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COMPARATIVE ANALYSIS ON THE ANTIMICROBIAL ACTIVITY OF HERBAL EXTRACTS (*GARCINIA KOLA* AND *AZADIRACHTA INDICA*) AND CONVENTIONAL ANTIBIOTICS AGAINST BACTERIAL PATHOGENS ISOLATED FROM ORTHOPEDIC WOUND INFECTIONS

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ABSTRACT

This study was designed to evaluate and compare the efficacy of some selected antibiotics and herbal leaf extract of *Garcinia kola* (bitter kola) and *Azadirachta indica* (Neem) against bacteria pathogens isolated from patients with wound infections in National Orthopedic Hospital Enugu (NOHE). A total of ninety-six (96) wound swab samples were collected from patients in NOHE using sterile swab stick. The isolated bacteria were characterized using standard microbiology techniques; and the pathogens were subjected to antibiotic susceptibility testing using the disc diffusion method. Extraction of herbal plant leaf extract with ethanol, methanol, hot and cold water was carried using standard techniques. Susceptibility studies using ethanol, methanol, hot and cold water extract against isolated bacteria pathogens was determined at different extract concentration of stock, 50µg, 25µg, 12.5µg, 6.125µg and 3.125µg using the agar well diffusion method. The result of this study revealed that out of the 96 wound swab collected 15(21.7%) bacteria pathogen was isolated in the following order *E. coli* 9(60%), *Pseudomonas aeruginosa* 4(26.6%), *Klebsiella* spp 1(6.6%) and *Staphylococcus aureus* 1(6.6%). All the bacterial isolates of *E. coli, P. aeruginosa, K. pneumoniae* and *S. aureus* were multiply resistant to the tested antibiotics used for this study. However, some of the organisms were susceptible to gentamicin, aztreonam, piperacillin, ciprofloxacin and sulphamethoxazole-trimethoprim. It was generally observed that the bacterial isolates from the wound infections were highly resistant to the tested antibiotics. Similarly, the test bacteria were multiply resistant to the antibacterial action of *A. indica* and *G. kola*. No zones of inhibition was recorded for any of the test organisms excluding

some *E. coli* isolates that showed some level of susceptibility to the ethanol, methanol, cold water extract and hot water extracts of *A. indica* leaf extracts. Some isolates of *P. aeruginosa* and *S. aureus* were also susceptible to the antibacterial action of *A. indica* and *G. kola*. In all, the bacterial pathogens from the wound infections used for this study were multiply resistant to the conventional antibiotics used; and these organisms sowed little or no susceptibility to the leaf extracts of the herbal plants (*A. indica* and *G. kola*) used. Nevertheless, the search for novel antimicrobial agents should be a continuous process – due to the continuous emergence and spread of drug resistant pathogens in both the community and hospital environment. Plants including *A. indica* and *G. kola* still hold the potential to transform medicine especially in the area of chemotherapy – since these natural substances posses in them great healing powers still untapped.

Keywords: *Garcinia kola, Azadirachta indica,* Antimicrobial Activity, Antibiotics, Drug Resistance

INTRODUCTION

Antibiotic-resistant bacteria are extremely important to human health because these organisms continue to defy the efficacy of some available antimicrobial agents. And this phenomenon calls for concerted efforts to contain the situation especially through the discovery of novel antimicrobial agents from plants with putative antimicrobial potentials. Plants are by far the richest resource of drugs of traditional systems of medicines, modern medicines, food supplements, pharmaceutical intermediates and chemical entities for synthetic drugs (Joshi *et al*., 2011; Esimone *et al*., 2007; Rahal *et al*., 2014; Iwu, 2014). Medicinal plants or herbs have been a valuable source of medication in virtually all cultures and societies worldwide due to their important antimicrobial principles and phyto-constituents with known wider therapeutic potentials. The plant kingdom has always been the preferred source of medication in all healing traditions since antiquity, and herbal plants including *Garcinia kola* (bitter kola)and *Azadirachta indica* (Neem) make available the starting raw materials for the synthesis of most conventional drugs used in clinical medicine today (Esimone *et al*., 2007 and Prabakaran *et al*., 2011). *Garcinia kola* (bitter kola) is a tropical plant which grows in moist forest. The seeds of *G. kola* are edible and are consumed as stimulant (Iwu, 2014; Okunji *et al*., 2002). *G. kola* is a tree that grows in the rain forests of west Africa; and the fruit, seeds, nuts and bark of the plant have been used for centuries in folk medicine to treat ailments from coughs to fever (Okoko, 2009; Okunji *et al*., 2002; Iwu, 2014; Ozoy *et al*., 2008). *G. kola* has purgative, anti-parasitic, and antimicrobial properties; and the seeds are also used for the treatment of bronchitis, throat infections, colic, head or chest colds, and cough. Bitter kola is also used for liver disorders and as a chewing stick. *Azadirachta indica* (Neem) is an evergreen tree that is endemic to the Indian subcontinent; and it is also native to Burma, Bangladesh, Sri Lanka, Malaysia and Pakistan (Mishra *et al*., 1995). Neem has spread from India to other tropical and semi-tropical countries of the world where they serve several medicinal and nutritional purposes. It is an indigenous plant that is

