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Original Article

Antibacterial Efficacy of *Garcinia kola* (Heckel) Seeds against Bacteria Involved in Throat Infections

Elizabeth Tomilayo Idowu*, Olubukola Olayemi Olusola-Makinde, Muftau Kolawole Oladunmoye

Department of Microbiology, the Federal University of Technology, P.M.B. 704, Akure, Ondo State, Nigeria

*Corresponding Author E-mail: idowutomilayo@yahoo.com

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ABSTRACT

Throat infections are common causes for visiting health care centres. This study is aimed at investigating the antibacterial efficacy of *Garcinia kola* (commonly called bitter kola) seeds against bacteria involved in throat infections. Twenty clinical and 4 typed bacterial isolates associated with throat infections were obtained. Extraction from *G. kola* seeds using ethanol, methanol, ethyl acetate, acetone, hot and cold water as extraction solvents was done. Phytochemical analyses of extracts, antibacterial efficacy, MIC (minimum inhibitory concentration) and MBC (minimum bactericidal concentration) of extracts on test isolates using ciprofloxacin as positive control and mechanisms of action of extract were carried out. Ethyl acetate extract was most effective against *Streptococcus pneumoniae* with 29±0.58 mm zone of inhibition while hot water extract was the least with 6.00±0.58 mm against *S. pyogenes*. Ethyl acetate extract again showed the lowest MIC and MBC at 6.25 mg/mL and 12.5 mg/mL against *S. aureus*, respectively. The mechanism of action of ethyl acetate extract is attributed to cell wall disruption with highest leakages of Na^+ and K^+ in *K. pneumoniae* obtained at 350 Cmol/kg and 82 Cmol/kg, respectively, while the highest leakage of proteins was obtained in *S. aureus* at 88.55±5.34 mg/mL. Time-kill kinetics of the extract also showed reduction in number of bacterial cells at 30 min intervals. These results prove that the ethyl acetate extract of *G. kola* seeds is of best efficacy against the tested bacteria involved in throat infections.

Key words: Bactericidal concentration, Inhibition, Phytochemicals, Solvent extraction

Introduction

Throat infections are prevalent in all seasons of the year and transmitted easily over a wide range of people. They have been reported as one of the commonest causes of visiting health care physicians (Anitha *et al.*, 2016) and are often associated with mild to severe pains, fever, headache, running or stuffy nose and fullness of the ear (Ahmad *et al.*, 2016). The major infections of the upper respiratory tract are pharyngitis, nasopharyngitis, tonsillitis, otitis media and sinusitis (Wang *et al.*, 2016). More than two hundred and twenty-five (225) pathogens are responsible for upper respiratory tract infections; the most common bacteria involved are *Streptococcus pyogenes*,

Klebsiella pneumoniae, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, *Citrobacter koseri* and *Acinetobacter baumanii* (Anitha *et al*., 2016). *Streptococcus pyogenes* is a Gram-positive, non-spore forming, facultative anaerobic bacteria that is capable of invasion through broken skin or mucous membrane. *S. pyogenes* or Lance-field group A beta-hemolytic Streptococcus (GAS) is the commonest bacterial pathogen that causes acute pharyngitis among school-aged children living in lower socio-economic conditions (WHO, 1981). Pharyngitis is a sore throat caused by inflammation of the throat. Throat may be scratchy and swallowing can be painful. Usually, a sore throat is the sign of another illness, such as a cold or the flu (Aamir *et al*., 2011). With increasing severity, there may be severe pain that increases with swallowing plus cervical lymphadenopathy with or without fever (Aamir *et al*., 2011). The conventional antibiotics that are used for the treatment of these infections are becoming costly, locally inaccessible, having certain side effects and are likely faced with bacterial resistance.

Antibiotics are secondary metabolites produced by a variety of microorganisms and are used as antimicrobial chemotherapeutic agents (Neu and Gootz, 2014). Unfortunately, using antibiotic has turned out to be insufficient in the fight against infection and disease development. The accumulation, persistence and inappropriate use of these antibiotics and the survival adaptation of microorganisms contribute to resistant microorganisms, turning the environment to a gigantic reservoir for antibiotic resistant genes (Huang *et al*., 2016). Antibiotic resistance is one of the major reasons for the exploration and utilization of antimicrobial potentials of medicinal plants and other natural sources. New resistance mechanisms are emerging and spreading globally, resulting in prolonged illnesses, disability and death (WHO, 2018). Also, without effective antimicrobials for prevention and treatment of infections, medical procedures such as organ transplantation and cancer chemotherapy become highly risky. Other major reasons for the exploration and utilization of antimicrobial potentials of medicinal plants and other natural sources include the high costs of conventional antibiotics, its non-availability especially in rural areas as well as the appearance of undesirable side effects of certain antibiotics (Odeyemi and Oluwajobi, 2011). These has necessitated the search for new organic molecules having antimicrobial activity, which in turn could be potential sources for starting materials for the semi-synthesis of new drugs (Akaochere *et al*., 2002).

