COMPARATIVE ANTIMICROBIAL ACTIVITIES OF EXTRACTS OF GARCINIA KOLA, KOLA ACUMINATA AND KOLA NITIDA SEEDS ON ISOLATES OF RESPIRATORY TRACT AND OTHER INFECTIONS

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Abstract
The methanolic extracts of the powdered seeds of *Garcinia kola* (Guttiferae), *Kola acuminata*, and Kola nitida (Sterculiaceae) were tested individually for antimicrobial activity using isolates from respiratory tract infections and other sites of infection such as High vagina swab, wounds, urethra and skin. The isolates included *Staphylococcus aureus* (5 strains), 2 strains each of *Escherichia coli*, *Proteus mirabilis* and *Streptococcus pneumoniae*; 1 strain each of *Streptococcus viridians*, *Entercoccus faecalis, Streptococcus pyogenes, Bacillus subtilis, Haemophillus influenzae, Candida albicans, Asp. niger*, Asp. *Eumigatus* and *Trichophyton* specie, and 3 strains each of *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. The antimicrobial susceptibility test of the extracts showed zones of growth inhibition ranging from 9mm to as large as 35mm, depending on the organism being tested. The minimum inhibitory concentration (MIC) was determined by agar-diffusion method, and ranged from 0.23mg/ml to 30mg/ml for the three extracts.
The crude extracts of *Garcinia Kola* showed a stronger antimicrobial activity against most organisms particularly the Gram-positive bacterial isolates from the respiratory tract, than the other two extracts, while *Kola nitida* was the least active.
Key Words: Kola seeds, antimicrobial activity

Introduction

Several medicinal plants have been identified having folklore claims of curative effects on different ailments. Most of them have provided suitable agents for the development of synthetic drugs. Examples include digoxin from *Digitalis* spp. leaf; opium alkaloids from papaver somniferum; anthraquinones from senna leaf; Atropine from belladonna leaf; Quinine and Quinidine from cinchona plant; physostigmine from *Physostigma venenosum* leaf; colchicines from *Colchium* corm; Vinca alkaloids (vincristine and vinblasstine) from *Catharathus* spp. root; strychnine from *Strychnosnux vomica* seed, reserpine from *Rauwolfia* root and leaves and nicotine from *Nicotiana* leaves (Sofowora,1993).

Phytomedicines derived from plants have shown great promise in the treatment of intractable microbial infectious diseases including opportunistic AIDS infections. Plants containing protoberberines and related alkaloids, picralima-type indole alkaloids and Garcinia biflavonones used in traditional African system of medicine, have found to be active against a wide variety of microorganisms (Iwu *et al.*, 1999). Hence the prominence currently being accorded traditional herbal medicine in healthcare delivery. According to *WHO et al.*, (1999), over 80% of the world's population still depend on medicinal plants for preventive and curative medicines.

Garcinia Kola (Guttiferae), *Kola acuminata* and Kola nitida (both of the family *Sterculiaceae*) are found in Africa: *K.* nitida and *K.* acuminata in the lowland rainforest of several West African or Indonesian countries, while *G. kola* is widely distributed in West and Central Africa. Their seeds are well known for edible, ceremonial and ritualistic applications. The commercial value of kola nuts is attributed to their caffeine content which has stimulant properties.

G. kola seeds are used in folk medicine and in many herbal preparations for the treatment of ailments such as laryngitis, liver disorders and bronchitis (Iwu, 1982), prevention or relief of colic, curing of chests colds and relieving cough (Iwu, 1993). The seed has been reported to have anti-inflammatory, antimicrobial, antidiabetic and antiviral (Iwu, 1986), as well as anti-ulcer properties (Ibironke *et al.*, 1997). Of the 148 crude alcoholic extracts from 115 plant species tested for antimicrobial activities, 22 extracts including those from *G. kola* and *K. nitida*, were found very effective on the Gram-positive bacteria and *Candida albicans* (Afindehou et al., 2002).

Adeniyi et al., (2004) worked on the *in vitro* anti-mycobacterial activities of three species of kola plant extracts (Sterculiaceae), *Kola* acuminata, Kola nitida and Kola milleni, and found that the methanolic extracts were effective on the mycobacteria. Adeleke *et al.*, (2006) also reported the methanolic extract of *G. kola* seeds as being the must effective. In a comparative study on the ethanol and water extracts of *Cajanus cajan, Garcinia kola* and *Xylopia aethiopica* for antimicrobial activity, the ethanol extract had higher activity on *S. aureus, P. aeruginosa, E. coli and C. albicans*. The present study is a comparative work on the antimicrobial activities of *G. kola, K. acuminata* and *K. nitida*.