widely distributed in Nigeria; and its medicinal value has made it a household plant. Neem possesses both antibacterial and antifungal activity, and the plant also contains some anti-inflammatory and anti-fever compounds (Niharika *et al*., 2010). It is considered as a very important part of the household and Neem is very much respected for its medicinal value. Neem is used to treat skin diseases, inflammations and fevers, and more recently, it has also been applied in the management of rheumatic disorders (Mishra *et al*., 1995; Ozoy *et al*., 2008). Neem is also used as insect repellent due to its insecticide effects. The biological evaluations of medicinal plants such as Neem and bitter kola for their antimicrobial activity are critical to the development of novel antimicrobial agents especially now that there are skyrocketing cases of antibiotic resistance in both the community and hospital environments. Medicinal plants including Neem and bitter kola possess a wide variety of phytochemicals such as alkaloids, tannins, flavonoids, steroids, saponins, and phenols – that are responsible for the antimicrobial proprieties of these plants. It is in view of this that this present day study evaluated the antibacterial activities of herbal extracts of *Azadirachta indica* (Neem) and *Garcinia kola* (Bitter kola) compared to some conventional antibiotics against pathogenic bacteria isolated from wound infections from patients in National Orthopedic Hospital, Enugu (NOHE).

MATERIALS AND METHODS

Collection and preparation of plants: Fresh leaves of *Garcinia kola* and *Azadirachta indica* were collected from secondary forests and open fields in Nsukka, Enugu State and Abakaliki, Ebonyi State, Nigeria respectively. The plant extracts were authenticated by Dr. C.O. Attama, a taxonomist in botany Department of University of Nigeria, Nsukka, Enugu State, Nigeria. The leaves of the plants were washed under running tap water and air dried for 7 days; and the dried leaves were homogenized to fine powder using a mechanical grinder. The powdered and/or homogenized plant materials were stored in airtight containers until use.

Collection and bacteriological analysis of clinical samples: A total of 96 swab surface samples of orthopedic wound with pus were collected with sterile swab sticks and inoculated onto nutrient broth (Oxoid, UK) for 18-24 hrs. Samples which showed microbial growth (indicated as turbidity) was subcultured onto blood agar, chocolate agar, MacConkey agar, cetrimide selective agar, and Mannitol salt agar plates and incubated at 37^{o}C for 18-24 hrs. All isolated pathogenic bacteria were identified using standard microbiological identification techniques. All test bacteria were standardized before use by inoculating a 5 ml normal saline in sterile test tubes with loopful of a 24 hr overnight culture of the test organism from a nutrient agar slant (Cheesbrough, 2006).

Antimicrobial susceptibility studies: Antibiogram was determined by the Kirby-Bauer disk diffusion method as recommended by the NCCLS, now CLSI (NCCLS, 1999). An overnight culture of the test bacteria (adjusted to 0.5 McFarland turbidity standards) was aseptically inoculated on the surface of Mueller-Hinton (MH) agar plate(s) using sterile swab sticks.

And commercially available single antibiotic disks [including amoxicillin/clavulanic acid, gentamicin, cefuroxime, tetracycline, cefepime, aztreonam, ampicillin, ceftazidime, sulphamethoxazole/trimethoprim, cefoxitin, piperacillin, ceftriaxone, ciprofloxacin, erythromycin, clindamycin, oxacillin, vancomycin, and lincomycin (Oxoid, UK)] were aseptically placed on the surface of the inoculated MH agar plates. The plates were incubated at 37^{o}C for 18-24 hrs, and the inhibition zone diameters (IZDs) were measured with a meter rule and recorded as recommended by the NCCLS (NCCLS, 1999; Ejikeugwu *et al.*, 2016).