The discovery and understanding of medicinal plants is still an on-going process, as the constituent chemicals with pharmacological potentials are being studied and used in the development of drugs (Avwoiro *et al*., 2014). *G. kola* (Heckel) belongs to the family Clusiaceae and is commonly known as bitter kola (English language). In Nigerian traditional languages, it is called Mijingoro (Hausa language), Agbilu (Igbo) and Orogbo (Yoruba language) (Tcheghebe *et al*., 2016). *G. kola* seed is known as a traditional remedy for patients with cough, laryngitis and liver diseases (Avwoiro *et al*., 2014). It has also been reported to contain phytochemical constituents such as saponins, tannins, flavonoids, proteins, glycosides, reducing sugars, starch, sterols and triteroenoids (Esimone *et al*., 2007). Extraction of phytochemicals from plant tissue is necessary to archive effective drug development, while the efficiency of the extraction depends on the extraction procedure. In solvent extraction, the nature, quality and quantity of phytochemicals extracted from a plant tissue depends on the temperature and polarity of the solvent used. Solvation generally increases with temperature, with limitations such as, phytochemical denaturisation and/or volatilisation above threshold temperatures, while solvent polarity may select phytochemicals (Altemimi *et al*., 2017). Solvent used in this study can be arranged from least to most polar as follows; ethyl acetate<acetone<methanol<ethanol<water. This research therefore aimed at assessing the

antibacterial efficacy of *G. kola* seeds extracts obtained using different solvent, against bacteria involved in throat infections.

Experimental

Collection of bacterial isolates

The clinical bacterial isolates involved in throat infections were collected from culture collection unit of Microbiology Department, Federal University of Technology, Akure. The typed isolates were collected from Federal Institute of Industrial Research, Oshodi (FIIRO), Lagos State, Nigeria. The isolates were confirmed in the laboratory by colonial, morphological and biochemical tests.

Preparation of *G. kola* seeds extract

This was carried out according to the method of (Seanago and Ndip, 2012). *G. kola* seeds were purchased commercially from the main markets in Akure. The seeds were washed, air-dried for 14 days and blended at low speed (in an electric blender) into powder. Extraction was done by separately soaking 100 g of the powder in different solvents for 48 h with intermittent agitation. Solvents included: ethanol, methanol, ethyl acetate, acetone and water, all at room temperature (27±2 °C), and then hot (70 °C) water (to evaluate the solubility and stability of the *G. kola* seeds bioactive components in hot and cold water). The extracts were sieved using clean muslin cloth and filtered using Whatman No. 1 filter paper. The recovery rate of extracts were calculated using the formula below:

$$\text{Percentage recovery of extract} = \frac{\text{Weight of extract recovered after extraction}}{\text{Initial weight of plant before extraction}} \times \frac{100}{1}$$

The extracts were concentrated to dryness using rotary evaporator (RE-52A, Union Laboratories, England) under reduced pressure at 37 °C. The extracts were preserved in air-tight containers at 4 °C.

Phytochemical screening of *G. kola* seed extracts

The plant extracts were evaluated for the presence of tannin, steroids, phenols, saponins, alkaloids etc using the methods described by (Odutayo *et al.*, 2017), briefly as follows;

Alkaloids: extracts (0.5 g) were stirred in 5 mL of 1% aqueous hydrochloric acid (HCl) on a steam water bath, 1 mL of the filtrate was treated with a few drops of Dragendorf reagent. The presence of a blue-black turbidity was taken as preliminary evidence for the presence of alkaloids.

Saponins: extracts (0.5 g) were shaken with distilled water in a test tube. Frothing which persist on warming was taken as preliminary evidence for the presence of saponins.

Tannin: extracts (0.5 g) were stirred with 100 mL of distilled water, filtered and ferric chloride reagent was added to the filtrate. Formation of blue black green or blue green precipitate indicates the presence of Taninin.

Phlobatannins: the extracts were boiled with 1% aqueous HCl. Formation of red precipitate indicates phlobatannins.

Anthraquinones: According to Borntrager's test, *G. kola* seeds extracts (0.5 g) were shaken with 10 mL of benzene, filtered and 5 mL of 10% ammonia solution was added to the filtrate. The mixtures were shaken and the presences of pink red or violet colour in the ammonia layer indicated the presence of free anthraquinones.

