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Full Length Research Paper

Inhibitory effects of Neem (*Azadirachta indica* Linn.) and Bitter Kola (*Garcinia kola* Heckel) leaves on selected pathogenic bacteria

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Concoctions of *Azadirachta indica Linn* (Neem) and *Garcinia kola* Heckel (Bitter kola) are commonly used for medicinal purposes in most Nigerian rural communities, because they are believed to possess some healing properties. The antibacterial activity of both neem and bitter kola was evaluated by agar well diffusion method against *Escherichia coli, Pseudomonas aeruginosa*, *Klebsiella pneumoniae, Staphylococcus aureus* and *Streptococcus pneumoniae*. Both neem and bitter kola leaf extracts in varying concentrations showed moderate antibacterial activity against the clinical pathogens. Cold water and hot water extracts showed better activity as compared to the methanol and ethanol extracts. Gram positive bacteria (*S. aureus* and *S. pneumoniae*) were more susceptible to the plant extracts than the Gram negative pathogens (*K. pneumoniae* and *P. aeruginosa*). The MICs of the plant extracts were determined at varying effectiveness of 25 and 12.5 mg/ml. The results obtained in this study give credence to the traditional use of neem and bitter kola in the treatment of infectious diseases in Nigerian rural communities.

Key words: *Azadirachta indica* Linn., *Garcinia kola* Heckel, clinical pathogens, resistance, Nigeria.

INTRODUCTION

The growing prevalence of antibiotic resistance amongst clinically important pathogens necessitates the search for potential healing powers in herbal plants, as a way of containing the menace. The use of herbs with healing powers for the treatment of infectious diseases is as old as mankind, and this form of therapy is practiced in most cultures especially in the rural areas (Izzo et al., 2009; Iwu et al., 1999). Majority of these herbal plants contain substances which are precursors for the synthesis of conventional drugs, or substances that can be used for therapeutic purposes. According to the World health Organization (WHO), about 80% of individuals from developing countries meet their primary health care needs through the use of traditional medicine that incorporates one herb to another as the main therapeutic agent (WHO, 2001). Nigeria and the rest of African continent are endowed with plethora of plants with putative medicinal properties which are yet to be tapped for the development of novel antimicrobial agents. Plant parts (including leafs, stem, bark and seeds) contain a variety of phytochemical compounds that gives impetus to their extensive usage in all healing traditions; and these plants are either used alone or in combination as concoctions with other plants of similar effect (Srivastava and Shukla 2000; Sibanda and Okoh, 2008). Plants with well documented antimicrobial effect include *Azadirachta indica*

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Linn (Neem) and *Garcinia kola* Heckel (Thakurta et al., 2007; Sibanda and Okoh, 2008; Srivastava and Shukla, 2000). Though advances in medicinal chemistry and pharmaceutical sciences have helped in producing many antimicrobial agents with little or no origin from plants, plant-derived substances still holds promise to revolutionize medicine, and many herbal plants have recently become of great interest owing to their versatile applications in the treatment of a variety of infectious diseases in most parts of the world (Baris et al., 2006; Barnes et al., 2007). The high cost of conventional drugs, drug inaccessibility in the rural areas and the mounting resistance of pathogens to available drugs has further justified the need for the use of plants for therapeutic purposes in many remote areas. Plants (including *A. indica* Linn and *G. kola* Heckel) have contributed immensely in meeting the primary health care needs of people in the developing countries (Ayogu and Amadi, 2009; Esimone et al., 2010; Saseed and Khan, 2008). It is because of this that this study was designed to evaluate and update on the antibacterial efficacy of *A. indica* (Neem), and *G. kola* against selected pathogenic microorganisms.

MATERIALS AND METHODS

Clinical isolates

The test microorganisms used in the present study included *Staphylococcus aureus*, *Klebsiella pneumoniae, Pseudomonas aeruginosa, Escherichia coli*, and *Streptococcus pneumoniae.* These were obtained from the culture collection unit of Microbiology Laboratory Department of Federal Teaching Hospital, Abakaliki (FETHA), Ebonyi State, Nigeria. All test isolates were subcultured, purified and biochemically identified based on standard microbiological techniques (Cheesbrough, 2006).

Collection and identification of plant

Leaves of *A. indica* Linn and *G. kola* Heckel were collected from Abakaliki Metropolis, and they were identified by Prof. A. Okafor of the Applied Biology Department, Faculty of Biological Sciences, Ebonyi State University, Abakaliki, Nigeria.

Preparation of plants extract

The plants were washed with water, shade-dried, grounded to powdery form and soaked in 250 ml flask of cold water, hot water, ethanol and methanol, respectively. Fifty grams of each ground plant material was consecutively soaked in 250 ml of water (cold and hot), ethanol and methanol for 24 h in a conical flask and allowed to stand on shaker for extraction (Ayogu and Amadi, 2009).