Extraction of herbal materials (aqueous extraction): A 20 grams sample of air-dried leaf powder was added to 100 ml of distilled water and boiled on slow heat for 2 hours. It was filtered through 6 layers of muslin cloth and centrifuged at 5000 rpm for 10 minutes and the supernatant was collected. This procedure was repeated twice; and after 6 hours, the supernatant collected at an interval of 2 hours was pooled together and concentrated to make the final volume of the plant extract. This was autoclaved at 121^{o}C for 15 minutes and was stored at 4^{o}C for further use. This procedure was repeated using cold water as the solvent for extraction.

Methanol extraction: A 20 gram of air dried leaf powder was added to 100 ml of 90% methanol in a conical flask plugged with cotton wool and was kept on rotator shaker at 190-220 rpm for 24 hours. After 24 hrs incubation the supernatant was allowed to cool and the solvent allowed to evaporate to make the final volume (one-fourth of the original volume); and this was stored at 4^{o}C in airtight bottles. These procedures were repeated using ethanol as the solvent for extraction.

Determination of antimicrobial activity of plant extracts: Test organisms isolated from wound infections of patients in NOHE were inoculated into nutrient broth tubes and incubated at 37^{o}C for 4-6 hours. Then 0.2 ml (10^{5} cfu/ml) of the broth culture of the test bacteria was seeded into MH agar plates and spread evenly. Four wells of 6 mm in diameter were cut into the seeded agar plates using a sterile cork borer. Four of the wells were each filled with hot and cold water leaf extracts; ethanol and methanol leaf extracts of *G. kola* and *A. indica* and a control antibiotic disc ciprofloxacin (5 µg) were placed at the center of the plates. This was repeated subsequently with other test organisms; and the plates were incubated at 37^{o}C for 18-24 hours. The zones of inhibition were determined and recorded (Esimone *et al.*, 2007).

RESULTS

A total of ninety six (96) swab samples from wound infections of patients who attended the National Orthopedic Hospital Enugu (NOHE) for medical attention were bacteriologically analyzed in this current day study. The distribution of the bacterial pathogens isolated from the clinical samples is shown in Table 1. The most frequent organism isolated was *Escherichia coli*; and this was followed by *Pseudomonas aeruginosa*. The antimicrobial susceptibility of the isolated bacterial pathogens to some selected and commonly used antimicrobial agents is shown in Table 2. The entire test organism showed varying levels of susceptibility to the test antibiotics. They were all resistant to amoxicillin-clavulanic

acid, ampicillin and ceftazidime. All the test organisms excluding *Pseudomonas aeruginosa* were also resistant to the antimetabolite, sulphamethoxazole-trimethoprim (Table 2).

Klebsiella pneumoniae was only susceptible to aztreonam, gentamicin and cefotaxime while *E. coli* was susceptible to cefotaxime, aztreonam, and gentamicin. Oxacillin and clindamycin had growth inhibitory effect on *Staphylococcus aureus* while *Pseudomonas aeruginosa* was susceptible to sulphamethoxazole-trimethoprim, ciprofloxacin, gentamicin and aztreonam (Table 2). The antimicrobial activity of the leave extracts of *G. kola* and *A. indica* is shown in Table 3.

Table 1: Frequency of isolation of bacterial pathogens from orthopedic wound infections

Bacteria	Source	No (%)
Klebsiella pneumoniae	**Wound swab**	**1 (6.6)**
Escherichia coli	**Wound swab**	**9 (60)**
Staphylococcus aureus	**Wound swab**	**1 (6.6)**
Pseudomonas aeruginosa	**Wound swab**	**4 (26.6)**

Table 2: Antibiogram of the bacterial pathogen to some selected antibiotics

Organisms	Resistant	Susceptible
K. pneumoniae	**AMC, AMP, CAZ, SXT, PRL, CXM, CIP, TE, FEP**	**ATM, CN, CTX**
E. coli	**AMC, AMP, CAZ, SXT, PRL, CXM, CIP, TE, FEP**	**CTX, ATM, CN**
S. aureus	**AMC, ATM, AMP, CAZ, SXT, CTX, DA, OX, CIP, E, VA, MY**	**PRL, CRO**
P. aeruginosa	**AMC, AMP, CAZ, CTX, PRL, CXM, TE, FEP**	**SXT, CIP, CN, ATM**