Flavonoids: The extracts (0.5 g) were stirred with 20 mL of dilute ammonia solution and a yellow colouration was observed. The disappearance of the yellow colour after the addition of 1 mL conc. H_2SO_4 indicated the presence of flavonoids.
Steroids: Acetic anhydride (20 mL) was added to 0.5 g of the extracts and filtered. Concentrated H_2SO_4 (2 mL) was added to the filtrates. A colour change from violet to blue or green indicates the presence of steroids.
Terpenoids: The extracts (0.5 g) were mixed with 20 mL of chloroform and filtered. Concentrated H_2SO_4 (3 mL) was added to the filtrates to form a layer. A reddish brown colour at the interface indicates the presence of terpeniods.
Cardiac Glycosides were determined using 4 separate methods. Legal's test: The extracts (0.5 g) were dissolved in pyridine and a few drops of 2% sodium nitroprusside with few drops of 20% NaOH were added. A deep red colouration which faded to a brownish yellow indicates the presence of cardenolides. Lieberman's test: acetic anhydride (20 mL) was added to 0.5 g of extracts and filtered, then, 2 mL of conc. H_2SO_4 was added to the filtrate. Colour change from violet to blue or green indicates the presence of steroids nucleous [*i.e.* aglycone portion of the cardiac glycosides]. Salkowski's test: The extracts (0.5 g) were mixed with 20 ml of chloroform and filtered. Concentrated H_2SO_4 (3 mL) was added to the filtrates to form another phase layer. A reddish brown colour at the interface indicates the presence of steroidal ring. Keller- killiani's test: The extracts (0.5 g) were dissolved in 2 mL of glacial acetic acid containing one drop of ferric chloride solution. Another phase layer was formed when 1 mL of concentrated H_2SO_4 was added. Brown colour at the interface indicates the presence of deoxy sugar.

Standardization of bacterial isolates

The standardization of bacterial isolates was carried out by diluting a 6 hours old broth cultures of the bacterial isolates in test tubes and comparing with a 0.5 McFarland Standard to adjust their suspension to a density equivalent to approximately 10^8 CFU/mL (Cheesbrough, 2006).

Antibacterial susceptibility testing of extracts against clinical and typed isolates of bacteria involved in throat infections

The antibacterial susceptibility testing was carried out using agar well diffusion method as described by (Okonkwo *et al.*, 2017). The standardized bacterial isolates were aseptically inoculated on the surface of sterile Mueller hinton agar (MHA) plates with the aid of a sterile swab stick by spread method. Four wells of 6 mm in diameter were punctured in the culture medium with sterile cork borer. Half a millilitre of the extracts was used to fill the wells, with ciprofloxacin [10 µg] as positive control and water as the negative control. The plates were allowed to stand on the laboratory bench before incubation at 37 °C for 24 hours. Production of zones of inhibition were observed and measured with meter rule and recorded appropriately.

Determination of minimum inhibitory concentration (MIC) of extracts against clinical and typed isolates of bacteria involved in throat infections

The minimum inhibitory concentration of the extracts from *G. kola* seeds was determined using the method adopted by (Bosso and Innalegwu, 2018). The MIC was obtained using the double fold dilution. One millilitre of the extracts reconstituted with 30% dimethyl sulphoxide (DMSO) at a concentration of 100 mg/mL was diluted serially to give different concentrations of 50 mg/mL, 25 mg/mL, 12.5 mg/mL, 6.25 mg/mL and 3.125 mg/mL in test tubes. One millilitre of 18 hour culture of the standardized bacterial isolates was added to each of the test tubes and mixed thoroughly. The

tubes were then incubated at 37 °C for about 18 hours. Another tube containing 30% DMSO with no extract was used as negative control while another tube containing ciprofloxacin was used as the positive control. The lowest concentration of the extracts that showed no visible turbidity of growth was recorded as the MIC.

Determination of minimum bactericidal concentration (MBC) of extracts against clinical and typed isolates of bacteria involved in throat infections

The MBC of the extracts from *G. kola* seeds were determined according to the method adopted by (Bosso and Innalegwu, 2018). The test tubes from the MIC test above that is without visible growth were aseptically inoculated on different sterile MHA plates and incubated for 24 hours at 37 °C. The MBC was taken as the lowest concentration of extracts that produced no visible growth of the bacterial isolates on the plate.

Determination of rate of killing of extracts

The rate of killing of ethyl acetate extract of *G. kola* seeds on bacterial isolates was carried out using pour plate technique described by (Akinyemi and Odundare, 2014). Five millilitres of the standardized test isolates were added to 5 mL of 50 mg/mL of the extract. One millilitre of the mixture was poured into empty sterile petri dish and sterile Mueller Hinton agar was poured into the plate and swirled gently to mix properly. The plates were incubated immediately at 37 °C for 24 hours. The same procedure was repeated for all the test isolates at every 30 minutes intervals till the third hour. The colonies in the plates after incubation were counted using a colony counter and recorded.

