ($p < 0.05$) between the anti-microbial activities of the plant extracts and those of the standard (i.e., conventional toothpaste) whereby the extracts relatively recorded wider inhibitory zones against the microbes (especially *B. subtilis*) could explain Okwu (2005)'s investigations that *G. kola* chewing-sticks are very efficient, reliable and effective against microbes in teeth-cleaning in the absence of toothpaste which has been the situation among some Southern Nigerian communities. According to Cetin and Gurler (1989), micro-organisms show variable sensitivity to chemical substances in relation to different resistance levels between strains. They reported that gram -ve bacteria generally are more resistant to extracts than the gram +ve largely due to their cell wall lipopolysaccharide. Omar *et al.* (2000) also observed that the wood and bark extracts were generally more active against gram +ve bacteria than their gram -ve counterparts. The present study also revealed that *E. coli* (gram -ve) resisted the *G. kola* bio-active ingredients (AI = 2.00 maximum) more than *B. subtilis* (gram +ve) (AI = 0.64 maximum).

Similarly, several other researchers including Nikaido and Nakae (1979) and Parekh and Chanda (2007) have established that gram +ve bacteria are more susceptible towards plant extracts than the gram -ve types. Yao and Moellering (1995) explained that the cell wall of gram +ve bacteria is of a single layer whereas the gram -ve bacteria are well protected with a multi-layered cell wall. Moreover, according to Nikaido and Nakae (1979), gram -ve bacteria have the outer membrane composed mainly of lipopolysaccharide which is rather impermeable to lipophilic molecules and hydrophobic dyes and acts as a selective barrier to hydrophilic molecules. However, the much thicker peptidoglycan layer of gram +ve bacteria is not an efficient permeable barrier to the movement of hydrophilic solutes. Thus, the passage of the active compounds from

the *G. kola* extracts could have been more inhibited across the gram -ve cell wall than that of the gram +ve cell. Unsurprisingly, *E. coli* could resist the extracts and Standard paste better than *B. subtilis* although other constituents could exert antagonistic effects on the active ingredients. In addition to their antibacterial activity, the extracts have the potential against other microbes which nonetheless were not presently tested (Lindsey *et al.*, 1999; Adegboye *et al.*, 2008).

Phyto-chemical analysis: Nature as a source of medicinal agents is endowed with plants with unlimited active constituents which continue to be invaluable in the treatment of many human diseases. For instance, isolation of chemical constituents from *Suregada multiflorum* (the Euphorbiaceae) from the tropical rain forests (Daubenfeld *et al.*, 2005) around Central, Eastern and Southern Thailand, similarly provided various compounds of alkaloids, cardiac glycosides, flavonoid and saponin as well as terpenoids, lactone and gelonin. Several of these secondary metabolites (e.g., flavonoids, tannins, saponins, steroids and alkaloids) which play important roles in the bio-activity of medicinal plants during the phyto-chemical screening of *G. kola* extracts. Tanimowo *et al.* (2011) reported in Leven that antibacterial activities of the plant extracts have frequently been attributed to metabolites such as tannins, saponins, alkaloids and glycosides which are useful in the treatment of diseases caused by microbes including bacteria. Cardiac glycosides are used to treat congestive heart failure and cardiac arrhythmia. They inhibit the Na⁺/K⁺ pump in animal cells which increases the amount of Ca²⁺ ions available for heart muscle contraction, improves cardiac output and reduces heart distention.

Thus, their absence in *G. kola* extracts would affect the fight against certain heart-related human diseases. However, this could be compensated for by the presence of other secondary metabolites. These include flavonoids which exhibit biochemical and pharmacological activities in mammalian and other biological systems.

They have health benefits and contribute to fight several diseases by providing a defense system against UV radiation, insects and microbes decrease allergic reactions and reduce blood platelet stickiness or blood clot formation (i.e., it is anti-thrombic) and certain cardiovascular disease risks. These are to some extent, due to their anti-oxidant properties which assist in neutralizing damaging chemicals in the body such as free radicals that contribute to arteriosclerosis, cancer, degenerative brain diseases like Alzheimer and premature ageing and many other diseases (Moolla, 2005). Besides they are anti-inflammatory, hepato-protective and antiviral

(Okaraonye and Ikewuchi, 2009) and have 2-5 fold greater anti-oxidant and free radical scavenging activities than vitamins C and E on an equimolar basis (Du Toit *et al.*, 2001). Tannins also exert anti-microbial activities by iron deprivation, hydrogen bonding or specific interactions with vital proteins such as enzymes in microbial cells (Scalbert, 1991).

