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Susceptibility of Multi Drug Resistant Bacteria Associated with Respiratory Tract Infection To Methanolic Extract of *Garcinia kola* Heckel (Bitter Kola)

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Abstract: The study was carried out to determine the effects of methanolic extract of bitter kola on bacterial isolates from sputum of patients with respiratory tract infections. One hundred and sixty patients, made up of 85 males and 75 females, attending chest clinics at Ekiti State University Teaching Hospital and General Hospital, both in Ado Ekiti, Nigeria, were recruited for this study. Biodata of the subjects were obtained and bacteriological investigations were carried out on the sputum samples. The subjects were 7-71 (41.27 ± 13.73) years old. Microscopical examination of the sputum following Gram staining of the smear revealed the presence of yeast cells (30.85%), epithelial cells (35.11%), white blood cells (13.83%), red blood cells (10.64%), hyphae and pseudohyphae (5.32%). Bacteria isolated from sputum were *Klebsiella pneumoniae*, *Corynebacterium argentoratense*, *Staphylococcus spp*, *Vibrio fluvialis*, *Enterobacter aerogenes*, *Burkholderi*a *cepacie, Escherichia coli*, *Pseudomonas aeruginosa*, *Vibrio mimicus*, *Vibrio vulnificus*, *Corynebacterium accolens*, *Luteococcus peritonei*, *Kingella denitrificans, Pseudocitrobacter spp, Aggregatibacter actinomycetemcomitan,s Sphingomonas paucimobilis, Citrobacter spp, Blastobacter denitrificans, Tatumella ptyseos* among others. The bacterial isolates showed varied degree of multidrug resistance (MDR). All the tested organisms were susceptible to imipenem, an indication of lack of carbapenemase production. Twenty-four (96%) of the 25 bacterial isolates tested were positive for extended spectrum beta-lactamases (ESBL) production. All the extended beta-lactamase positive bacterial isolates were susceptible to the methanolic extract of bitter kola at low concentration. The methanolic extract bitter kola was found to contain saponins, tannins, cardiac glycosides, alkaloids and steroids

Key words: Respiratory infection · Bacteria · Multidrug resistance · *Garcinia kola* · Methanolic extracts

INTRODUCTION

Respiratory tract is the part of the human system that plays a vital role in breathing processes. The respiratory tract is constantly exposed to microbes due to the extensive surface area, for instance, the lungs have an exposed internal surface area of approximately 500m^2 [2].

Acute lower respiratory tract infection is a common cause of hospital admission in Nigeria; however no comprehensive study on the prevalence of pneumonia in adults is readily available. Among patients attending a tuberculosis clinic in South Western Nigeria, 6.4% had streptococcal pneumonia [4]. Few studies have also investigated the aetiology of pneumonia in Nigerian adults. Upper respiratory tract infections are mostly caused by viruses. Group A beta hemolytic *Streptococci* (GABHS) cause 5 to 10% of cases of pharyngitis in adults [5]. Thus transmission occurs more commonly in crowded conditions.

Plant-derived substances have recently attracted great research interest owing to their versatile applications [6]. Specifically, medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical inter-mediates and chemical entities for synthetic drugs [6]. This is due to the presence of secondary metabolites which accumulate in the various parts of these plants conferring on them their pharmacological relevance [7]. Generally, some of these plants especially the edible ones are eaten habitually without any knowledge of their pharmacological effect.

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Garcinia kola Heckel (Clusiaceae), commonly known as bitter kola (English) and orogbo (Yoruba) is a widespread tree of evergreen forest, valued in Nigeria for its medicinal nuts which has led to its exploitation in the natural forests in recent times [8]. The stem, bark and the seeds are used for acute fever, inflammation of the respiratory tract and throat infections. It is chewed extensively in Southern Nigeria as a masticatory to cause nervous alertness and has been proven to exhibit pharmacological uses in treating coughs and throat infections [8]. *Garcinia kola* stem bark has been shown to contain a complex mixture of phenolic compounds such as tannins, guttiferin, biflavonoids, xanthenes, benzophenone, kola flavanone and garcinia flavanone all of which have antimicrobial activity. Besides, *Garcinia kola* exhibits purgative, antiparasitic, anti-inflammatory, anti-bacterial and antiviral properties [9, 10].

In addition, the plant possesses hepatoprotective, analgesic and hypo-glycemic activities [11, 12]. *G. kola* enjoys a folk reputation in the management of sickle cell disease, as poison antidote and in the preservation of lipid food products prone to rancidity [13]. Considering the enormous relevance of *Garcinia kola* in folkloric medicine, the study was conducted to determine the antimicrobial activity of *Garcinia kola* against bacterial isolate of the respiratory tract and the susceptibility patterns of the isolates.

The study was carried out to assess microorganisms associated with respiratory tract infectionsand evaluate the antibacterial potential of extracts of *Garcinia kola* especially on multidrug resistant bacteria.

MATERIALS AND METHODS

Study Area and Population: The research was carried out on patients visiting Ekiti State University Teaching Hospital (EKSUTH), Ado- Ekiti, Ekiti State, Nigeria over a period of six months (February-July, 2015). The population studied was a heterogeneous population of different age group, ethnicity and educational status.

Ethical Consideration: Approval was sought and collected from the Research/Ethics Committee of the Ekiti State University Teaching Hospital. Biodata and other information were collected after obtaining informed consent from each patient with the assurance that all information obtained would be treated confidentially.