Determination of mechanisms of action of extract

Protein leakage: Two millilitres of 50 mg/mL concentration of ethyl acetate extract of *G. kola* seeds were added to 2 mL of a standardized test organism and incubated for 18 hours. The suspension was centrifuged at 5000 rpm for 15 minutes and the supernatant was collected. From the supernatant collected, 0.2 mL was pipetted into clean test tubes. The modified Lowry method as described by Hartree (1972), was used for the protein determination. One millilitre of potassium sodium tartrate and sodium carbonate was added and incubated for 10 minutes at 50 °C. The mixtures were cooled to room temperature and 1 mL of $KNaC_4H_4O_6.4H_2O$ and copper sulphate pentahydrate and left for another 10 minutes. Finally, 3 mL of Folin-Ciocalteu phenol reagent in water was added after incubation for 10 minutes at 50 °C. The mixtures were allowed to stand at room temperature for 1 hour and the absorbance was read at 700 nm against the blank. Three hundred (300) micrograms of BSA were used as standard protein.

Sodium and potassium ions leakage from cells of bacterial isolates by ethyl acetate extract of *G. kola* seeds were determined according to the method described by (Akinyemi and Ogundare, 2014). Two millilitres of 50 mg/mL were added to 2 mL of a standardized test isolates and incubated for 18 hours. The suspension was centrifuged at 5000 rpm for 15 minutes and the supernatant was collected. The supernatant was diluted by adding 0.1 mL into 10 mL sterile distilled water. Sodium and potassium ions leakage was obtained using a flame photometer at 589 nm and 766 nm respectively.

Statistical analysis

Statistical analysis was carried out using SPSS version 20. The one-way ANOVA test was used to determine the statistical significance in the zones of inhibition of the extracts. $P<0.05$ was considered significant.

Results

The bacterial isolates obtained are Gram-positive bacteria namely; *Streptococcus pneumoniae* (n=5), *Streptococcus pyogenes* (n=5), *Staphylococcus aureus* (n=5), *Streptococcus pyogenes* ATCC12384, *Staphylococcus aureus* NCTC6571, and Gram-negative bacteria namely, *Klebsiella pneumoniae* (n=2), *Haemophilus influenzae* (n=2), *Escherichia coli* (n=1), *Klebsiella pneumoniae* ATCC13888, *Escherichia coli* ATCC25922.

Table 1 shows the percentage recovery of extracts from *Garcinia kola* seeds. The highest yield of 12.2% was obtained with acetone while the least yield of 3.4% was obtained with methanol as extraction solvent.

Table 1. *Garcinia kola* seed extract yield

Solvent	Original Weight/input [g]	Extracted Weight/output [g]	Percentage recovery [%]
Ethanol	100	12	12
Methanol	100	3.4	3.4
Ethyl acetate	100	9.3	9.3
Acetone	100	12.2	12.2
Hot water	100	5.3	5.3
Cold water	100	8.1	8.1

Table 2 shows the phytochemical constituents present in extracts from *G. kola* seeds. Tannins, phenol, terpenoids and cardiac glycosides were present in all the six extracts from *G. kola* seeds. The phytochemical constituents absent in all the six extracts were phlobatannins and steroids. Alkaloids and saponins were present in only in hot and cold water extracts of *G. kola* seeds.

Table 2. Phytochemical constituents of extracts

Phytochemical constituent	Ethanol extract	Methanol extract	Ethyl acetate extract	Acetone extract	Hot water extract	Cold water extract
Saponin	+	-	-	-	+	+
Tannin	+	+	+	+	+	+
Phlobatannin	-	-	-	-	-	-
Flavonoid	+	-	+	+	+	+
Steroid	-	-	-	-	-	-
Terpenoid	+	+	+	+	+	+
Alkaloid	-	-	-	-	+	+
Phenol	+	+	+	+	+	+
			Cardiac Glycosides			
Keller kiliani	+	+	+	+	+	+
Salkwoski	-	-	-	-	-	-
Lieberman	+	+	+	+	+	+

Key: +: present, -: absent

Table 3 shows the antibacterial activity of *G. kola* seeds extracts against twenty clinical isolates involved in throat infections. The highest zone of inhibition of 29.00 $\pm$ 0.58 mm was observed with

ethyl acetate extract of *G. kola* seeds against one of the *Streptococcus pneumoniae* while *Streptococcus pyogenes* was resistant to cold and hot water extracts with zones of inhibition of 0.00 ± 0.00 mm. All the bacterial isolates used were susceptible to ciprofloxacin, a broad-spectrum antibiotic. According to the results of the statistical analysis obtained, there were significant differences in the zones of inhibition produced by the extracts from *G. kola* seeds against the bacteria involved in throat infections that were used in this study.

