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Research Article

IN VITRO ANTIMICROBIAL STUDY OF THE EFFICACY OF A TOOTHPASTE FORMULATED FROM *GARCINIA KOLA* STEM WOOD EXTRACT

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ABSTRACT

The stem wood and twigs of *Garcinia kola* are extensively used in Ghana and other West African countries as "chew-sticks" for personal oral hygiene and the decoction of various parts is used to treat infections and other diseases. In this study we investigated the *in vitro* antimicrobial properties of a methanolic extract of *G. kola* stem wood and its toothpaste formulation against *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Klebsiella pneumoniae*, *Bacillus subtilis*, *Staphylococcus aureus*, α-haemolytic *Streptococcus pneumoniae*, *Streptococcus pyogene*s and *Candida albicans* by the standard agar diffusion and broth dilution methods. The cytotoxic activity of the extract against a normal human cell lines (SVK-14) and three human cancer cell lines (DLD-1, MCF-7 and M14) was also assessed by the standard MTT Colorimetric method. All the micro-organisms were significantly susceptible ($p<0.01$) to the extract (MIC range 0.25 - 2.0 mg/ml) and the toothpaste (zones of growth-inhibition 8 – 17 mm) indicating compatibility of the extract with the excipients employed in the formulation. The antimicrobial activity appears to be biostatic as shown by a Survivor-time study. The extract however was not cytotoxic against both the human normal and cancer cell lines tested. The results provide supportive evidence for the safe use of this plant as chew-stick for personal oral hygiene and in the treatment of infectious conditions in traditional medicine in Ghana.

Keywords: *Garcinia kola*, Chew-stick, Toothpaste, Formulation, Biostatic.

INTRODUCTION

Chew-sticks are widely used in Africa and Asia as a means of maintaining oral hygiene[1,2]. It is believed that chew-stick usage among Africans produces reduction in carious lesions than toothbrushes, and hence their use has been encouraged by the World Health Organization[3,4].

Garcinia kola Heckel (Gutiferae) is a forest tree of West Africa. The stem wood and twigs are extensively employed as chew-stick for personal oral hygiene[5]. Small pieces of the wood are frayed into soft fibre at one end and used to clean the teeth daily mostly in the rural communities. Decoction of the twigs, stem bark and seeds of the plant are also employed to treat ailments such as urinary tract infections, diarrhoea, dysentery, laryngitis, skin diseases and cough[6-8]. However in the urban settings toothpaste formulations are preferred. In this study we therefore investigated the antimicrobial properties of *G. kola* stem wood extract and its toothpaste formulation as well as the cytotoxic properties of the extract in order to establish its health benefits in comparison with commercial fluoride toothpastes.

MATERIALS AND METHODS

Plant Material

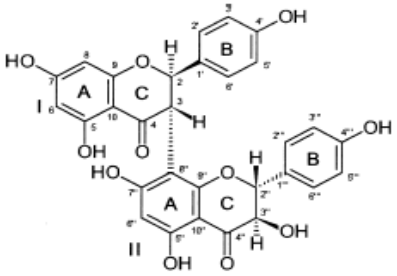
The *G. kola* stem wood was obtained from the Bobiri forest in the Ashanti Region of Ghana in January 2008 and authenticated by the Department of Pharmacognosy, KNUST, Kumasi, Ghana where a voucher specimen (No FP / PHM / 0030) has been deposited. The bark was peeled off, the wood chopped into small pieces and dried in the open for seven days. The dried material was milled into a coarse powder for extraction.

Extraction

Two kilograms of the powdered *G. kola* wood was soxhlet extracted exhaustively with 70% methanol and concentrated under reduced pressure using a Buchi Rotavapor R-114. The concentrate was evaporated to dryness *in vacuo*. The dry extract (114 g) was kept in a desiccator until used further.

Bioassay-guided Isolation

Four grams of the extract was fractionated by flash column chromatography[9] using silica gel 60H (Merck) and eluted with chloroform and graded quantities of ethyl acetate. Analytical TLC was employed to determine the chemical profile of the various fractions. An antimicrobially active compound was isolated from the active fraction and purified by recrystallization in 50%v/v aqueous ethyl acetate. Spectroscopic analysis revealed the isolate to be II-3-4'-I-4'-5-II-5-I-7-II-7-heptahydroxy-3,8-biflavanone(GB1) previously isolated from the bark and fruits of *G. kola*[10,11].