Screening for antimicrobial activity

Twenty milliliters each of molten agar (Muller Hinton) was poured aseptically into sterile Petri dishes and then allowed to gel. The surface of the agar plates were then streaked with standardized inoculum of the test bacteria that was adjusted to 0.5 McFarland turbidity standards. Thereafter, a sterilized 6 mm cork borer was used to bore 5 holes on the agar plate(s), and 4 of the holes were filled with equal volumes of the respective plant extracts that was diluted with 0.5% dimethyl sulfoxide (Esimone et al., 2010). Dimethyl sulfoxide was also tested on test organisms at different concentration to ensure they did not show antibacterial activity. The plates were allowed to stand for about 30 min for pre-diffusion of the plant extracts, and these were incubated at 37°C for 24 h. The inhibition zone diameters were determined after incubation (Onyeagba et al., 2004; Esimone et al., 2008).

Minimum inhibitory concentrations (MIC)

MIC was evaluated for only ethanol and methanol extracts. Varying concentration of each extract (100, 50, 25, 12.5 and 6.25 mg/ml), were prepared. 0.1 ml of each concentration was added to each 5 ml of nutrient broth containing 0.5 ml of standardized test organism of bacterial cells. The tubes were incubated aerobically at 37°C for 24 h. A tube containing no antibiotics and no plant extract was used as a positive control. The tube with least concentration of extracts without growth after incubation was taken as the MIC (NCCLS, 1990).

RESULTS

The plant extracts of bitter kola and Neem plant produced varying levels of antimicrobial activity against the test organisms including *S. aureus*, *K. pneumoniae, P. aeruginosa, E. coli*, and *S. pneumoniae.* All the extracts of *G. kola* Heckel (Bitter kola) leaf showed antibacterial effect against the tested pathogens except for *K. pneumoniae*. The ethanol and methanol leaf extracts of bitter kola had no activity against the *P. aeruginosa* used in our study. The antibacterial activity of *A. indica* Linn leaf extracts was found to be better against tested clinical pathogens than the bitter kola leaf extracts. The test clinical pathogens were susceptible to all the extracts of Neem except for the ethanolic extract where no inhibitory activity was observed for *K. pneumoniae*. The test pathogens were successfully inhibited at MICs of 25 mg/ml and 12.5 mg/ml.

DISCUSSION

Discovering and harnessing the hidden potentials of plants with medicinal properties is the basis for unleashing novel antimicrobials for the fight against antimicrobial resistance. In the present study, the antibacterial activity of two medicinal plants (neem and bitter kola) was evaluated against clinical isolates of *E. coli, P. aeruginosa*, *K. pneumoniae*, *S. aureus* and *S. pneumoniae*. Table 1 shows the antibacterial properties of *G. kola* Heckel (Bitter kola) leaf extracts against pathogenic microorganisms. From this Table 1, it was observed that the cold water, hot water, ethanol and methanol leaf extracts of bitter kola were ineffective against the Gram-negative organism *K. pneumoniae*. However, *S. aureus, E. coli* and *S. pneumoniae* were susceptible to all extracts of *G. kola*. The largest zones of inhibition (18, 17 and 16 mm) were obtained with the cold and hot water

Table 1. Antibacterial properties of *Garcinia kola* Heckel leaf extracts (100 mg/ml) against different bacterial pathogens.

Organism	Inhibition zone diameter (mm)				Control (ampicillin, 1 mg/ml)
	Cold water	Hot water	Ethanol	Methanol	
Staphylococcus aureus	18	17	11	13	27
Klebsiella pneumoniae	NI	NI	NI	NI	23
Escherichia coli	18	16	09	11	28
Streptococcus pneumoniae	14	16	07	09	33
Pseudomonas aeruginosa	10	12	NI	NI	30

NI = No Inhibition.

Table 2. Antibacterial activity of *Azadirachta indica Linn* leaf extracts (100 mg/ml) against different bacterial pathogens.

Organism	Inhibition zone diameter (mm)				Control (ampicillin, 1 g/ml)
	Cold water	Hot water	Ethanol	Methanol	
Staphylococcus aureus	10	14	12	13	28
Klebsiella pneumoniae	09	10	NI	08	22
Escherichia coli	16	12	14	13	27
Streptococcus pneumoniae	17	16	11	14	32
Pseudomonas aeruginosa	14	16	08	07	29

NI = No Inhibition.