Table 3. Antibacterial activity of *G. kola* seeds extracts against clinical isolates from throat infections

Isolates	Ethanol [mm]	Ethyl Acetate [mm]	Methanol [mm]	Acetone [mm]	Cold water [mm]	Hot water [mm]	Ciprofloxacin [mm]
A1	28.00±0.58^{a}	29.00±0.58^{a}	14.67±0.33^{a}	25.00±0.00^{a}	21.00±0.00^{a}	15.33±0.33^{c}	15.67±0.33^{gh}
A2	22.67±0.33^{c}	27.00±0.58^{b}	12.67±0.67^{bcd}	20.00±0.00^{de}	16.33±0.33^{b}	20.67±0.33^{a}	11.67±0.33^{j}
A3	24.33±0.67^{b}	26.67±0.33^{b}	14.67±0.33^{a}	22.33±0.33^{b}	14.67±0.33^{c}	20.00±0.58^{ab}	12.00±0.58^{j}
A4	21.33±0.33^{d}	21.67±0.33^{d}	11.67±0.33^{defg}	18.00±0.58^{f}	15.00±0.58^{c}	19.33±0.33^{b}	11.33±0.88^{j}
A5	19.33±0.33^{e}	24.67±0.33^{c}	12.00±0.00^{cdef}	19.00±0.00^{ef}	0.00±0.00^{i}	0.00±0.00^{j}	20.67±0.33^{bc}
B1	12.33±0.33^{ghi}	18.33±0.33^{e}	14.67±0.33^{a}	18.33±0.33^{f}	9.00±0.58^{fg}	6.00±0.58^{i}	18.00±0.58^{de}
B2	11.67±0.33^{hij}	17.00±0.58^{ef}	11.67±0.33^{defg}	20.33±0.33^{d}	6.33±0.67^{h}	10.00±0.58^{ef}	18.67±0.33^{d}
B3	10.67±0.67^{jk}	14.33±0.33^{hij}	10.33±0.33$^{g\ defg}$	21.67±0.3^{bc}	0.00±0.00^{i}	9.00±0.58^{fg}	19.00±0.58^{d}
B4	12.33±0.33^{ghi}	17.33±0.33^{ef}	13.33±0.88^{abc}	19.00±0.58^{ef}	8.00±0.58^{g}	11.00±0.58^{e}	15.67±0.33^{gh}
B5	11.00±0.58^{ijk}	17.00±0.58^{ef}	14.00±0.58^{ab}	20.67±0.33^{cd}	8.33±0.33^{g}	6.00±0.58^{i}	21.00±0.58^{b}
C1	13.67±0.33^{fg}	11.67±0.33^{k}	11.00±0.58^{efgh}	16.67±0.33^{g}	12.67±0.33^{d}	0.00 ±0.00	16.00±0.58^{gh}
C2	14.67±0.33^{f}	13.67±0.67^{ij}	12.33±0.67^{cde}	16.00±0.58^{g}	9.67±0.33^{f}	8.67±0.33^{gh}	19.33±0.33^{cd}
C3	13.00±0.58^{gh}	11.67±0.33^{k}	11.67±0.33^{defg}	14.00±0.58^{h}	12.00±0.58^{de}	10.33±0.33	18.33±0.33^{de}
C4	10.33±0.33^{jk}	9.67±0.33^{l}	11.00±0.58^{efgh}	12.33±0.33^{ij}	11.00±0.00^{e}	9.00±0.58^{fg}	16.67±0.33^{fg}
C5	12.33±0.33^{ghi}	9.33±0.33^{l}	9.00±0.58^{j}	11.33±0.33^{j}	9.67±0.33^{f}	7.67±0.33^{h}	18.00±0.58^{de}
D1	11.00±0.58^{ijk}	13.00±0.58^{j}	10.67±0.33^{fghi}	12.67±0.33^{i}	12.00±0.58^{de}	10.33±0.33^{ef}	14.67±0.33^{hi}
D2	10.00±0.58^{k}	11.00±0.58^{k}	9.67±0.33^{hij}	14.67±0.33^{h}	12.00±0.00^{de}	13.67±0.33^{d}	23.00±0.00^{a}
E1	14.67±0.33^{f}	16.00±0.00^{fg}	9.00±0.33^{j}	14.00±0.58^{h}	11.67±0.33^{de}	10.67±0.33^{e}	16.00±0.58^{gh}
E2	13.00±0.33^{gh}	14.67±0.33^{hi}	9.67±0.33^{hij}	11.67±0.3^{ij}	11.67±0.33^{de}	11.33±0.33^{e}	14.00±0.58^{i}
F	13.33±0.33^{fg}	15.33±0.33^{gh}	9.33±0.3^{ij}	11.33±0.33^{j}	12.33±0.33^{d}	10.33±0.33^{ef}	17.00±0.58^{efg}

Key: A1 – 5 = Isolates of *Streptococcus pneumoniae*, B1 - 5 = Isolates of *Streptococcus pyogenes*, C1 - 5 = Isolates of *Staphylococcus aureus*, D1 - 2 = Isolates of *Klebsiella pneumoniae*, E1 - 2 = Isolates *Heamophilus influenza*, F: Isolate of *Escherichia coli*. Values are presented as mean±SE of duplicates, values in the same column carrying same superscript are not different significantly ($p<0.05$) according to new Duncan's Multiple Range test

Table 4 shows the antibacterial activity of *G. kola* seeds extracts against typed isolates of bacteria involved in throat infections. Statistical analysis revealed no significant difference in the effect of acetone extract of *G. kola* seeds against the test bacterial isolates. Ethyl acetate extract of *G. kola* seeds was highly effective against the four test isolates. *K. pneumoniae* was resistant to the extracts except ethanol, ethyl acetate and acetone extracts of *G. kola* seeds.