Sampling Techniques: Early morning sputum specimens "before eating" were aseptically collected into sterile containers from 100 patients attending Ekiti State University Teaching Hospital, between March and July, 2015. All samples were transported from the hospital to the Microbiology laboratory of Afe Babalola University, Ado-Ekiti (ABUAD).

Sample Processing: Using a sterile wire loop, well-mixed sputum was inoculated on blood agar and nutrient agar. The plates were incubated aerobically at 37 °C for 24 hours and plates were sub-cultured for further tests.

Sputum was examined macroscopically for the physical appearances, whether mucoid, purulent, watery, blood stained etc. It was also examined microscopically using Gram-staining technique and observed with oil immersion lens (x100) for the presence of red blood cells, epithelial cells, white blood cells, fungal hyphae, pseudohyphae and yeast cells as described by World Health Organization [14].

Inoculation, isolation, characterization and identification of isolates.

All isolates were characterised using standard microbiological and biochemical tests as described by Barrow and Feltham [15] and Cheesbrough [16]. Bacterial isolates were identified with the help of online Gideon informatics [17] with reference to Barrow and Feltham [15] and Garrity *et al.* [18].

Antibiotic Susceptibility Test: All the isolated organisms were tested for antibiotic susceptibility by Kirby-Bauer disc diffusion method on Mueller-Hinton agar. This was carried out by making an even spread of the pure isolates on prepared Mueller-Hinton agar using sterile swab sticks and aseptic placement of the antibiotics discs using sterile forceps. The plates were incubated aerobically at 37 °C for 24 hours after which the zones of inhibition were measured and interpreted according to Clinical and Laboratory Standards Institute [19]. Antibiotics used are: Ceftazidime (30µg), Cefuroxime (30µg), Gentamicin (10µg), Cefixime (5µg), Ofloxacin (5µg), Augmentin (30ìg), Ciprofloxacin ((5µg), Nitrofurantoin (300ìg), Cotrimoxazole (25µg), Nalidixic acid (30ìg),, Amoxycillin (25ìg), Tetracycline (25ìg) and Imipenem (10µg) for gram negative isolates; and Ceftazidime (30µg), Cefuroxime (30µg), Gentamicin (10µg), Ceftriaxone (5µg), Erythromycin (15µg), Cloxacillin (5µg), Ofloxacin (5µg), Augementin (30µg) for gram positive isolates.

Assay for ESBL Production: Assay for production of extended spectrum β lactamase production by the bacterial isolates from sputum was determined by the

double disc method as described by Clinical and Laboratory Standards Institute [19]. Antibiotics used are: Ceftazidine (30µg) and Ceftazidine/Clavulanic acid (30/10µg); Cefuroxime (30µg) and Cefurozime/ Clavulanic acid (30/10µg); Ceftriazone (30µg) and Cetriaxone/ Clavulanic acid (30/10µg). A = 5mm increase in a zone diameter for either antimicrobial agent tested in combination with clavulanic acid versus the zone diameter of the agent when tested alone indicates ESBL production (eg, ceftazidime zone =16; ceftazidime-clavulanic acid zone=21) [19].

Plant Sample Collection: The seed of *Garcinia kola* used in the research work was obtained from a public market in Ado-Ekiti, Ekiti State, Nigeria and then transported to the Microbiology Laboratory of Afe Babalola University, Ado-Ekiti (ABUAD).

Preparation of *Garcinia kola* Extract: Before extraction, the seeds of *Garcinia kola* were removed from the seed coat, washed to remove sand, after which it was cut into bits to aid fast drying. It was dried at room temperature and then blended with a blender (the blender was first washed with distilled water and then cleaned with methylated spirit before blending). The powdered forms of the seed of *Garcinia kola* were placed in different containers and were properly labelled. Extracts were obtained using the methods of Iwaki and co-workers [20].

Extraction: Methanol was used as solvent, for extraction of active compounds in the plant material. 25g of processed plant material (*Garcinia kola*) was soaked in methanol in vacuum jar. This was shaken vigorously and allowed to stand for 48 hours to effect proper extraction of active ingredient. The suspension was filtered with Whatman's filter paper to obtain the supernatant while the debris was discarded. The mixture was loaded into a Soxhlet extractor and extracted at 60°C for 24 h. All extracts were stored at -40°C.

Phytochemical Analysis: The methanolic extract of bitter kola was tested for the presence of the following plant secondary metabolites such as alkaloids, saponins, tannins, flavonoids, steroids, glycosides and phenols. All tests are performed as described by Sofowora [21].

Test for alkaloids. About 2ml of the extract was added to 1ml of aqueous hydrochloric acid. A few drops of saturated picric acid solution were added. A creamish precipitate obtained indicated the presence of alkaloid.

Test for tannins. Two hundred milligrams of plant material was dissolved in 10ml of distilled water and filtered. 2ml of the extract was added to 2ml of $FeCl_3$. If a blue- black precipitate was obtained, this indicated the presence of tannins.

Test for Saponins (Frothing Test): From the extract, 0.5ml was added to 5ml of distilled water. Frothing persistence indicated presence of saponins.

Test for Flavonoids: About 2ml of plant extract was weighted in a test tube and dissolved diluted NaOH and diluted HCl were added. The presence of yellow solution that turns colorless indicates the presence of flavonoids.

