

## Comparative Antibacterial Studies of *Loranthus Micranthus* (African Mistletoe) from Different Host Trees against Urinary Tract Infection Bacterial Isolates

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### Abstract

Natural plant products have been used for therapeutic purposes since time immemorial and their use is of greater demand nowadays due to increasing multiple antimicrobial resistances among bacterial pathogens. The aim of this work was to assess the antibacterial activities of the leaf extracts of *Loranthus micranthus* (African mistletoe) parasitic on five different host trees viz; *Garcinia kola* (Bitter kola), *Vitis vinifera* (grape), *Kola acuminata* (Kolanut), *Pentaclethra macrophylla* (Ugba), and *Dialium guinnense* (Icheke). The test organisms included fifty *Escherichia coli* and *Staphylococcus aureus* each, isolated from patients with urinary tract infection. These were identified by cultural, microscopic and biochemical tests. Antibiotic sensitivity studies were determined using the Kirby Bauer disc diffusion method and the multi-drug resistant isolates ascertained. The ethanolic and aqueous extracts of *Loranthus micranthus* from the different host plants were subjected to antimicrobial evaluation using the multidrug resistant *E. coli* and *S. aureus* isolates. The minimum inhibitory concentration (MIC) was determined using the tube dilution method and the phytochemical contents of the extract were also determined. Results obtained showed that the isolates were highly sensitive to amoxiclav (18mm) and Amikacin (15mm) and highly resistant to Nalidixic acid (17mm). Comparatively, the ethanolic extracts of *Loranthus micranthus* parasitic on *Garcinia kola* and *Vitis vinifera* had the highest activity for *E. coli* and *S. aureus* isolates at 10mm and 15mm respectively, while the aqueous extracts of those parasitic on *Dialium guinnense* had the least activity (0mm) for both organisms. Phytochemical analysis revealed the presence of alkaloids, tannins, flavonoids and cardiac glycosides. The leaf extracts of *Loranthus micranthus* parasitic on *Garcinia kola* demonstrated antibacterial potentials and could be an alternate source of treatment for UTIs caused by *E. coli* and *S. aureus*.

**Keyword:** Antibacterial activity, *E. coli*, *Loranthus micranthus*, urinary tract infection, multi-drug resistance, *S. aureus*

### 1. Introduction

Urinary tract infection (UTI) is an infection of any part of the urinary tract caused by microorganisms (Ani and Mgbechi, 2008). They are mostly caused by bacteria although fungi and viruses can also be implicated (Griebing, 2007). They are the second most common type of infection in the body after viral respiratory illness and acute enteric disease, accounting annually for about 150 million and 8.3 million visits to hospitals worldwide and in

Nigeria respectively (Ojo and Anibijuwon, 2012). More than 95% UTI are caused by single bacterial species, *E. coli* which is the most frequently infecting organism, though other bacteria such as *Klebsiella*, *Pseudomonas*, *Staphylococcus*, *Strept. pneumoniae*, *Proteus vulgaris*, *Chlamydiae* and *Niesseria* spp. can also cause UTIs (Amit, Jhadwal, Lal, and Singh 2012; Ali, Ravikumar, Saravanan, Anuradha and Valliammal., 2013). UTIs account for a large proportion of the global antibiotic consumption and may contribute to progressive bacterial resistance (Toner, Papa, Aliyu, Dev, Lawrentschuk. And Al-Hayek, 2017).

The vast majority of UTIs are caused by organisms usually originating from the bowel flora with *E. coli* being the most common uropathogen. It is responsible for over 80% of community acquired and approximately 50% of hospital acquired UTIs as well as 60 – 90% of uncomplicated UTIs (Silvio, 2015). Uropathogenic *E. coli* (UPEC) can infect the urinary tract by the expression of virulent factors such as auto-transporter proteins that may function as motility mediators, adhesions, flagella, O antigens, invasions, proteases and serum resistance factors that enables them to adhere to and colonize the perineum and urethra and migrate to the urinary tract where they establish an inflammatory response in urothelial cells (Silvio 2015). Symptoms include dysuria, urgency, frequency and cloudy urine (Ojo and Anibijuwon, 2010). Antibiotics are the standard treatment for UTIs, but some strains of *E. coli* called extended spectrum beta-lactamase (ESBL) producers are resistant to most drugs and appear to be causing more UTIs now than ever before (Sanchez *et al.*, 2012).