Table 4. Antibacterial activity of *G. kola* seeds extracts against typed isolates of bacteria involved in throat infections

Isolates	Ethanol [mm]	Methanol [mm]	Ethyl Acetate [mm]	Acetone [mm]	Cold water [mm]	Hot water [mm]
S. pyogenes ATCC 12384	13.00±0.58^{c}	17.33±0.33^{a}	20.67±0.33^{b}	13.00±0.58^{a}	10.00±0.58^{b}	11.33±0.33^{b}
S. aureus NCTC 6571	22.67±0.33^{a}	15.00±0.58^{b}	24.67±0.33^{a}	14.00±0.58^{a}	14.33±0.33^{a}	13.00±0.58^{a}
K. pneumoniae ATCC 13888	12.33±0.33^{c}	0.00±0.00^{d}	16.00±0.58^{d}	13.33±0.33^{a}	0.00±0.00^{c}	0.00±0.00^{c}
E. coli ATCC 25922	14.33±0.33^{b}	10.00±0.58^{c}	18.00±0.58^{c}	13.33±0.33^{a}	11.00±0.58^{b}	10.00±0.58^{b}

Values are presented as mean±SE of duplicates, values in the same column carrying same superscript are not different significantly [$p<0.05$] according to new Duncan's Multiple Range test

Table 5 shows minimum inhibitory concentration (mg/mL) of extracts from *G. kola* seeds on clinical and typed isolates involved in throat infections. The lowest MIC of 6.25 mg/mL obtained was found in ethyl acetate extract against both clinical and typed isolates of *S. aureus* while the highest MIC of 100 mg/mL was found in cold water extract of *G. kola* seeds against clinical *E. coli*. The MIC of cold water, hot water and methanol extracts of *G. kola* seeds were not found because the bacteria were resistant to the initial 100 mg/mLconcentration used in this study.

Table 5. Comparative minimum inhibitory concentration [mg/ml] of extracts from *G. kola* seeds on clinical and typed isolates involved in throat infections

Isolates	Ethanol	Ethyl acetate	Acetone	Methanol	Cold water	Hot water
S. pyogenes ATCC 12384	12.5	12.5	25	25	50	25
S. pyogenes	12.5	25	25	25	50	50
S. aureus NCTC 6571	25	6.25	25	50	25	25
S. aureus	12.5	6.25	25	50	25	50
E. coli ATCC 25922	25	12.5	25	50	25	50
E. coli	25	12.5	50	25	100	50
K. pneumonia ATCC 13888	25	12.5	25	NF	NF	NF
K. pneumonia	25	25	12.5	25	NF	NF

Key: NF: Bacteria were not susceptible to extracts from *G. kola* seeds at 100 mg/mL

Table 6 shows the minimum bactericidal concentration (MBC) mg/mL of extracts from *G. kola* seeds on clinical and typed isolates involved in throat infections. The MBC of cold

water, hot water and methanol extracts of *G. kola* seeds was not found because the bacteria were resistant to the initial 100 mg/mL concentration used in this study. The lowest MBC of 12.5 mg/mL was obtained in ethyl acetate extract against clinical and typed *S. aureus* and in ethanol extract against clinical *S. aureus*.

Table 6. Comparative minimum bactericidal concentration (MBC) mg/mL of extracts from *G. kola* seeds on clinical and typed isolates involved in throat infections

Isolates	Ethanol	Methanol	Ethyl acetate	Acetone	Cold water	Hot water
S. pyogenes ATCC 12384	12.5	25	25	25	100	50
S. pyogenes	25	50	25	50	100	50
S. aureus NCTC 6571	25	50	12.5	50	50	25
S. aureus	12.5	50	12.5	25	25	50
E. coli ATCC 25922	50	100	25	50	50	100
E. coli	50	25	25	100	100	100
K. pneumonia ATCC 13888	50	NF	50	50	NF	NF
K. pneumonia	50	50	50	25	NF	NF

Key: NF: Bacteria were not susceptible to extracts from G. kola seeds at 100 mg/mL

Figure 1 shows the rate of killing of bacterial isolates by 100 mg/mL ethyl acetate extract of *G. kola* seeds. There was a significant reduction in the number of bacterial cells at every 30 minutes of inoculation of the test bacteria mixed with ethyl acetate extract of *G. kola* seeds.