Test for Steroids: Two hundred milligrams of plant material was dissolved in 10ml chloroform and filtered. 2ml of the filtrate was added to 2ml acetic anhydride and concentrated H_2SO_4. Blue-green ring obtained indicated the presence of terpenoids.

Test for cardiac glycosides (Keller-Kiliani test). A volume of 2ml of the extract was added to 1ml glacial acetic acid, 1ml $FeCl_3$ and 1ml concentrated H_2SO_4. Absence of green-blue colour indicated that cardiac glycosides were absent.

Antimicrobial Activity of *Garcinia kola* Extracts Using Agar-well Diffusion Method: Susceptibility of the isolated organisms to methanolic extract of bitter kola was determined by agar well diffusion technique using Mueller-Hinton agar. Seven millimetre (7mm) diameter wells were prepared on agar containing a suspension of each isolated organisms. The methanol extract was diluted into two folds, using dimethyl sulfoxide (DMSO) as diluents and different concentrations were added to the wells. The plates were left at ambient temperature for 15 minutes and then incubated at 37 ° C for 24hours, after which the zones of inhibition were measures and recorded.

Statistical Analysis: The distributions of bacteria isolated from sputum along age and sex were analysed for significant association using SPSS V-16.0.

RESULTS

Sputum samples were collected from one hundred and sixty patients attending Ekiti State Teaching Hospital Ado- Ekiti, aged 41.27 ± 13.73 (7-71) years and made up of 85 males and 75 females. Microscopical examination of the

sputum following Gram staining of the smear reveals presence of yeast cell cells (30.85%), epithelial cells (35.11%), white blood cells (13.83%), red blood cells(10.64%), fungal hyphae and pseudohyphae (5.32%).

A total of 80 bacteria made up of 34 different bacteria species were isolated from the sputum collected. The predominant organisms are *Klebsiella pneumoniae* (27.5%), *Corynebacterium argentoratense* (8.8%), *Staphylococcus* spp (5.0%), *Vibrio fluvialis* (6.3%), *Enterobacter aerogenes* (5.0%), *Burkholderia spp* (5.0), *Escherichia coli* (3.0%), *Pseudomonas aeruginosa* (3.0%), *Vibrio mimicus* (3.0%), *Vibrio vulnificus* (3.0), *Corynebacterium accolens* (3.0), *Luteococcus peritonei* (3.0%). Others are *Kingella denitrificans, Streptococcus australis, Lautropia mirabilis, Pseudocitrobacter* spp., *Aeromonas bestiarum, Acinebacter* spp., *Tatumella ptyseas, Pantoea agglomerans, Proteus vulgaris, Vibrio metschnikovii, Sphingomonas paucimobillis, Blastobacter denitrificans, Proteus mirabilis, Arthrobacter sanguinis, aggregatibacter* spp., *Arthrobacter scleromas, Aerococcus viridians* and *Luteococcus sanguinis* (Figures 1). No significant association was obtained in the distribution of bacteria isolated from sputum along age and sex ($p>0.05$) (Figures 2 and 3).