extracts of *G. kola* against *S. aureus, S. pneumoniae* and *E. coli*. *P. aeruginosa* was not susceptible to the ethanol and methanol extracts of bitter kola (Table 1). Bitter kola leaf and seed is used in most tradition as a masticatory agent, where they are chewed, and the plant is believed to provide some level of healing to dental and oral diseases (Esimone et al., 2007; Schwach-Abdellaoui et al., 2000; Sibanda and Okoh, 2008). The moderate antibacterial effect of bitter kola against clinical pathogens as obtained in our study (Table 1) is comparable to other studies conducted to elucidate the antimicrobial properties of *G. kola* leaf and seed (Ndukwe et al., 2004; Sibanda and Okoh, 2008; Esimone et al., 2007). Cold water and hot water extracts of bitter kola produced better inhibitory effect against the tested pathogenic microorganisms (*E. coli, P. aeruginosa*, *K. pneumoniae*, *S. aureus* and *S. pneumoniae*) as against the ethanol and methanol extracts, which are both believed to be better solvents for the extraction of the bioactive constituents of medicinal plants (Ogueke et al., 2006; Onuoha and Obi, 2000). The antibacterial effect of *A. indica* Linn (neem) against the tested pathogenic microorganisms is shown in Table 2. In this study, the hot water and cold water leaf extracts of neem showed reasonable inhibitory effect, and inhibited the growth of all the test clinical pathogens to a high degree. The susceptibility of the test pathogens to the cold water and hot water extracts of neem was also observed for the ethanol and methanol extracts except for *Klebsiella* species, where no inhibitory activity was recorded for ethanol extract. The test pathogens (*E. coli, P. aeruginosa*, *K. pneumoniae*, *S. aureus* and *S. pneumoniae*) used in our study have been implicated in a number of bacterial-related infections including tooth decay, urinary tract infections (UTIs), typhoid fever and stomach-related illnesses (Taiwo and H-X Lee, 1999; Ayogu and Amadi, 2009); and they are mostly treated in Nigerian rural communities with herbal plants believed to possess some form of healing powers (Neem and bitter kola inclusive). The antibacterial effects of the duo of Neem and bitter kola leaves as observed in our study further gives credence to the traditional use of neem and bitter kola plant parts for the treatment of some infectious diseases in this area. The level of antibacterial activity of the tested plant extracts (leaves of *A. indica* Linn and *G. kola* Heckel) against the clinical pathogens was however expected, owing to their continued use in most African rural traditions (Nigeria inclusive) to meet certain primary healthcare needs. Comparable antibacterial activity of leaf extracts of neem has been reported by Thakurta et al. (2007), Faiza et al. (2009), Taiwo and H-X Lee. (1999) and Saseed and Khan, (2008). The *in vitro* effectiveness of neem and bitter kola as depicted in our study has been attributed to some phytochemical constituents and bioactive compounds that these plants are known to possess, which justifies there usage for medicinal purposes. The MICs for the ethanol and methanol leaf extracts of neem and bitter kola are shown in Table 3. The ethanol extract of neem leaf inhibited the growth of the test clinical pathogens at a concentration of 100 and 50 mg/ml, 25 mg/ml, and 12.5 mg/ml except for *K. pneumoniae* where no inhibitory activity was recorded

Table 3. Minimum inhibitory concentration (MIC).

Extract	Conc. (mg/ml)	*E. coli*	*S. aureus*	*P. aeruginosa*	*S. pneumoniae*	*K. pneumoniae*
AIE	100	-	-	-	-	-
	50	+	+	+	+	-
	25	+	+	+	+	-
	12.5	+	+	+	+	-
	6.25	-	-	-	-	-
AIM	100	-	-	-	-	+
	50	-	-	-	-	+
	25	-	+	+	+	-
	12.5	-	+	+	+	-
	6.25	-	-	-	-	-
GKE	100	-	-	-	-	-
	50	-	-	-	-	-
	25	+	+	+	+	+
	12.5	+	+	+	+	+
	6.25	-	-	-	-	-
GKM	100	-	-	-	+	+
	50	-	-	-	+	+
	25	+	+	-	-	-
	12.5	+	+	-	-	-
	6.25	-	-	-	-	-

AIE: *Azadirachta indica Linn* ethanol, AIM: *A. indica* Linn methanol, GKE: *Garcinia kola* Heckel ethanol, GKM: *G. kola* Heckel methanol.

(Table 3). The MICs for the methanol extract were 25 mg/ml against *E. coli, S. aureus, P. aeruginosa*, *S. pneumoniae* and *K. pneumoniae*; and 50 mg/ml against *S. aureus, Pseudomonas* species, and *Streptococcus* species. The MICs for the ethanol extract of *G. kola* were 25 and 12.5 mg/ml against the tested clinical pathogens (*E. coli, P. aeruginosa, K. pneumoniae*, *S. aureus* and *S. pneumoniae*). Methanol extracts of bitter kola successfully inhibited the growth of the test pathogens except for the Gram negative organism *P. aeruginosa* where no inhibitory effect was recorded (Table 3). Further research into the molecular characterization of the bioactive components of neem and bitter kola and other medicinal plants used for traditional medicine is therefore advocated as a way of identifying potent and putative constituents that will serve as a foundation for the development of new drugs that can be used to fight the mounting cases of drug resistance in the health sector. Our study further gives impetus to the health benefits of neem and bitter kola in the treatment of some bacterial related diseases in Nigerian rural communities.

Conclusion

The results of our study indicated that leaf extracts of *A. indica* Linn. (Neem) and *G. kola* Heckel (Bitter kola) possess antibacterial activity, which give good reason for their continued usage for the treatment of some bacterial related infections in this region.

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