Materials and Methods

Microorganisms

The organisms used in this study were obtained on slant as pure cultures from the pharmaceutical microbiology laboratory, University of Ibadan (UIPHM), Medical microbiology unit of the University college hospital (UCH), Ibadan, Oyo State, and the Medical microbiology unit of Olabisi Onabanjo University Teaching Hospital (OOUTH), Sagamu, Ogun state. Twenty six strains of microorganisms were collected out of which 15 strains (57.7%) were isolated from respiratory tract infections, 6 (23.1%) from four other sites of infections, namely, skin, urethral discharge, wound swab and high vagina swab, 2(7.7%) from unknown clinical sources while 3 (11.5%) were typed strains. The microbial isolates were subjected to purity check by means of Gram staining and some conventional biochemical tests.

Preparation of Plant Materials

The three different seeds were weighed separately, and in the case of *Garcinia kola*, the seeds were first peeled before weighing. They were then sliced and air-dried until fully dried each to a constant weight and the percentage moisture content determined. The dried samples were pulverized with an electric grinding machine into powdered form, labeled and stored for the study

Soxhlet Extraction

The extraction of the active ingredients of the plant seeds was carried out using the method described by Harbone (1973). The pulverized seeds of each plant weighing 100g were soxhlet-extracted using 250ml of methanol separately for 6 hours. The extracts obtained were concentrated by evaporation using water-bath at 100°C and then evaporated to dryness in an oven at a temperature of 60°C until constant weights were obtained. The dried extracts were stored in airtight jars in a refrigerator at a temperature of 4°C until when needed.

Antimicrobial Susceptibility Testing

Screening for antimicrobial activity was carried out on each of the extracts collected against the microbial isolates using agar-cup diffusion method (Singleton, 1999).

Each seed extract was prepared into a 30mg/ml solution by dissolving 3g of the individual powdered seed extract in 100ml of 40% methanol. This solution was then serially diluted to give solutions of 15mg/ml and 7.5mg/ml concentrations. Also 1.5g of each powdered seed extract were blended together at a ratio 1.5:1.5 for combinations of two different extracts (i.e. GK:KA, GK:KN, KA:KN) and 0.75g of each powdered seed extract at a ratio 0.75:0.75:0.75 for the combination of the three powdered seed extracts (i.e GK:KA:KN) and were reconstituted with 100ml of methanol (40%). From each of the combinations, 10ml were also serially diluted as stated above to obtain three different concentrations of the combined powdered extracts at ratios 15:15, 7.5:7.5 and 3.75:3.75:3.75 for combinations involving two extracts and ratios 7.5:7.5:7.5, 3.75:3.75:3.75 and 1.88:1.88:1.88 for combinations of the three extracts.

A volume of 0.1ml of each of the concentrations was introduced into the wells (three well per sample) of the already prepared seeded agar plates with bored wells using calibrated Pasteur pipette. Gentamicin at 2μg/ml concentration was introduced into one of the wells to serve as the negative control.

Ketoconazole at 10mg/ml was added to seeded plates containing fungi. The plates were left for one hour at room temperature to allow for diffusion of the extracts and controls in the wells through the agar medium after which they were incubated at 37°C for the bacteria and at room temperature for 3 days for fungi. This was done in duplicates. Zones of growth of inhibition of the various concentrations of the extracts and the control were measured and recorded for the duplicate plates. Average values were then recorded.

Minimum Inhibitory Concentration (Mic) Determination

The powdered extracts were reconstituted by solubilising 6g of the individual powdered extract in 100ml of methanol (40%) to give a 60mg/ml concentration of the powered extract concentrates, these were then diluted serially with methanol (40%) such that the concentrations were halved in each container in a series, with the concentrations ranging from 60mg/ml to 0.12mg/ml, giving 9 decreasing graded concentrations. A 1 in 100 dilution of an overnight broth culture of each of the bacteria was prepared from which 0.1ml was seeded into 20ml of melted and cooled agar medium for pour-plates. Similar volume of 10^{-2} fungal dilution from a 2 day-old fungal culture was used to prepare fungal pour-plates, with Sabouraud's dextrose agar. The surface was dried for 20 minutes in an already disinfected hot air oven at 37°C in an inverted position. Five wells were bored into the individual culture plates and the plugs removed. Each of the wells was filled with the different concentrations of the test extracts and after 1hr of pre-incubation diffusion, the plates were incubated at 37 C for 24hrs for bacteria and at room-temperature for 3 days for fungi. The diameters of zones of concentration preventing growth were taken as the minimum inhibitory concentration (MIC) of the extract. The procedure was done in duplicates and the average values were recorded.