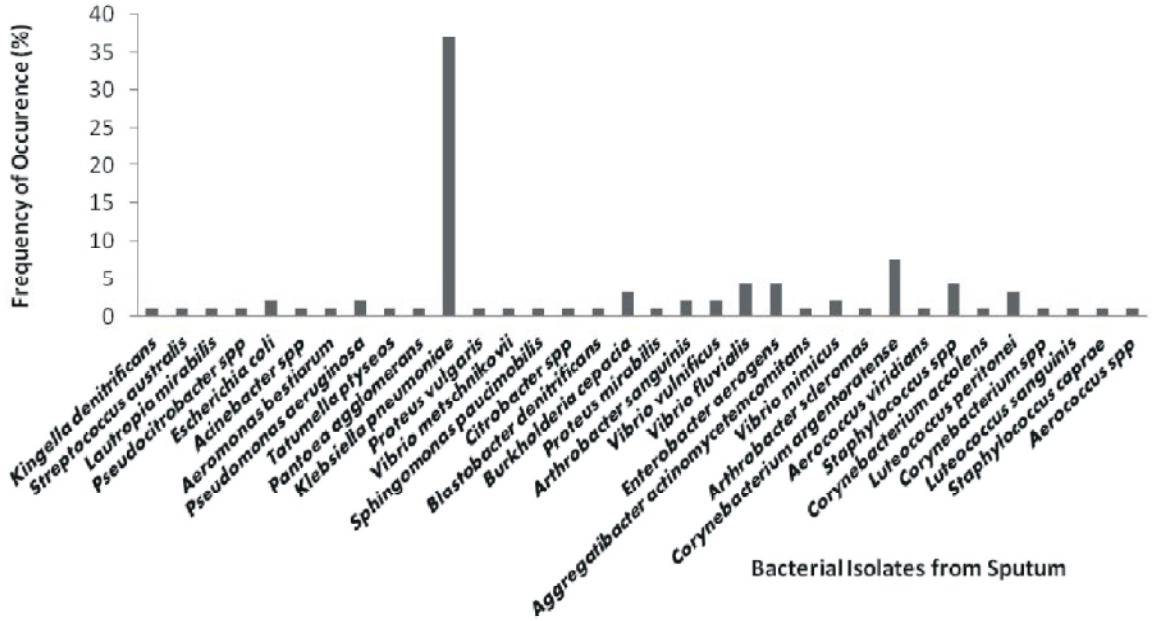

Fig. 1: Frequency of isolation of aerobic bacteria from sputum

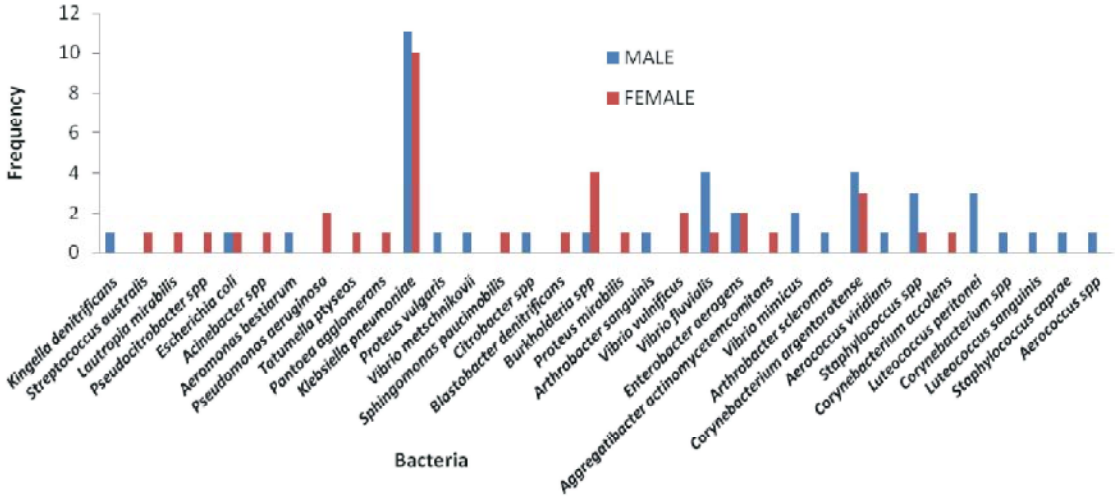

Fig. 2: Distribution of bacteria isolated from sputum along sex

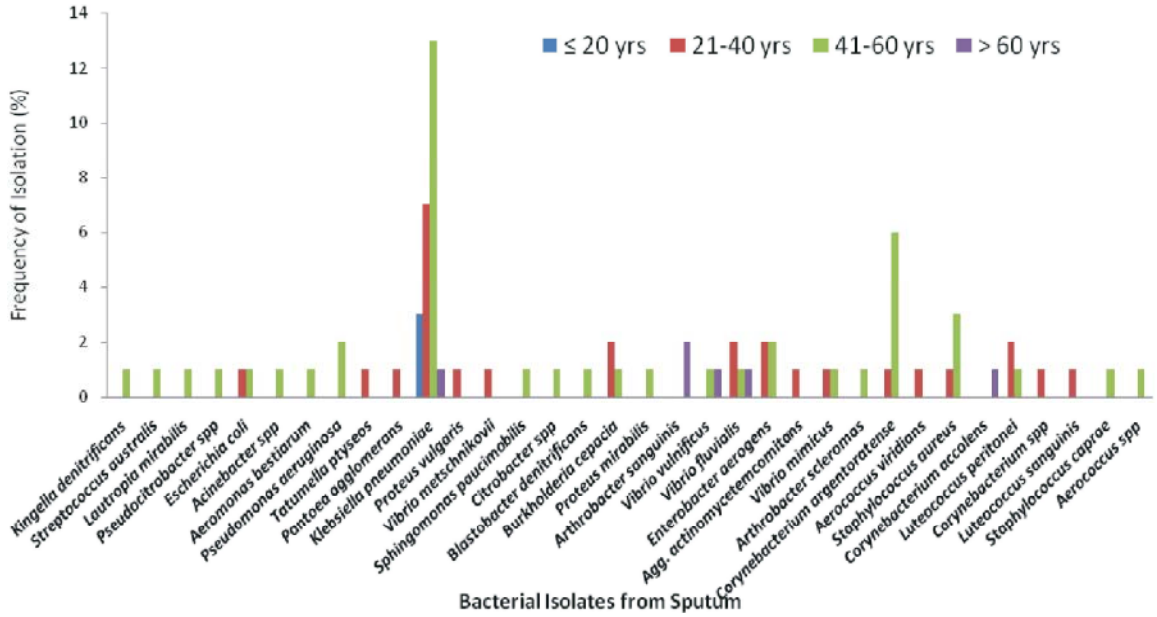

Fig. 3: Distribution of bacteria isolated from sputum along age

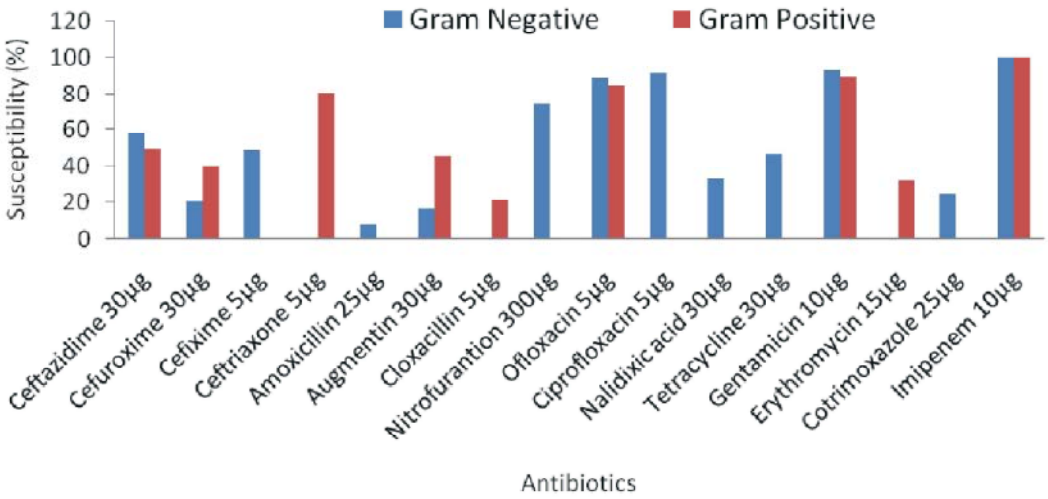

Fig. 4: Susceptibility of bacteria isolated from sputum to antibiotics

The Gram negative bacterial isolates were highly susceptible to ciprofloxacin (91.78%), gentamicin (92.89%), ofloxacin (88.67%) and nitrofurantoin (74.22%). High resistance were obtained for Augmentin, cefuroxime, ceftazidime Cotrimixazole, Amoxicillin, tetracycline, nalixilic acid and cefixime with 2.22%, 21.00%, 49.56%, 25.00%, 8.33%, 46.83%, 33.33% and 58.56% susceptibility respectively (Figures 4). The Gram positive bacterial isolates were susceptible to Gentamicin (89.30%), Ofloxacin (84.96%) and Ceftriazone (80.43%), while low susceptibility were obtained from Cloxacillin (21.74), Erythromycin (32.61%), Cefuroxime (40.30%) and Ceftazidime (50.17%). All the tested organisms were susceptible to imipenem (Figures 4).

Twenty-four (96%) of the 25 isolates tested were positive for extended spectrum beta-lactamases (ESBLs) production (Tables 1). All the antibiotic resistant and extended beta-lactamase producing bacterial isolates were susceptible to the methanolic extract of *Garcinia kola* at low concentrations (Tables 2 and 3). The methanolic extract of bitter kola was found to contain saponins, tannins, cardiac glycosides, alkaloids and steroids.

Table 1: Extended spectrum beta-lactamase production profile of bacterial isolates from sputum

Codes	Organisms	Ceftazidime 30 µg	Ceftazidime/Clavulanic acid 30µg/10 µg	Cefuroxime 30µg	Cefuroxime/ Clavulanic acid 30µg/10 µg	Ceftriaxone 30µg	Ceftriaxone/Clavulanic acid 30µg/10 µg
