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J-F T.K. Akoachere, BSc, MSc, PhD, Assistant Lecturer, R.N. Ndip, BSc, MSc, PhD, Lecturer, E.B. Chenwi, BSc, MSc, L.M. Ndip, BSc, MSc, Assistant Lecturer, T.E. Njock, BSc, MSc, PhD, Lecturer and D.N. Anong, BSc, MSc, Assistant Lecturer, Department of Life Sciences, Faculty of Science, University of Buea, P.O. Box 63, Cameroon

Request for reprints to: Dr. R.N. Ndip, Department of Life Sciences, University of Buea, P.O. Box 63, Cameroon

ANTIBACTERIAL EFFECT OF *ZINGIBER OFFICINALE* AND *GARCINIA KOLA* ON RESPIRATORY TRACT PATHOGENS

J-F T.K. AKOACHERE, R.N. NDIP, E.B. CHENWI, L.M. NDIP, T.E. NJOCK and D.N. ANONG

ABSTRACT

Objective: **To investigate the antibacterial activity of *Zingiber officinale* (ginger) *Garcinia kola* (bitter kola) on four respiratory tract pathogens.**
Design: **A prospective study based on laboratory investigations.**
Setting: **Department of Life Sciences, University of Buea. Throat swabs were collected from 333 individuals with running nostrils, cough and / or catarrh in three localities of Buea namely Bokwango, Molyko and Bolifamba. *Staphylococcus aureus, Streptococcus pyogenes, Streptococcus pneumoniae* and *Haemophilus influenzae* were isolated from the specimens using standard microbiological procedures. The antibacterial activity of ethanolic extracts of ginger and bitter kola, were investigated on these pathogens using the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) assays.**
Results: **The extracts exhibited antibacterial activity against the pathogens. The MIC of extracts ranged from 0.0003µg/ml to 0.7µg/ml for ginger and 0.00008µg/ml, to 1.8µg/mL for bitter kola, while MBC ranged from 0.1.35µg/ml to 2.04µg/ml for ginger and 0.135µg/ml to 4.2µg/ml for bitter kola.**
Conclusion: **Results indicated that extracts of ginger root and bitter kola may contain compounds with therapeutic activity.**

INTRODUCTION

Respiratory tract infections (RTIs) continue to be a major cause of morbidity and mortality worldwide. Infections, which are prevalent during the cold months, are of particular importance in children, elderly people and in those persons with reduced host defences(1). Disease states like pneumonia, meningitis, sinusitis and chronic bronchitis have been associated with *Staphylococcus aureus, Streptococcus pyogenes, Streptococcus pneumoniae* and *Haemophilus influenzae*(2,3). Cough and colds mirror the onset of respiratory illness, which occur in developed and developing countries, and are far more frequent in young children in developing (20%) than in developed countries (4%)(4). In the tropics, a large family size correlates with the frequency of RTIs(5). Although there is a wide range of antibiotics for the treatment of bacterial infections, the development of resistance to chemotherapeutic agents, is increasingly becoming a pressing problem(6). Other limitations of modern chemotherapeutic drugs are their high costs and non-availability, especially in rural areas. As a consequence, it is necessary to search new organic molecules with antibacterial activity; which, in addition, could be potential sources for starting materials for the semi-synthesis of new drugs.

African plants, in particular medicinal plants, constitute a rich but still largely untapped pool of natural products(7). WHO estimates indicate that 80% of the population (mostly in developing countries) still relies on plant-based medicines for primary health care(8). Ginger and bitter kola have been used in Cameroon for such clinical conditions as bronchitis, partial impotence, cough, laryngitis, etc. by the local population, but without supporting scientific evidence(9). Ginger has been shown to possess other interesting pharmacological and physiological properties. For instance, it acts as an anti-inflamanatory, analgesic, antipyretic, antihepato-toxic and cardiotonic substance(10).

This study is aimed at evaluating the antibacterial activity of two commonly used plant products ginger and bitter kola in Cameroon: against 288 clinical isolates of respiratory tract pathogens commonly affecting individuals of all ages in our environment.