Results

The seeds were weighed before air-drying using 300 seeds of *G. kola* , 80 seeds of *K. acuminata* and 100 seeds of *K. nitida* . After drying, *G. Kola* weighed 1,640.8g (41.2g moisture content). The percentage moisture contents of the dried seeds were then calculated (Table 1).

The yields of the methanolic extracts of *Garcinia kola, Kola acuminata* and *kola nitida* seeds were determined, with *G. kola* having the highest yield while *K. nitida* had the least (Table1).

The results of the antimicrobial susceptibility testing of the extracts against the micro-organisms are presented in Tables 2 and 3. Most of the organisms were sensitive to the extracts to varying degrees and the extracts were more active on the Gram –positive organisms than on the Gram-negative organisms. *Garcinia Kola* extract was the most active on the *Staphylococcus* spp. and *Streptococcus* spp. while *Kola acuminata* and *Kola nitida* had almost equal activity on them. The activities were seen to slightly increase with increase in concentration on some organisms, especially the Gram-positives particularly those isolated from the respiratory tract infections and the typed strains. The extracts were also found to be reasonably active on the *Pseudomonas* spp. used in this study with *G. kola* as the most active and K. nitida the least active on the species. All the typed organisms used in this study were more susceptible to the different extracts better some of the clinical isolates.

Reflecting the minimum inhibitory concentration (MIC) of the methanolic extracts (Table 3), most of the Gram-positive bacteria were found to be susceptible at lower concentration compared to the Gram-negative bacteria except for *Haemophillus influenzae* and Pseudomonas aeruginosa ATCC27853 that were also found to be susceptible at low concentrations compared to some others. The MICs for the three extracts against the Gram-positive bacterial strains isolated from respiratory tract infections ranged from 0.23mg/ml-3.75mg/ml for G. *Kola*, 0.94mg/ml – 7.5mg/ml for k. *acuminata* and 0.94mg/ml - 15mg/ml for *K. nitida* while those isolated from other sources ranged from 3.75mg/ml – 7.5mg/ml for *G. Kola*, 7.5mg/ml – 15mg/ml for *K. acuminata and K.*

nitida. The MICs for the three extracts against the Gram-negative bacterial strains isolated from the respiratory tract infections ranged from 1.88mg/ml – 7.5mg/ml for *G. Kola*, 3.75mg/ml – 15mg/ml for *K. acuminata* and 1.88mg/ml – 30mg/ml for *G. kola* and 15mg/ml – 30mg/ml for both K. *nitida*.

Candida albicans was seen to be susceptible to the various extracts at lower concentrations especially to *G. kola* and *K. nitida*, and so also was the *Trichophyton* spp. to *K. acuminata* compared to other fungi. The MICs of the three extracts against the fungi isolated from the respiratory tract infections ranged from 0.47mg/ml-1.88mg/ ml for *G. kola*, 0.94mg/ml-1.88mg/ml for *K. acuminata* and 0.47mg/ml-7.5mg/ml for *K. nitida* while those isolated from other sources ranged from 3.75mg/ml-7.5mg/ml for *G. kola*, 0.47mg/ml-3.75mg/ml for *K. acuminata* and 0.94mg/ml-15mg/ml for K. nitida. Remarkably, one strain each of *Staph. aureus* and *Escherichia coli* were not sensitive entirely to any of the extracts but were well inhibited by gentamicin used as control.