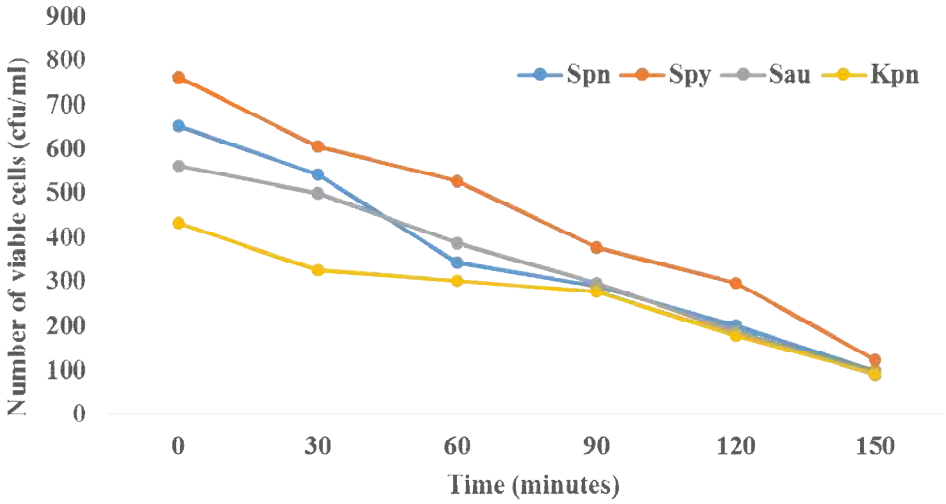

Figure 1. Rate of Killing of Bacterial Isolates by 100 mg/mL Ethyl Acetate Extract of *G. kola* Seeds

Key: Spn: *S. pneumoniae*, Spy: *S. pyogenes*, Sau: *S. aureus* and Kpn: *K. pneumoniae*

Table 7 shows the amount of protein leakage from bacterial isolates by ethyl acetate extract of *G. kola* seeds. Statistical analysis revealed that there is no significant difference in the amount of protein leakage from *S. pneumoniae* and *S. pyogenes*. There is no significant

difference in the amount of protein leakage from *S.aureus* and *K. pneumoniae*. The highest mean protein leakage of 88.55 ± 5.34 mg/mL was found in *S. aureus*.

Table 7. Protein leakage from bacterial isolates by ethyl acetate extract of *G. kola* Seeds

Isolates	Protein concentration (mg/ml)
Streptococcus pneumoniae	66.31±0.48^{b}
Streptococcus pyogenes	61.39±0.90^{b}
Staphylococcus aureus	88.55±5.34^{a}
Klebsiella pneumoniae	84.35±2.10^{a}
Control	2.98±0.65^{c}

Values are presented as mean±SE of duplicates, values in the same column carrying same superscript are not different significantly ($p<0.05$) according to new Duncan's Multiple Range test

Figure 2 shows the amount of leakage of sodium (Na^+) and potassium ions (K^+) from bacterial isolates by ethyl acetate extract of *G. kola* seed. There was a higher amount of leakage of sodium ions than potassium ions in all the bacterial isolates. The highest leakages of both Na^+ and K^+ were observed in *K. pneumoniae*.

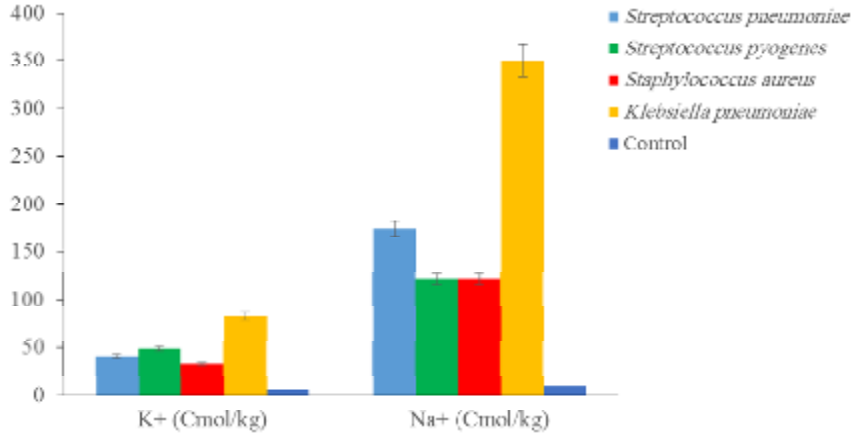

Figure 2. Leakage of sodium (Na^+) and potassium ions (K^+) from bacterial isolates by ethyl acetate extract of *G. kola* seeds

Discussion

The human ear, nose and throat (ENT) are closely related parts of the body. Therefore, infections related to the ENT are jointly studied and managed (Ahmad *et al.*, 2016). Throat infections are prevalent in all seasons of the year and transmitted easily over a wide range of people, both young and old. They can lead to the development of more complicated health conditions if not properly managed (Anitha *et al.*, 2016).