MATERIALS AND METHODS

Study Area and Subjects: The study area was Buea, in the South West Province of Cameroon, about 1000 metres above sea level; temperatures are low (15°C) with a relatively high humidity, which are predisposing factors for RTIs.

A total of 333 individuals from three localities; Bokwango, Molyko and Bolifamba, aged between 2 to 48

years, were sampled. Participants were informed of the aim of the study and their written consent was obtained.

Collection of Specimen: Throat swabs were taken from persons with running nostrils, cough and/or catarrh. Samples were collected following a previously reported method(11), and they were immediately transported to the laboratory in polythene bags containing ice packs at a temperature of approximately 4°C.

Micro-organism and Media: The following media (Oxoid, Basingstoke, England) were used to isolate the pathogens: crystal violet blood agar for *S. pyogenes;* blood agar for *S. pneumoniae,* nutrient agar (supplemented with 7.5% sodium chloride) for *S. aureus* and chocolate agar for *H. influenzae.* After innoculation, crystal violet blood agar plates were incubated anaerobically between 35-37°C for two to seven days. Other media were incubated aerobically for 24-48 hours at 37°C. Identification of isolates was based on their cultural, morphological and biochemical characteristics(12).

Preparation of Ginger and Bitter Kola Extracts: The extracts were prepared by using the method of Osuinde and Esiovwa(13). Briefly, 3 grams of Ginger rhizomes and bitter kola seeds were macerated separately in a clean mortar and 6ml of 95% ethanol added in the ratio 1:2(w/v). The mixtures were allowed to stand overnight, after which extracts were drained and evaporated to powder at 50°C overnight. Yields of crude ginger and bitter kola extracts were 0.0584g and 0.0407g respectively.

Determination of Minimum Inhibitory Concentration (MIC): The MIC was determined by the tube dilution method following the scheme reported by Koneman *et al(14)*. Briefly, two sets of ten tubes were prepared for each extract and isolate. One millilitre of nutrient broth was put into the first tubes and 9ml to the remaining nine tubes. Ten μg each of ginger and bitter kola were added to the first tubes respectively. The tubes were well mixed and 1ml transferred serially until the 10th tube. 1 x 10^8CFU/ml of each isolate was added to each tube and incubated at 37°C for 24 hours. Controls containing only nutrient broth without extracts were included. The MIC was regarded as the lowest concentration (highest dilution) of extract that inhibited visible growth (no turbidity).

Determination of Minimum Bactericidal Concentration (MBC): From each tube that did not show visible growth in the MIC, 0.01ml was transferred into extract free nutrient broth. After 18 hours of incubation, a loopful containing approximately 10^8 CFU/ml of organism was aseptically transferred onto nutrient agar plates. The plates were incubated at 37°C for 24 hours. The MBC was recorded as the lowest concentration (highest dilution) of extract that had less than 99% growth on nutrient agar plates.

Susceptibility Testing of Pathogens to Plant Extracts and Commercial Antibiotics: The disc diffusion method was used as previously reported(13). The surfaces of nutrient agar plates were flooded with 10^8 CFU/ml of each isolate and drained. Sterile filter paper disc (5mm in diameter), impregnated with extracts (10µg) were aseptically transferred onto the surface of innoculated agar extract. Sterile discs soaked in distilled water served as control.

Commercial antibiotic discs containing penicillin 10µg, clindamycin 2µg, erythromycin 15µg, cefazoline 30µg, chloramphenicol 30µg and tetracycline 30µg were transferred onto agar plates already seeded with test organisms. Plates were incubated at 37°C for 24 hours and the diameters were then compared with recorded diameters of the control organisms, *E. coli* NCTC 10418 and Oxford *S. aureus* NCTC 6571 to determine susceptibility (≥ 12mm) or resistance (≤ 6mm).

Statistical Analysis: The Chi square test was used for statistical analysis. P values of 0.05 was considered significant.