32a	*Klebsiella pnemoniae* I	21[a]	26[a]	0	0	0[b]	21[b]
4bii	*Klebsiella pnemoniae* II	25[a]	33[a]	28	31	21[b]	28[b]
15b	*Klebsiella pnemoniae* III	30	33	0	0	12[a]	28[a]
35	*Klebsiella pnemoniae* IV	25	26	0	0	0[a]	25[a]
6ai	*Klebsiella pnemoniae* V	15[a]	21[a]	25	29	11	12
4ai	*Klebsiella pnemoniae* VI	25[a]	30[a]	0	0	11[b]	30[b]
42	*Klebsiella pnemoniae* VII	13[a]	24[a]	0	0	0[b]	24[b]
45a	*Klebsiella pnemoniae* VIII	16	18	0	0	0[a]	25[a]
47a	*Klebsiella pnemoniae* IX	9[a]	27[a]	0[b]	22[b]	9[c]	30[c]
41	*Klebsiella pnemoniae* X	19	21	0[a]	20[a]	0[b]	30[b]
47b	*Klebsiella pnemoniae* XI	9[a]	29[a]	0[b]	16[b]	10[c]	31[c]
14	*Klebsiella pnemoniae* XII	22	24	0[a]	10[a]	20	24
17a	*Klebsiella pnemoniae* XIII	24	25	0	0	0[a]	24[a]
26	*Klebsiella pnemoniae* XIV	20[a]	27[a]	0	0	0[a]	40[a]
16a	*Klebsiella pnemoniae* XV	21	25	10[a]	17[a]	10	18
17b	*Klebsiella pnemoniae* XVI	15[a]	22[a]	10[b]	17[b]	0[c]	18[c]
37b	*Klebsiella pnemoniae* XVII	30	31	17[a]	26[a]	0	0
43	*Klebsiella pnemoniae* XVIII	18	20	0	0	0[a]	18[a]
29b	*Klebsiella pnemoniae* XIX	29	30	0	0	0[a]	22[a]
24b	*Escherichia coli*	0[a]	25[a]	21	32	0[b]	22[b]
45b	*Burkholderia spp*	17	20	0	0	0[a]	25[a]
3b	*Kingella denitifricans*	0[a]	17[a]	0	0	0[b]	20[b]
2	*Arthrobacter sanguinis*	14	18	10	11	0[a]	14[a]
27a	*Pseudomonas aeruginosa*	0[a]	27[a]	0	0	0	0
1ii	*Vibrio vulnificus*	24	25	0	0	0	0
6bii	*Staphylococcus spp*	28	28	0	0	0[a]	11[a]
8c	*Corynebacterium accolens*	13[a]	23[a]	0	0	0[b]	15[b]
36b	*Corynebacterium argentoratense*	0[a]	29[a]	0[b]	26[b]	0	0

[a,b,c] Zones of inhibition values within row with the same superscript indicates Extended spectrum beta- lactamase production

* Values are zones of inhibition in millimetres (mm)