Key: AMC = Amoxicillin-clavulanic acid (30 µg), ATM = Aztreonam (30 µg), AMP = Ampicillin (10 µg), CAZ = Ceftazidime (30 µg), SXT = Sulphamethoxazole/trimethoprim (25 µg), CTX = Cefoxitin (30 µg), PRL = Piperacillin (30 µg), CRO = Ceftriaxone (30 µg), CIP = Ciprofloxacin (10 µg), E = Erythromycin (10 µg), DA = Clindamycin (10 µg), OX = Oxacillin (5 µg), VA = Vancomycin (5 µg), MY = lincomycin (10 µg), FEP = Cefepime (30 µg), TE = Tetracycline (10 µg), CXM = Cefuroxime (30 µg), Gentamicin (10 µg).

Table 3: Antibacterial Activity of *Garciniakola* and *Azadirchtaindica* Leaves extractAgainst Selected Bacterial Pathogens Isolated from Orthopedic wound infections At Stock Concentration.

Bacterial isolates	Inhibition zone diameters of herbal plants (mm)								CONTROL CIP (5µg)
	A1	A2	A3	A4	G1	G2	G3	G4	
E1	**NI**	**NI**	**NI**	**NI**	**NI**	**NI**	**NI**	**NI**	**NI**
E2	**NI**	**NI**	**NI**	**NI**	**NI**	**NI**	**NI**	**NI**	**NI**
E3	**NI**	**NI**	**NI**	**NI**	**NI**	**NI**	**NI**	**NI**	**NI**
E4	**18**	**10**	**13**	**17**	**NI**	**NI**	**NI**	**NI**	**20**
E5	**NI**	**NI**	**NI**	**NI**	**NI**	**NI**	**NI**	**NI**	**NI**
E6	**NI**	**NI**	**NI**	**NI**	**NI**	**NI**	**NI**	**NI**	**NI**
E7	**NI**	**NI**	**NI**	**NI**	**NI**	**NI**	**NI**	**NI**	**NI**
E8	**NI**	**NI**	**NI**	**NI**	**NI**	**NI**	**NI**	**NI**	**NI**
E9	**NI**	**NI**	**NI**	**NI**	**NI**	**NI**	**NI**	**NI**	**NI**
P1	**NI**	**NI**	**NI**	**NI**	**NI**	**NI**	**NI**	**NI**	**NI**
P2	**NI**	**NI**	**NI**	**NI**	**NI**	**NI**	**NI**	**NI**	**NI**
P3	**NI**	**NI**	**12**	**14**	**NI**	**NI**	**NI**	**NI**	**10**
P4	**NI**	**14**	**NI**	**15**	**NI**	**NI**	**NI**	**NI**	**NI**
S1	**NI**	**NI**	**NI**	**14**	**NI**	**NI**	**NI**	**NI**	**25**
K1	**NI**	**NI**	**NI**	**NI**	**NI**	**NI**	**NI**	**NI**	**NI**

Key: A1 = *Azadirachta indica* ethanol extract, A2= *Azadirachta indica* methanol extract, A3 = *Azadirachta indica* Hot Water extract, A4 = *Azadirachta indica* Cold Water extract, G1 = *Garcinia kola* Ethanol Extract, G2 = *Garcinia kola* Methanol Extract, G3 = *Garcinia kola* Cold Water Extract, G4 = *Garcinia kola* Hot Water Extract, NI = No Inhibition, mm = millimeter, µg = microgram, S = *Staphylococcus aureus*, E = *Escherichia coli*, P = *Pseudomonas aeruginosa.*