RESULTS

Out of the 333 individuals sampled, 288 isolates of the following organisms were isolated: *S. aureus* (28.5%), *S. pneumoniae* (26.4%), *S. pyogenes* (20.1%) and *H. influenzae* (25.0%) (Table 1).

Table 1

Prevalence of micro-organisms

Organism	Localities Bokwango	Bolifamba	Molyko	Total(%)
S. aureus	16	21	45	82 (28.5)
S. pneumoniae	34	25	17	76 (26.4)
S. pyogenes	24	20	14	58 (20.1)
H. influenzae	26	26	20	72 (25.0)

Table 2

Antibacterial activity of ginger extract (10µg) and bitter kola extract (10µg)[A]

Locality	*S. aureus*		*S. pneumoniae*		*H. Influenzae*		*S. pyogenes*	
	Ginger	Bitter kola	Ginger	Bitter kola	Ginger	Bitter kola	Ginger	Bitter kola
Bokwango	6	5	2	6	1	1	6	6
Molyko	4	4	3	2	4	3	5	4
Bolifamba	2	2	4	9	2	1	3	2

A= Zone of inhibition (mm)

Table 3

Antibacterial activity of some commercial antibiotic discs[A]

Locality	*S. aureus*						*S. pneumoniae*						*H. influenzae*						*S. pyogenes*					
	P	ERY	CZ	CC	CXM	CHR	TET	P	ERY	CZ	CC	CXM	CHR	TET	P	ERY	CZ	CC	CXM	CHR	TET	P	ERY	CZ	CC	CXM	CHR	TET
Bokwango	4	3	12	5	13	6	5	2	4	14	3	12	4	4	3	4	14	4	12	4	4	2	2	13	5	12	4	4
Molyko	3	3	12	5	12	4	5	2	4	14	3	12	4	3	3	4	12	4	12	4	3	2	2	13	4	13	4	3
Bolifamba	4	4	13	4	12	5	5	2	4	13	3	12	4	3	4	4	13	4	12	3	3	2	2	12	4	12	3	3

P = penicillin (10μg); ERY= erythromycin (15μg); CZ = cefazoline (30μg); CC = clindamycin (2μg); CXM = cefuroxime (30μg); CHR = chloramphenicol (30μg); TET = tetracyline (30μg); A, Mean ± standard deviation of diameter of zone of inhibition (mm)

Table 4

MIC of ginger and bitter kola extract (µg/mL)

Locality	*S. aureus*		*S. pneumoniae*		*H. influenzae*		*pyogenes*	
	Ginger	Bitter kola	Ginger	Bitter kola	Ginger	Bitter kola	Ginger	Bitter kola
Bokwango	0.0003	1	0.4	0.0003	0.316	0.667	0.0005	0.0005
Molyko	0.4	1.66	0.7	1.7	0.001	0.001	0.083	0.25
Bolifamba	0.6	1.8	0.001	0.00008	0.2	1.053	0.389	0.44

Table 5

MBC of ginger and bitter kola extracts (ug/mL)

Locality	*S. aureus*		*S. pneumoniae*		*H. influenzae*		*S. pyogenes*	
	Ginger	Bitter kola	Ginger	Bitter kola	Ginger	Bitter kola	Ginger	Bitter kola
Bokwango	0.15	3.4	0.16	0.16	0.73	1.17	0.15	0.9
Molyko	1.73	3.64	2.04	3	0.67	1	1.25	1
Bolifamba	0.24	4.2	0.15	0.135	0.63	1.42	1.44	1.11

The plant extracts showed antibacterial activity against the isolates with zones of inhibition ranging from 1mm to 9mm (Table 2). The results of antibacterial activity of commercial discs are shown in Table 3. The pathogens showed resistance to five of the antibiotics tested as indicated by the diameters of inhibition zones. The MIC of the plant extracts ranged from 0.0003µg/ml, to 0.7µg/ml, for ginger extract, and 0.00008µg/ml to 1.8µg/ml for bitter kola extract (Table 4). The UBC ranged from 0.15µg/ml to 2.04µg/ml for ginger extract and 0.135 µg/ml for bitter kola extract (Table 5).