Table 1: PERCENTAGE MOISTURE CONTENTS AND EXTRACT YEILDS OF THE SEEDS OF *G. KOLA*, *K. ACUMINATA* AND *K. NITIDA*

SEED	SEED WT(g)	SEED DRY WT	MOIST CONT (g)	% MOIST CONT	POWDER WT (g)	YEILD WT (g)	% YEILD
Garcinia kola	1,682g	1,640.8g	41.2g	2.45%	1000g	120.33g	12.03%
Kola acuminata	1,553g	1,502g	50.5g	3.25%	1000g	92.71g	9.27%
Kola nitida	1,813g	1,765.3g	47.7g	2.63%	1000g	51.37g	5.14%

Key:
MOIST = Moisture, CONT. = content

Table 2: SUSCEPTIBILITY PATTERN OF THE MICROBIAL ISOLATES TO THE METHANOLIC EXTRACT CONCENTRATES OF *G. KOLA. K. ACUMINATA* AND *K. NITIDA*.

ORGANISM	GK (mg/ml) 30	15	7.5	Ka (mg/ml) 30	15	7.5	Kn (mg/ml) 30	15	7.5	Control G	K	M
SA UCH 142	24[a]	20	18	22	19	16	20	17	14	19	ND	-[b]
SA OOUTH206	20	17	13	17	14	11	16	13	11	20	ND	–
SA OOUTH422	19	16	14	18	15	-	17	14	14	20	ND	–
SA UCH173	-	-	-	-	-	-	-	-	-	25	ND	–
SA NCIB8588	35	31	27	19	15	12	16	14	12	35	ND	–
SP UCH320	24	21	18	22	18	15	22	20	16	25	ND	–
BS UCH032	25	19	15	19	15	11	18	15	12	20	ND	–
SN UCH022	19	16	14	15	12	10	13	10	-	26	ND	–
SN UIPHM	18	15	12	15	12	10	14	11	9	20	ND	–
SV UIPHM	16	14	10	12	11	10	12	10	-	12	ND	–
SF UIPHM	16	14	12	14	11	10	13	11	-	11	ND	-
PA OOUTH431	13	13	10	13	11	11	15	11	-	22	ND	–
PA ATCC27853	17	14	11	17	14	12	15	13	11	26	ND	–
PA UCH189	15	13	11	14	12	-	13	10	8	23	ND	–
EC UCH065	13	12	11	13	12	-	12	11	11	15	ND	–
EC OOUTH321	-	-	-	-	-	-	-	-	-	20	ND	–
PM OOUTH072	11	-	-	11	10	9	11	10	9	27	ND	–
PM UCH130	12	-	-	18	18	17	18	18	14	27	ND	–
KN UCH898	-	-	-	12	12	11	11	11	-	23	ND	–
KN ATCC13883	26	24	20	19	16	14	19	17	15	23	ND	–
KN UCH052	11	-	-	11	-	-	11	-	-	15	ND	–
HF UCH311	22	19	16	18	15	13	18	15	14	26	ND	–
CA UCH2010	17	15	12	16	14	12	15	12	10	ND	40	–
AN UCH0321	11	11	10	12	12	12	11	10	10	ND	20	–
AF UCH288	12	10	10	12	11	11	10	10	-	ND	-	-
T.Sp UCH035	11	11	11	12	12	12	10	10	10	ND	48	-

Key:
SA = *Staph aureus*　SP = *Strep pyogenes*　KN = *Kleb pneumoniae*
SN = *Strep pneumoniae*　BS = *Bacillus subtilis*　HF = *Haemophillus infuenzae*
SV = *Strep viridians*　SF = *Enterococcus faecalis*　PM = *Proteus mirabilis*
PA = *Pseud aeruginosa*　EC = *Esch coli*　CA = *Candida albicans*
AN = *Asper niger*　AF = *Asper fumigatus*　T.Sp = *Trichophyton spp*.
Gk = *Garcinia kola*　Ka = *Kola acuminata*　Kn = *Kola nitida*
NCIB = National centre for Industrial bacteria
ATCC = America Typed Culture Collection
OOUTH = OLabisi Onabanjo University Teaching Hospital, Sagamu, Nigeria
UCH = University College Hospital, Ibadan, Nigeria
UIPHM = University of Ibadan Pharmaceutical Microbiology Laboratory.
a = Zone of growth inhibition (mm)　b = No antimicrobial activity　ND = Not done
G = Gentamicin (mg/ml)　K = Ketoconazole　M = Methanol (40%)

Table 3: MINIMUM INHIBITORY CONCENTRATIONS (MIC) OF METHANOLIC EXTRACTS (CONCENTRATES) OF *G. KOLA, K. ACUMINATA AND K. NITIDA* AGAINST THE MICROBIAL ISOLATES.