Plants with tannins are astringent in nature and are used for the treatment of intestinal disorders such as diarrhoea and dysentery (Adegboye *et al.*, 2008). Microbiologically, they have remarkable anti-cancer properties (Li *et al.*, 2003; Adegboye *et al.*, 2008) and are useful in the treatment of inflamed or side ulcerated tissues (Olajide *et al.*, 2004; Adegboye *et al.*, 2008). Thus, the presence of tannins in *G. kola* extracts supports its traditional medicinal use in the treatment of ailments (especially dental microbial infections). Just *et al.* (1998) reported the inhibitory effects of saponins on inflamed cells.

The steroidal components are also of vital importance due to their relationship with compounds such as sex hormones (Okwu, 2001). According to Otani *et al.* (2005), alkaloids comprise one of the largest groups of plant secondary metabolites. Many exhibit strong biological activities in both traditional and modern clinical medicines.

Vincristine and tasol are widely used as anti-cancer drugs and morphine as indispensable analgesic. They are employed as endogenous biological barriers against microbes and also comprise anti-fungal (Iwasa *et al.*, 1997, 1998; Mahady *et al.*, 2003) and anti-insect characteristics (Steppuhn *et al.*, 2004). These substances contribute to account for the health related properties of *G. kola* which are based on its anti-oxidant, anticancer, anti-tumor, anti-viral, anti-inflammatory and anti-allergic activities and support its popular utilization as herbal medicine in South Eastern Nigeria (Okwu, 2005). The findings therefore support *G. kola*'s superior position among medicinal plants and its usefulness in folklore remedies for the treatment of several microbial infections. The growth inhibition against *E. coli* and *B. subtilis* indicates its chemical potential against bacteria or other disease-causing microbes. The excellent anti-microbial properties of its aqueous extracts have demonstrated that its bio-degradable and environmentally-friendly chewing-sticks could continue to be as effective as the conventional toothpastes in maintaining oral hygiene.

The bark which exhibited the greatest inhibiting anti-bacterial properties could be properly refined into oral hygiene products by the pharmaceutical and related industries. The most active and efficaciously established

fractions could be employed in toothpaste formulations and tested against pathogenic oral diseases since pure fractions exhibit more effective anti-microbial inhibitions than crude extracts. Such combined complementary active components of herbal remedies with inactive substances give plants an efficient safety and much superior to their isolated and pure active components (Parekh and Chanda, 2007). The chemicals could also be tried in the control of plant pathogens to replace many conventional chemicals against disease-causing microbes.

Although, *G. kola* ranks well among the medicinal plants used routinely in some parts of Africa for the treatment of microbial infections due to the slow-growing nature of its seedlings and rarity coupled with potential levels of exploitation, a recent Ghanaian inventory revealed that it is severely threatened and are close to commercial extinction. It is now harvested mainly from the wild. Its status in the International Union for Conservation of Nature and Natural Resources (IUCN) red list of threatened species was re-assessed in 2004 as Vulnerable. Bio-diversity conservation has been a great concern to natural resource scientists. Thus, this medicinally valuable woody species needs protection which could be easily achieved through plantation establishments.

CONCLUSION

This study validates folkloric utilization of *G. kola* chewing-sticks as effective oral hygiene tool as they possess anti-microbial properties. However, *B. subtilis* showed less resistance to the extracts (AI = 0.36-0.64) than *E. coli* (AI = 0.00-2.00). *G. kola* also contains substantial amounts of phyto-chemicals (e.g. tannins, saponins, steroids, flavonoids and alkaloids) with antimicrobial, physiological, biochemical and pharmacological activities in human systems.

Chewing-stick utilization in developing countries conforms to the objectives of Primary Health Care Approach (PHCA). Thus, alongside toothpastes, *G. kola* chewing-sticks could continue to be of great value in local communities with limited oral health-care facilities. With scientific and rational method of preparation, the discarded bark, particularly established to be more potent against the bacterial isolates than the stem-wood could be promoted in the maintenance of oral hygiene when efficiently refined into toothpaste brands and pharmaceutical formulations. This would require the isolation and elucidation of the structure of the chemical composition of the compounds which confer the anti-microbial and pharmacological activities of the bioactive ingredients of the plant.

ACKNOWLEDGEMENTS

Researchers express the deep gratitude to staff of the Faculty of Bio-Sciences, Kwame Nkrumah University of Science and Technology [KNUST], Kumasi-Ghana for the provision of the isolates, Mr. Eric Acheampong (Department of Applied Biology, College of Sciences, KNUST) for his technical guidance throughout the anti-microbial and phyto-chemical analyses and Miss Agnes Ankomah Danso (Biometrician, Crop Research Institute, Centre for Scientific Industrial Research [CSIR], Kumasi) for the statistical assistance.

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