Table 2: Antimicrobial effect of methanolic extract of *Garcinia kola* on Gram negative bacterial isolates

Codes	Organisms	200 mg/ml	100 mg/ml	50 mg/ml	25(mg/ml)	12.5 mg/ml	Antibiotics Resistance Clusters
32a	*Klebsiella pnemoniae* I	13	9	5	0	0	AUG/TET/AMX/COT/NIT
4aii	*Klebsiella pnemoniae* II	14	13	12	9	5	CFM/AUG
15b	*Klebsiella pnemoniae* III	9	8	8	7	5	CAZ/CXM/CFM/AUG/NIT
35	*Klebsiella pnemoniae* IV	8	7	6	6	5	CAZ/CXM/AUG/NIT
6ai	*Klebsiella pnemoniae* V	19	14	9	9	6	CXM/AUG
4ai	*Klebsiella pnemoniae* VI	24	19	14	14	13	CXM/CFM/AUG/NIT
42	*Klebsiella pnemoniae* VII	14	9	8	5	0	NAL/AUG/TET/AMX/COT
45a	*Klebsiella pnemoniae* VIII	6	6	4	0	0	NAL/AUG/TET/AMX/COT/NIT
47a	*Klebsiella pnemoniae* IX	14	8	7	5	0	NAL/OFL/AUG/TET
41	*Klebsiella pnemoniae* X	9	7	5	0	0	NAL/OFL/AUG/AMX/COT/NIT
47b	*Klebsiella pnemoniae* XI	14	13	4	0	0	NAL/AUG/TET/AMX/COT/CIP
14	*Klebsiella pnemoniae* XII	11	9	6	6	4	CAZ/CXM/CFM/AUG/CIP
17a	*Klebsiella pnemoniae* XIII	9	6	5	0	0	CAZ/CXM/CFM/AUG/NIT

Table 2: Continued

Codes	Organisms	200 mg/ml	100 mg/ml	50 mg/ml	25(mg/ml)	12.5 mg/ml	Antibiotics Resistance Clusters
26	*Klebsiella pnemoniae* XIV	14	11	8	6	4	CXM/CFM/AUG/NIT
16a	*Klebsiella pnemoniae* XV	20	16	9	9	5	CXM/AUG/NIT
17b	*Klebsiella pnemoniae* XVI	14	9	5	0	0	CAZ/CXM/CFM/AUG/NIT
37b	*Klebsiella pnemoniae* XVII	31	14	11	11	10	GEN
43	*Klebsiella pnemoniae* XVIII	13	10	5	4	0	NAL/AUG/TET/AMX/COT/NIT
29b	*Klebsiella pnemoniae* XIX	8	6	6	0	0	CAZ/CXM/AUG
36b	*Klebsiella pnemoniae* XX	13	10	9	8	5	CAZ/CXM/AUG
45b	*Burkholderia cepacia* I	24	18	16	10	5	NAL/AUG/AMX/COT/NIT
12a	*Burkholderia cepacia* II	13	9	9	9	7	CXM/CFM/AUG
1ii	*Vibrio vulnificus*	10	8	7	5	5	CXM/AUG
10aii	*Enterobacter aerogenes* I	18	14	9	5	0	CXM/AUG
7bi	*Enterobacter aerogenes* II	24	19	11	8	0	CAZ/CEF/AUG
7aii	*Proteus mirabilis*	14	13	12	9	9	CAZ/CXM/AUG
11	*Pantoea agglomerans*	20	19	14	10	0	CXM/CFM/OFL
29a	*Tatumella ptyseos*	10	10	7	6	0	AMX/COT/GEN
16b	*Vibrio fluvialis*	24	19	19	14	11	CXM/OFL/AUG
15a	*Pseudomonas aeruginosa* I	14	9	5	0	0	CAZ/CXM/CFM/AUG/NIT
27a	*Pseudomonas aeruginosa* II	7	6	6	6	5	CAZ/CXM/CFM/OFL/AUG/NIT/CIP
24b	*Escherichia coli* I	19	18	15	14	9	AUG/NIT
8a	*Escherichia coli* II	19	18	16	14	13	CXM/CFM/AUG/CIP
12b	*Vibrio mimicus*	16	9	9	6	0	CXM/CFM/AUG
2	*Arthrobacter sanguinis*	28	21	21	15	11	CAZ/CXM/CRO/ERY/CLX
7bii	*Blastobacter denitrificans*	14	11	10	9	6	CAZ/CXM/CFM/OFL/AUG/NIT
22	*Aggregatibacter actinomycetemcomitans*	20	14	6	5	4	CXM/OFL/AUG
TP1	*K. pnemoniae* (ATCC 13883)						
TP2	*Proteus mirabilis* (ATCC 12453)	10	5	3	0	0	

Table 3: Antimicrobial effect of methanolic extract of *Garcinia kola* on Gram positive bacterial isolates

Codes	Organisms	200 mg/ml	100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml	Antibiotics Resistance Clusters
20	*Luteococcus sanguinis*	28	15	13	9	5	CXM/CRO/ERY/CLX/AUG
40	*Corynebacterium argentoratense* I	15	9	8	5	4	CAZ/AUG
33	*Corynebacterium argentoratense* II	10	9	4	0	0	CAZ/ERY
32b	*Luteococcus peritonei* I	18	15	15	12	10	CXM/ERY/CLX/AUG
10c	*Luteococcus peritonei* II	21	20	19	18	10	ERY/CLX/AUG
44	*Luteococcus peritonei* III	13	9	8	0	0	CAZ/CXM/CLX/AUG
39a	*Staphylococcus caprae*	11	11	6	5	3	CAZ/AUG
8c	*Corynebacterium accolens*	20	19	18	15	12	CAZ/CRO/CLX
46b	*Corynebacterium argentoratense*	24	19	10	8	5	CAZ/CXM/ERY/AUG
TP3	*Staphylococcus aureus* (ATCC 25923)	15	9	5	0	0	