The use of bacteria only in this study is because of the reports of (Anitha *et al.*, 2016; Ahmad *et al.*, 2016) stating that pathogenic microorganisms isolated from the throat samples of infected patients are mostly bacteria. According to (Moirangthem and Gurung, 2013), bacteria such as *S. aureus*, *S. pyogenes*, *Pseudomonas aeruginosa* and *Proteus* spp were responsible for throat infections in a Referral Hospital in Sikkim, India.

The different percentage recovery observed with the ethanol, methanol, ethyl acetate, acetone, hot water and cold water shows the difference in the polarity of the solvents which in turn affects the solubility of the constituents of *G. kola* seeds in the solvents (Ibrahim *et al*., 2017).

The phytochemical constituents present in all the six extracts of *Garcinia kola* seeds as obtained in this study includes tannins, saponins, terpenoids, phenols and alkaloids. Similar results were obtained by (Okwulehie *et al*., 2017) who reported the presence of tannin, phenols, saponin, flavonoid, alkaloid in *G. kola* seeds collected from Ubani market in Umuahia, Anambra state, Nigeria.

The aqueous extracts of *G. kola* seeds showed the least antibacterial activity compared to the four other tested extracts. This could mean that the active ingredients in the plants are not so soluble in water as in ethanol, methanol, ethyl acetate and acetone (Muna *et al*., 2011).

At 100 mg/mL, ethyl acetate extract of *G. kola* seeds was most effective against *S. pneumoniae* with 29±0.58 mm zone of inhibition while hot water extract was least effective with 6±0.33 mm against *S. pyogenes*. This is contrary to the findings of (Seanago and Ndip, 2012), where methanol extract of *G. kola* seeds obtained from a local market in Cameroon, produced higher antibacterial activity at 100 mg/ml against the test bacterial isolates than acetone, ethyl acetate, ethanol and aqueous extracts. This may be due to the longer extraction time (48 hours) that was used in this study, which has enhanced the extraction potential of ethyl acetate over methanol or the different locations where *G. kola* seeds were obtained in this study and that of (Seanago and Ndip, 2012).

Gram-positive bacteria were more susceptible to the *G. kola* seed extracts than the Gram-negative bacteria which agrees with report of (Seanago and Ndip, 2012). This has been explained by the difference in cell wall composition of Gram-positive bacteria with that of Gram-negative bacteria, being complex (Vaghasiya *et al*., 2011).

According to (Bosso and Innalegwu, 2018), the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of a plant extract is an indication of the activity of the extract. The lower the MIC and MBC value, the higher the potency of the plant. It can be inferred that ethyl acetate extract of *G. kola* seeds having the lowest MIC and MBC in this study could be the most potent antibacterial agent against the tested bacterial isolates.

The MBC values were higher than the MIC values in this work. This suggests that the extracts were bacteriostatic at lower concentration and bactericidal at higher concentrations (Seanago and Ndip, 2012).

At 30 minutes interval, there was a reduction in the number of viable bacterial cells using ethyl acetate extract of *G. kola* seeds. This is in agreement with the report of (Ogundare and Akinyemi, 2011). Therefore, the longer the exposure of the bacterial cells to *G. kola* seeds extracts, the higher the efficacy of the extract.

The highest leakage of proteins was observed in *S. aureus* at 88.55±5.34 mg/mL concentration. The protein leakage from the cells of the bacterial isolates is an indication of the bacterial cell rupture by the extracts (Tang *et al*., 2016).

The leakages of sodium ions [Na^+] and potassium ions [K^+] from the cells of the test isolates by ethyl acetate extract of *G. kola* seeds means that the extract may have induced antibacterial effects through the leakage of intracellular constituents as a result of damage to the cell membrane of the test bacteria. Similar to the work of (Akinyemi and Ogundare,

2014), potassium ions did not leak as much as sodium ions from the cells of the test organisms. The highest leakages of sodium ions may be caused by the molecular mass of the ions, which might have resulted from the high proportion of sodium to escape the cells than the potassium ions (Ryan and Ray, 2004). Sodium and potassium ions have been known to affect osmotic balances in the cell and their leakages might cause cell lyses and death.

Conclusions

The phytochemical constituents in *G. kola* seeds extract included saponins, tannins, flavonoids, terpenoids, alkaloids, phenols, and cardiac glycosides. However, no single solvent extract showed the presence of all bioactive compounds. Typed isolates of bacteria involved in throat infections are susceptible to extracts of *G. kola* seeds than the clinical isolates. Ethyl acetate is deemed as the better solvent for the extraction of bioactive compounds present in *G. kola* seeds compared to the other five solvents used in this study as it records the highest antibacterial activity against bacteria involved in throat infections. Mechanism of action and time-kill kinetics of the ethyl acetate extract reveals its potentials as an antibacterial agent in the treatment of throat infections caused by such bacteria as: *S. pneumoniae*, *S. pyogenes*, *S. aureus*, *K. pneumoniae*.

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