GB1

Toothpaste formulation

The toothpaste was formulated using Sodium lauryl sulphate (2 %w/w), sodium bicarbonate (37.5 %w/w), kaolin (9 %w/w), xanthan gum (2 %w/w), glycerine (25.22 %v/w), peppermint oil (0.7 %v/w), *G. kola* extract (0.5 %w/w) and water (q.s.). The ingredients were incorporated by trituration to obtain a smooth brown toothpaste.

Random samples of Close-up, Aquafresh, and Coalgate toothpastes were purchased from the Kumasi central market.

Cytotoxicity testing

The extract was evaluated for cytotoxicity against human colon adenocarcinima (DLD-1), breast adenocarcinima (MCF-7), melanoma (M14) and a normal human keratinocyte (SVK-14) cell lines by the standard MTT colorimetric bioassay[12,13]. Adriamycin and 5-Fluorouracil (Roche, UK) were used as positive controls. The cell lines, grown in complete RPMI 1640 medium, were inoculated into 96 well microtitre plates in 180µl at plating densities ranging between 5,000 and 40,000 cells per well. Aliquots of 20µl of the

extract and standard drugs and a drug contact time of 96 h were used.

Microorganisms

The following microorganisms: *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa*, (NCTC 10662), *Proteus mirabilis* (NCTC 3177), *Klebsiella pneumoniae* (ATCC 13883), *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (NCTC 10788), α-haemolytic *Streptococcus pneumoniae* (ATCC 49619), *Streptococcus pyogenes* (ATCC 19615) and *Candida albicans* (ATCC 102321), maintained at the Department of Pharmaceutics, KNUST, were used.

Antimicrobial assay

Zones of growth-inhibition: The agar-diffusion method was used to screen the *G. kola* wood extract and the toothpastes for antimicrobial activity. The dried extract was dissolved in 50% aqueous-methanol and 25 µl aliquots were applied to wells while the toothpastes were squeezed to fill completely their appropriately labeled wells on Mueller Hinton agar plates (BBL, Becton Dickinson and Co., Cockeysville, MD, U.S.A.), which had been inoculated with test microorganisms according to the standard protocol described by the National Committee of Clinical Laboratory Standards. The plates were incubated at 37 °C and the diameters of the inhibition zones were measured after 24 h. Gentamicin (Pharm-Intas, India) and Clotrimazole (Denk Pharma, Germany) were used as positive controls and 50% aqueous-methanol served as negative control.

Minimum Inhibitory Concentration (*MIC*): The MIC of the extract was determined by the broth dilution assay. A stock solution (8.00 mg/ml) of extract in 2 %v/v DMSO was two-fold diluted in 0.5 ml of Mueller Hinton broth. 50 µl broth culture of test organism (10^6 cfu/ml) was added. The tubes were incubated at 37 °C for 24 h. MIC was determined as the lowest concentration of the extract that completely inhibited visible growth of the test organism.

The survivor-time study: This was assessed by incubation of the organism in the presence of the extract at 5.0 and 10.0 mg/ml concentrations. Aliquots of 1 ml each were withdrawn at 0, 1, 2, 3, 4, 5, 6, 12 and 24 hour periods, neutralized by dilution and inoculated onto Nutrient Agar (Oxoid) plates. These were incubated at 37ºC for 24 hours and the number of colony forming units determined and plotted (Fig. 1: (a) *E. coli,* (b) *Staph. aureus,* and (c) *C. albicans*). The Survival-Time curves of the other organisms, (*Ps. aeruginosa, B. subtilis, P. mirabilis, K. pneumoniae, Strep. pyogenes* and ∝-haemolytic *Strep. pneumoniae*) followed a similar pattern.

Statistical analysis: Each experiment was repeated at least three times and the results were expressed as mean ± S.E.M. Statistical significances were compared between the replicates and analyzed by the Student's t-test. Data with $p < 0.01$ were considered statistically significant.