* Values are zones of inhibition in millimeters (mm)

DISCUSSION

Respiratory tract infections (RTIs) are among the most commonly prevalent infectious diseases in clinical history. This study described the antibiotic susceptibility profiles of bacterial isolates from sputum of RTIs patients and assessed the effect of methanolic extract of bitter kola on MDR and ESBLs producing bacteria. Bitter kola has been known for its broad spectrum against a wide range of microorganisms [22].

Bacteriological investigation of the sputum samples indicated high prevalence of *Klebsiella pneumonia*. The other bacteria isolated include *Escherichia coli, Burkholderia spp, Vibrio vulnificus, Vibrio fluvialis, Enterobacter aerogens, Aggregatibacter actinomycetemcomitans, Staphylococcus caprae, Aerococcus spp*, *Staphylococcus spp, Proteus vulgaris, Corynebacterium accolens, Luteococcus peritonei, Luteococcus sanguinis, Pantoea agglomerans, Aeromonas bestiarum, Pseudomonas aeruginosa,*

Kingella denitrificans, Pseudocitrobacter spp, Arthrobacter scleromas, Corynebacterium argentoratense Sphingomonas paucimobilis, Citrobacter spp, Blastobacter denitrificans, Tatumella ptyseos, Lautropia mirabilis among others. Although many of these bacteria are not commonly reported with respect to respiratory tract infection in Nigeria, they may play a significant role in epidemiology of respiratory tract infections in the country. Recent studies in Nigeria show an increasing involvement of *Klebsiella pnemoniae, Kingella denitrificans, Staphylococcus* spp, *Enterobacter aerogens, Pseudomonas aeruginosa and Proteus vulgaris* in respiratory tract infections [23].

The bacterial isolates showed a high degree of resistance to the antibiotics tested. The Gram negative bacteria were resistant to Augumentin and Amoxycillin, 97.78% and 91.67% respectively, while they were highly susceptible to Gentamicin (92.89%) and Ciprofloxacin (91.78%). The Gram positive bacterial isolates were highly susceptible to Gentamicin (89.30%), Ceftriaxone (80.43%) and Ofloxacin (84.96%).the results were consistent with the findings of Liebowitz and coworkers [24].

The bacteria isolated were found to be β-lactamase producer. The incidence of bacterial resistance mediated by β –lactamases has been reported in several African countries including Nigeria and South Africa [25, 26]. The extended beta-lactamases (ESBLs) production was high in *Klebsiella pneumoniae* compared to other organisms; this is because *Klebsiella* organisms are often resistant to multiple antibiotics [27].

The present study revealed that the methanolic extract of *Garcinia kola* inhibits the growth of gram positive and the gram negative bacterial isolates from the sputum of patients with respiratory tract infections. The antibacterial potency of methanolic extract of bitter kola could be attributed to the high active compounds present in the extract. Saponins are considered a key ingredient in traditional Chinese medicine and are responsible for most of the observed biological effects [28]. Adeleye and Opiah [29] had previously demonstrated the antibacterial potency of methanolic extract of bitter kola against pathogens such as *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus spp and Proteus spp.* The plant has been shown to possess anti-inflammatory, antimicrobial, antioxidant, antiviral anti-diabetic and anti-hepatotoxic activities [30, 31]. The seeds have shown a broad spectrum of antibacterial activities [32].

Klebsiella pnemoniae being the most prevalence in this study is one of the top organisms causing infections in hospitalized patients. This organism can cause serious blood stream infections and other intrusive infections that could be fatal [33]. It is found in the normal flora of the mouth, skin and intestines. It can cause destructive changes to human lung if aspirated, specifically to the alveoli resulting in bloody sputum [34]. Carriage of *Klebsiella pneumoniae* is frequently associated with colonization of the upper respiratory tract or gastrointestinal (GI) tract, with the potential for GI tract amplification of antibiotic resistant strains of *K. pneumoniae* following antibiotic therapies [35]. *Klebsiella pneumoniae* opportunistically infects a variety of mucosal surfaces with the primary sites of infection including the urinary tract and the lower respiratory tract (LRT) [36]. *K. pneumoniae* could cause disease with mortality rates of up to 22.7% [37]. *K. pneumoniae* being the most prevalence organism in this study was higly susceptible to methanolic extract of bitter kola at a concentration as low as 12.5mg/ml. Mukhatr and Shuaibu, [38] reported that at 200mg/ml, *K. pneumoniae* was susceptible to methanolic extract of bitter kola.