DISCUSSION

Surgical wounds sites with high bacterial contaminants constitute a serious problem in the hospital environment especially in surgical practice where clean operations can become contaminated and subsequently infected by pathogenic bacteria. The degree to which surface wounds are infected by surrounding bacteria contaminants have become clinically important owing to the resistance of some wound pathogens (Taiwo *et al*., 2002). In this present day study, a total of 15 (5.6%) bacteria pathogen isolated from wound infections of patients from NOHE

were bacteriologically examined and their susceptibility to some conventional antibiotics and herbal plants was also evaluated. *Escherichia coli* were the most frequently isolated organism; and this was followed by *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Staphylococcus aureus*. These organisms where also reported to be the most isolated bacterial pathogens from wound pathogens both within Nigeria and elsewhere (Sani *et al*. (2012; Mafikoya *et al*., 2009; Alok *et al*., 2008). However, Jonathan *et al.* (2008) reported that *S. aureus* is the predominant organism isolated from surgical wound infections, a report that is dissimilar to ours. Also in this study, the bacterial isolates recovered from the wound infections were tested for their antimicrobial susceptibility to some conventional antibiotics, and the result showed that *S. aureus* was resistant to virtually all the tested antibiotics with exception to piperacillin and ceftriaxone – to which the organism was susceptible to. Alok *et al.* (2008) reported *S. aureus* isolated from orthopedic wound infections in some Indian hospitals were susceptible to both piperacillin and ceftriaxone (as obtainable in this present day study). This report from India also shows that piperacillin and ceftriaxone are still effective in the treatment of wound infections and other infections in which *S. aureus* is implicated as causative agents (Alok *et al*., 2008). The *E. coli* isolates and *K. pneumoniae* isolates were also resistant to the tested antibiotics excluding cefotaxime, aztreonam and gentamicin – to which they were susceptible to. The *P. aeruginosa* isolates were susceptible to sulphamethoxazole-trimethoprim, gentamicin, ciprofloxacin and aztreonam. Varying rates of resistance and susceptibility of the test isolates including *E. coli, K. pneumoniae, S. aureus* and *P. aeruginosa* as obtainable in this study have been previously reported (Nwachukwu *et al*., 2009; Alok *et al*., 2008; Aisha *et al*., 2013). Increased level of bacterial resistance to some commonly available antibiotics used to treat microbial infections including wound infections has necessitated the need and search for novel antimicrobial agents (Ejikeugwu *et al*., 2012; Peter *et al*., 2012). A number of studies have validated the use of plants in the treatment of bacterial diseases or infections including wound infections. In this study, the antibacterial activity of leave extracts of *A. indica* and *G. kola* was evaluated on bacterial pathogens isolated from wound infections; and the result compared to conventional antibiotics used for the treatment of the same infection. The leaf extracts of *G. kola* and *A. indica* was evaluated on the wound pathogens including *E. coli, K. pneumoniae, S. aureus* and *P. aeruginosa* at varying concentrations including 50 μg/ml, 25 μg/ml, 12.5 μg/ml, 6.25 μg/ml and 3.123 μg/ml. The test plants (*A. indica* and *G. kola*) had very low inhibitory effect on the test bacterial pathogens. The ethanol extract, methanol extract, hot water extract and cold water leaf extracts of both *G. kola* and *A. indica* showed no antibacterial effect on the wound pathogens used in this study even though the antibacterial activity of *G. kola* and *A. indica* and other herbal plants have been previously reported by other researchers (Yerima, *et al*., 2012; Onyeagba *et al*., 2004; Iwu, 2014; Maragathavalli *et al*., 2012). However, the ethanol, methanol, hot water and cold water

extracts of *A. indica* showed appreciable level of antibacterial activity on only one *E. coli* isolate. This particular *E. coli* isolate was however, resistant to all the extracts of *G. kola*. Two isolates of *P. aeruginosa* and the *S. aureus* isolate used in this study were susceptible to the hot water extract and cold water extract of *A. indica*; but they were all resistant to the leaf extracts of *G. kola*. Previous studies have also reported that the extracts (aqueous, ethanol and methanol) of *A. indica* and *G. kola* showed very low antibacterial activity against bacterial pathogens (as obtainable in this present day study) [Hajera *et al*., 2013; Fuad *et al*., 2012; Al-Jiffri *et al*., 2011]. When compared to the conventional antibiotics used in this study, the test bacterial pathogens were multiply resistant to both the herbal plants (leaf extracts of *A. indica* and *G. kola*) and the antibiotics used in this study. Conclusively, the test bacterial pathogens from the wound infections including *E. coli, K. pneumoniae, S. aureus* and *P. aeruginosa* were multiply resistant to both the conventional antibiotics and traditional herbal plants of *A. indica* and *G. kola* used in this present day study; and this gives impetus to the reality of bacterial resistance to some available drugs in this region. Continued antibiotic stewardship and the search for novel antimicrobial agents especially from plant origin is critical in the fight against antimicrobial resistance.

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