RESULTS AND DISCUSSION

In the study we investigated the *in vitro* antimicrobial properties of a methanolic extract of *G. kola* stem wood and its toothpaste formulation. All the test micro-organisms were susceptible to the *G. kola* wood extract and the isolated compound (GB1) with MICs ranging between 0.25 mg/ml and 2.0 mg/ml (Tables 1 and 2).

Table 1. Zones of growth-inhibition exhibited by *G. kola* wood extract and GB1

	Zones of growth inhibition (Mean± SEM, mm)			
Organisms	**Extract**	**Gentamicin**	**Clotrimazole**	**GB1**
	2.50mg/ml	**0.25mg/ml**	**0.25mg/ml**	**0.5mg/ml**
Ps. aeruginosa	8±0.3	3±1.4	NT	12±0.6
K. pneumoniae	8±1.2	24±0.3	NT	11±0.2
P. mirabilis	10±0.6	28±0.1	NT	11±0.5
E. coli	16±0.2	28±0.2	NT	13±0.1
Staph. aureus	12±0.4	24±0.2	NT	25±0.2
B. subtilis	12±0.3	26±0.3	NT	22±0.4
Strep. pneumoniae	19±0.3	32±0.2	NT	27±0.1
Strep. pyogenes	18±0.1	33±0.5	NT	26±0.3
C. albicans	16±1.3	NT	32±0.1	28±0.3

Key: NT = Not Tested

Table 2. Minimum Inhibition Concentration of *G. kola* wood extract

Organisms	**Minimum Inhibitory Concentration (Mean±SEM)**	
	Extract (mg/ml)	**Gentamicin (µg/ml)**
Ps. aeruginosa	2.0±0.01	200±1.26
K. pneumoniae	2.0±0.10	0.125±0.41
P. mirabilis	2.0±0.31	1.0±0.20
E. coli	1.0±0.05	0.125±0.08
Staph. aureus	0.5±0.21	0.125±0.13
B. subtilis	0.5±0.20	0.125±0.05
Strep. pneumoniae	0.5±0.11	0.125±0.15
Strep. pyogenes	0.5±0.06	0.125±0.02
C. albicans	0.5±0.01	250±0.46*

*Clotrimazole was used instead.

The herbal toothpaste also inhibited all the test microorganisms whilst none of the commercial toothpastes employed showed any antimicrobial activity (Table 3). The extract produced a dose-dependent reduction of the exponential growth phase of the test microorganisms as shown by the 'flat nature' of the survivor-time curves (Fig.1). This suggested that the active constituents were biostatic in action[10]. The Gram positive bacteria and *C. albicans* showed higher susceptibilities (2 to 4 times higher) than the Gram negative bacteria employed. The results were significant ($p<0.01$) and consistent with those reported for the seed, root and stem bark extracts of this plant and other species such as *G. cowa* and *G. pedunculata*[14-17].

The extract at the tested concentrations showed no significant cytotoxic activity against the four types of human cell lines as compared to the standard agents employed (Table 4). Also, the plant has been found to possess antihepatotoxic properties[18]. The results also indicated that the activity of the *G. kola* toothpaste compared favourably with that of the extract (Tables 1 and 3) and demonstrated that the excipients had no negative influence on the drug release properties of the formulation. The antimicrobial properties of the herbal toothpaste could therefore be an added advantage over the commercial toothpastes in maintaining oral hygiene.

Table 3. Zones of growth-inhibition exhibited by the toothpastes

Organisms	**Zones of growth-inhibition (Mean±SEM, mm)**			
	CU	**AQ**	**CG**	**GK**
Ps. aeruginosa	--	--	--	8.0±0.01
K. pneumoniae	--	--	--	7.0±0.13
P. mirabilis	--	--	--	10±0.07
E. coli	--	--	--	10±0.10
Staph. aureus	--	--	--	14±0.04
B. subtilis	--	--	--	8.0±0.11
Strep. pneumoniae	--	--	--	17±0.21
Strep. pyogenes	--	--	--	15±0.06
C. albicans	--	--	--	16±0.02