Tatumella ptyseos human infections have been exceedingly rare worldwide. *Tatumella ptyseos* has been isolated from human respiratory clinical specimens (mainly sputum) or blood and it is likely an infrequent opportunistic pathogen. Clinical information on *T. ptyseos* infection has been very limited and isolates from sources other than sputum are uncommon [39]. Berka and co-researchers [40] reported that tracheobronchial/pulmonary infections (pneumonitis, asthmatic bronchitis, pharyngitis, Wegener granulomatosis, pneumonia, chronic lung disease and pulmonary oedema), infection associated with pulmonary tuberculosis and gastrointestinal infection (gastroenteritis) are agent associated with *Tatumella ptyseos.*

Lautropia mirabilis, a recently characterized motile gram-negative coccus, has been isolated from oral and pulmonary sites [41]. Its pathogenicity is unknown, as it has been recovered from both ill and healthy persons. Gerner- Smidt *et al.* [41](1994) reported that *L. mirabilis* has been isolated from the oral cavities of 32 of 60 (53.3%) children infected with human immunodeficiency virus (HIV) and3 of 25 (12.0%) HIV-uninfected controls; theassociation of *L. mirabilis* isolation with HIV infection is significant ($P < 0.001$). This association suggests a possible link between immunocompromise and oral colonization with *L. mirabilis*.

Aggregatibacter actinomycetemcomitans is a Gram-negative, facultative nonmotile, rod-shaped oral commensal often found in association with localized aggressive periodontitis, a severe infection of the

periodontium, although it is also associated with nonoral infections. It is one of the bacteria that might be implicated in destructive periodontal disease. Although it has been found more frequently in localized aggressive periodontitis [42], Prevalence in any population is rather high. It has also been isolated from actinomycotic lesions (mixed infection with certain *Actinomyces* species, in particular *A. israelii*). It possesses certain virulencefactors that enable it to invade tissues, such asleukotoxin. It has also been isolated from women withbacterial vaginosis [42]. *A. actinomycetemcomitans* serotypes, such as ATCC 29523, are frequently isolated in oral cavity.

Corynebacterium argentoratense is a cause of an acute and contagious infection characterized by pseudomembranes of dead epithelial cells, white blood cells, red blood cells and fibrin that form around the tonsils and back of the throat [43]. It is an uncommon illness that tends to occur in unvaccinated individuals, especially school-aged children, those in developing countries [44], the elderly, neutropenic or immunocompromised patients and those with prosthetic devices such as prosthetic heart valves, shunts, or catheters. This organism was the most prevalent among the gram positive bacteria in this study. This hasn't been reported in any study yet. According to Collins *et al.* [45], it can be spread within a hospital and effects of infection include granulomatous lymphadenopathy, pneumonitis, pharyngitis, skin infections and endocarditis.

Corynebacterium accolens is considered an inhabitant of upper respiratory tract. *C. accolens* from human clinical specimens (wound drainage, endocervix, sputum and throat swab specimens) collected over a 30-year period is a gram-positive bacillus and was originally characterized by its satellite growth around a *Staphylococcus aureus* streak on blood agar [34].

Vibrio fluvialis causes a variety of infections inimmunocompetent/HIV patients, including bacteremia, biliary tract infection and acute diarrhoea [46]. The other rarely reported infections caused by this pathogen include suppurative cholangitis, peritonitis, acute otitis and endophthalmitis. Large numbers of (29%) endophthalmitis patients were reported to have mixed infection with *V. fluvialis* [47]. A report from Cuba showed that *V. Fluvialis* was one of the predominantly identified pathogens from different extraintestinal samples clinical vibrios, antimicrobial resistance is largely reported in *V. fluvialis*. Majority of the *V. fluvialis* strains have been found to be resistant for β-lactams, azithromycin and sulfamethoxazole [48].

Burkholderia cepacia is an important pathogen of pulmonary infections in peoplewith cystic fibrosis (CF) [49]. Cystic fibrosis (CF) predisposes patients to bacterial colonization and infection of the lower airways. Several species belonging to the genus *Burkholderia* are potential CF-related pathogens, but microbiological identification may be complicated [50] (Burdge, 1995). The genetic defect causing CF predisposes patients to an aberrant pulmonary susceptibility to infectious disease. Several of the bacterial species encountering a suitable ecological niche in the lungs of CF patients are especially pathogenic for the host [50].

Pseudomonas aeruginosa is frequently isolated from non-sterile sites (mouth swabs, sputum, e.t.c) and under these circumstances, it often represents colonization and not infection. Resistance to antibiotics is common in *P. aeruginosa* infections. Phage therapy against *P. aeruginosa* has been investigated as a possible effective treatment, which can be combined with antibiotics, has no contraindications and minimal adverse effects [51]. The sceptible of *P. aeruginosa* to methanolic extract of bitter kola at low concentration has also been reported in the work of Farombi and co-workers [8].

Vibrio vulnificus is presently considered the most infectious and lethal of all human pathogenic *vibrios*. Septicemic infections by the organism usually result from the consumption of raw shell fish. Cirrhosis of the liver due to chronic alcoholism is considered a high risk factor for infection by this organism, presumably due to increased levels of serum iron released by damaged hepatocytes. The organism produces an unusually large number of extracellular virulence factors [39].

CONCLUSION

It is evident from this study that methanolic extract of bitter kola can serve as a suitable antimicrobial chemotherapeutic agent for the treatment of some respiratory tract infections especially *Klebsiella pneumoniae*. This could profound the necessary solution to the menance of multi-drug resistant bacteria in Nigeria.

It is therefore suggested that, bitter kola, which is widely chew worldwide irrespective of the age or sex should be made by pharmaceutical companies towards carrying out more research work especially in the development of new drugs containing more of pharmaceutical and biologically active compounds present in plant extracts.

Competing Interests: The authors declare that they have no competing interests.

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