ORGANISM	MIC (MG/ML)		
	G. Kola	*K. acuminate*	*K. nitida*
SA UCH142	0.47	0.94	0.94
SA OOUTH206	0.47	3.75	3.75
SA OOUTH422	0.94	15	.75 3
SA UCH173	-	-	-
SA NCIB8588	0.23	1.88	1.88
SP UCH320	1.88	1.88	1.88
BS UCH032	0.23	1.88	1.88
SN UCH022	1.88	7.5	15
SN UIPHM	3.75	7.5	15
SV UIPHM	7.5	7.5	15
SF UIPHM	3.75	7.5	15
PA OOUTH431	7.5	15	15
PA ATCC27853	1.88	1.88	3.75
PA UCH189	7.5	15	15
EC UCH065	-	-	-
EC OOUTH321	-	-	-
PM OOUTH072	-	-	-
PM UCH130	-	-	-
KN UCH898	-	-	-
KN ATCC13883	0.23	-	0.94
KN UCH052	30	30	30
HF UCH311	1.88	3.75	1.88
CA UCH2010	0.47	0.94	0.47
AN UCH0321	1.88	1.88	7.5
AF UCH288	7.5	3.75	15
T.Sp UCH035	3.75	0.47	0.94

Key:

SA = Staph aureus | *SP= Strep pyogenes* | *KN = Kleb pneumoniae*
SN = Strep pneumoniae | *BS = Bacillus subtilis* | *HF = haemophilllus influenzae*
SV = Strep viridians | *SF=Enter faecalis* | *PM= proteus mirabilis*
PA= Pseud aeruginosa | *EC=Esch coli* | *CA = Candida albicans*
AN =Asper niger | *AF =Asper fumigatus* | *T.Sp= Trihophyton spp.*

NCIB = National center for industrial bacterial
ATCC = America Typed Culture Collection
OOUTH= Olabisi Onabanjo University Teaching Hospital. Sagamu.
UCH = University College Hospital. Ibadan
UIPHM = University of Ibadan. Pharmaceutical Microbiology Laboratory.

Discussion

The results of the antimicrobial susceptibility tests of the extracts showed that the various extracts were active on most organisms especially the Gram-positive bacteria compared to the Gram-negatives. This is in general agreement with the observations of Leive (1974) and Atindehou *et al*., (2002) that Gram-positive bacteria were more sensitive to antimicrobial action of these agents than the Gram-negative bacterial. It also corroborates the ethno-medicinal claims for *G. Kola* used in throat infections like sore throat, an infection caused by Gram-positive bacteria. The activities of most the extracts were found to increase with increase in extracts concentrations as shown by their diameter of zones of growth inhibition in agreement with observation of Oshodi *et al. (2004)*. An observation in this study that one strain each of *Staph aureus* and *Escherchia coli* entirely not sensitive to the three extracts but were affected by gentamicin suggests the need for the isolation of the active antimicrobial constituents of the three plant seeds tested. Flournoy *et al., (1989) and Adeleke* (2007) reported a correlation between the clinical source of microorganisms and their antibiotic sensitivity. This has found support in this study whereby isolates from respiratory tract infection were more susceptible than those from other sources.

Considering the MIC values obtained, the *G. Kola* extract was found the most active among the three extracts. Interestingly, this observation was also pertinent to *Pseud aeruginosa*, notorious for its recalcitrance to most antimicrobial compounds. Moreover, it was the most susceptible among the Gram-negative bacteria tested.

All the fungal isolates used in this study were found to be susceptible to all the extracts, though poorly, but more against Candida *albicans* than any of the other fungi. This correlates with the observation of Adeleke *et al.*, (2006) on the activity of *Garcinia Kola* on some fungal isolates.

Whilst confirming the antimicrobial activites of the methanolic seed extracts of the plants tested, this study has identified *G. Kola* seed extract as the most active followed by *K. acminata.* It has also corroborated the observation that antibiotic sensitivity was dependent on clinical sources of microorganisms.

Conclusion

This study has confirmed the antimicrobial activity of the methanolic extracts of the seeds of *Garcinia kola* , *Kola acuminata and Kola nitida. G. kola was* found the most active followed by *K. acuminata*, depending on the test organism. Also, the activity of the extracts was seen to increase with increase in concentration particularly against the Gram-positive bacteria isolated from respiratory tract infections. Moreover, the findings confirm earlier observations that there was a relationship between clinical sources of the resistant strains of microorganisms and their susceptibility to the extracts.

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