Key: -- no inhibition zone observed; CU - Close-up, AQ - Aquafresh, CG - Coalgate and GK - *G. kola*, toothpastes

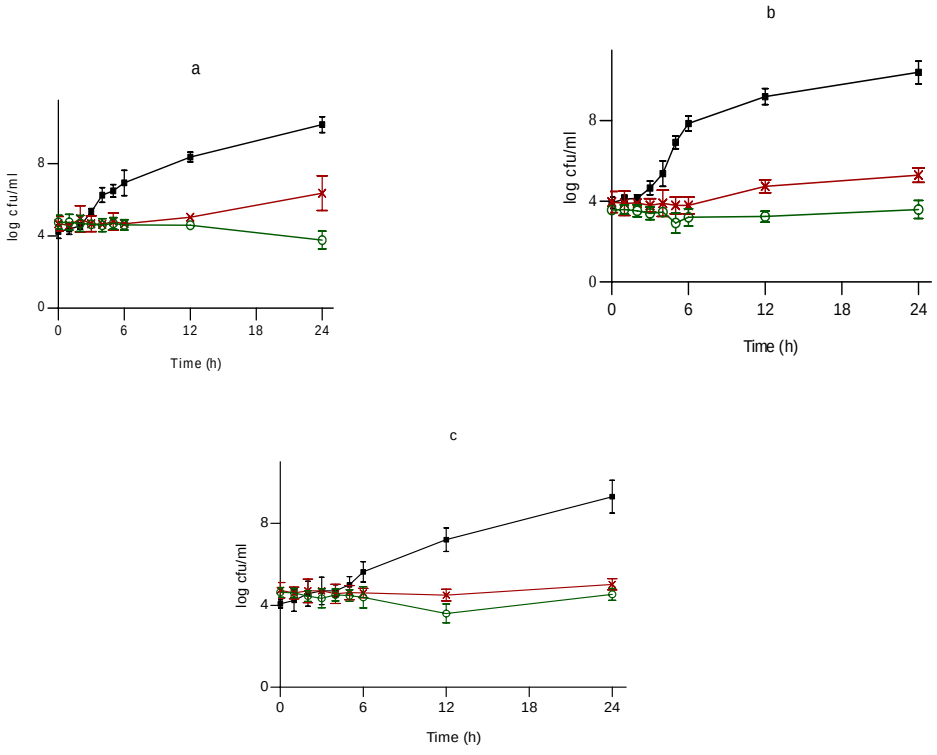


Fig.1: Survival of (a) *Escherichia coli*, (b) *Staphylococcus aureus* and (c) *Candida albicans* in broth containing 0 (■, control), 5 (x) and 10 (○) mg/ml of *Garcinia kola* stem wood extract.

Table 4. Cytotoxicity and growth-inhibitory activity of *G. kola* wood extract and standard cytotoxic drugs on human cell lines

Extract/Drug	Cell lines			
	DLD-1	MCF-7	M14	SVK-14
		[IC_{50} (µg/ml±SEM)]		
Garcinia kola	112±4.5	212±1.8	210±3.7	215±2.9
Adriamycin	0.6±0.1 [a]	0.4±0.2 [a]	1.0±1.2 [a]	514±1.2[a]
5-Fluorouracil	1.5±1.0	2.5±0.5	27.5±1.2	597±1.6

[a] value in ng/ml

As such, *G. kola* chew-stick can provide prophylactic and therapeutic effects due to the antimicrobial activity in addition to its mechanical cleansing ability. It is also reported that *G. kola* has antioxidant properties[19] as such its chew-sticks may be useful in controlling periodontal infections. The development of microbial resistance to commonly used antibiotics has triggered the search for new agents especially from plant sources. The activity of GB1 in this study and the reports of its effects against drug resistant bacteria including methicillin-resistant *Staph aureus*[15] indicate clearly the presence of antimicrobial constituents in *G. kola* which can serve as lead for the development of more effective antibiotics.

CONCLUSION

G. kola chew-stick and its toothpaste formulation have significant antimicrobial properties. One of the active constituents is GB1. This study provides a supportive evidence for the use of this plant for personal oral hygiene and in treatment of infectious conditions in traditional medicine in Ghana.

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