DISCUSSION

Plant extracts used in this study exhibited antibacterial activity against all the pathogens tested. *S. aureus* was most susceptible to ginger extract with an inhibition zone diameter of 6mm, while *S. pneumoniae* was most susceptible to bitter kola extract with an inhibition zone diameter of 9mm (Table 2). There was a significant difference ($p<0.05$) between the diameters of zones of inhibition of ginger and bitter kola extracts for each organism in the three localities. The reason(s) for this cannot be ascertained, but we speculate that the difference could be due to interactions between factors related to the host, pathogens and the environment as earlier suggested(15), as well as rate of diffusion of the extract, culture medium, depth of the medium and density of innoculation(16).

The activity of the ginger and bitter kola extracts was compared with that of commercial antibiotic discs (Table 3). Comparing the diameters of inhibition zones obtained from the commercial antibiotics, all test organisms were resistant to five (penicillin, erythromycin,

clindamycin, chloramphenicol and tetracycline) of the seven antibiotics tested. They were only susceptible to cefazoline and cefuroxime. The wide spectrum of activity of plant extracts compared to the commercial antibiotics is an indication of their antibacterial potential in medicine. There are no reports of severe toxicity in humans from eating ginger or bitter kola(10), thus marketers of the natural form of the compound need not demonstrate its safety as would be the case for a new pharmaceutical.

The observed resistance to most of the antibiotics was not unexpected because these drugs are relatively cheap, and therefore commonly available to the population who tend to abuse them heralding the emergence of resistance(17). Meanwhile, cefazolin and cefuroxime are more expensive and not affordable by the local population. The demonstrable antibacterial activity of the plant extracts to these pathogens is therefore an interesting and important finding since they are cheap, and easily affordable by the population.

The lowest MBC for ginger extract (0.0003µg/ml) was recorded for *S. aureus* (Table 4) while the highest MICs of ginger extract (0.7µg/ml) and bitter kola extract (1.8µg/ml) were recorded for *S. pneumoniae* and *S. aureus* respectively. There was no significant difference ($p>0.05$) between the MICs of ginger and bitter kola extracts respectively in the three localities with respect to each of the organisms. However, there was a significant difference ($p<0.05$) between the MICs of the extracts for *S. pneumoniae* and *S. aureus* each in the three localities.

The lowest MIC for ginger extract (0.15µg/ml) was recorded for *S. aureus* and *S. pyogenes*, while the highest value (2.04µg/ml) was recorded for *S. pneumoniae* (Table 5). *S. aureus* generally had low MBC values for ginger extract compared to the other test organisms. The highest MBC for bitter kola extract (4.2µg/ml) was recorded for *S. aureus*, while the least value (0.135µg/ml) was recorded for *S. pneumoniae*. There was no significant difference ($p>0.05$) in the MBC of ginger extract for *S. aureus, H. influenzae* and *S. pyogenes* each in the three localities. The difference between the MBC of bitter kola extract for *S influenzae* and *S. pyogenes* each in the three localities was not significant ($p>0.05$), but was significant ($p<0.05$) for S. *aureus.*

These differences observed could be attributed to the fact that serotypes of pneumococci vary geographically and are subject to antigenic changes as previously reported(18). In addition, the phenomenon of tolerance due to recurrent infections, caused by overcrowding which is a crucial variable associated with risk of streptococcal infections could be a contributing factor(19). Furthermore, this could be due to the age or diet of the subjects in this study since age and diet are apparent risk factors, which may change the adaptive response of an infecting micro-organism.

In conclusion, the results of this study indicate that ginger and bitter kola extracts could be better options to some of the antibiotics commonly used for respiratory tract infections in the environment of Buea, Cameroon. These findings, therefore, are of great significance and could be clinically exploited.

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