*Staphylococcus aureus* is a relatively uncommon cause of UTI in the general population, although isolation of *S. aureus* from urine samples is often secondary to *Staphylococcal* bacteremia arising elsewhere. In certain patients, *S. aureus* causes urinary tract colonization and infection (Muder *et al.*, 2006). Out of the known causative pathogens, *S aureus* is a nosocomial bacteria pathogen and is a relatively uncommon cause of UTI, resulting in up to 7% of such cases (Wagenlehner *et al.*, 2007). *S. aureus* bacteriuria can result from an ascending infection precipitated by urinary catheterization or from a haematogenous route of infection often resulting from intravascular device exposure and may present an undetected bacteremia (Toner *et al*, 2017). Urinary tract instrumentation and the presence of an indwelling catheter increase the risk of *S. aureus* carriage in the urinary tract. The majority of cases of *S. aureus* bacteriura are not associated with symptoms of UTI (Muder *et al.*, 2006).

Natural plant products have been used for therapeutic purposes since time immemorial and their use is of greater demand nowadays (Yusuf *et al.*, 2013). Many of the existing synthetic drugs are known to cause various side effects and increasing resistance by pathogens. In this regard, drug developments from plant based compounds are now being employed in meeting the demand for newer drugs with minimal side effects (Orhue *et al.*, 2014). Mistletoes therefore are one of such plants reported to possess several medicinal properties. It is a general term for short woody shoot parasites in several plant families especially in *Loranthaceae* and *Viscaceae* families. *Loranthus micranthus* represents the Eastern Nigerian mistletoe that grows mostly in the South East region of the country. It is a medicinal plant from the *Loranthaceae* family popularly called 'Kauchi' by the Hausa/ Fulani, "ewe amofon" by the Yoruba and 'awuruse/ Ibu' by the Igbos. It is an obligate semi parasitic tropical plant normally found growing on indigenous trees and a number of tree crops of economic importance including palm fruit, mahogany, *Kolaacuminata*, *Baphia nitida*, *Vitis vinifera*, *Pentaclethra macrophylla* etc. (Osadebe *et al.*, 2012). It has been widely used in ethno medicine for treatment of hypertension, diabetes, schizophrenia and as an immune system booster (Osadebe and Omeje 2009). The composition and activities of mistletoe are

dependent on host trees, harvesting period and might also be dependent on the type of solvent used for extraction (Nwankwo *et al.*, 2011). The aim of this work was to assess and compare the antimicrobial efficacy of mistletoe from different host trees on the multidrug resistant urinary tract infection *E. coli* and *S. aureus* isolates.

## **2. Materials and Methods**

### **2.1. Plant materials**

Fresh leaves of *Loranthus micranthus* were collected from five different host trees located at Mbaise and Nnewi North LGA of Imo and Anambra States respectively. These host trees were; *Garcinia kola* (Bitter kola), *Kola acuminata* (Kolanut), *Dialium guineense*, (Icheku), *Pentaclethra macrophylla* (Ugba) and *Vitis Vinifera* (Grape). The leaves identified and confirmed at the Crop Science Department of the Federal University of Technology, Owerri.

### **2.2. Preparation of ethanolic plant extracts**

The leaves were destalked, washed and air dried at room temperature ( $28^{\circ}\text{C}\pm 2$ ) for four weeks after which they were pulverized using a manual grinding machine into uniform powder and stored in well labeled air tight containers. Fifty grams of the ground plant material was extracted successively with 200ml of ethanol in a Soxhlet apparatus as described by Hussain *et al.*, (2010). After complete solvent evaporation, the extracts were reconstituted in 1% dimethyl sulfoxide (DMSO) to a concentration of 500mg/mL, put in labelled sterile screw capped bottles and stored in the refrigerator till further use.

### **2.3. Preparation of aqueous plant extracts**

The crude aqueous extraction procedure (CAEs) was used. This was carried out by soaking the powdered leaves in water. One hundred grams of the ground plant material was soaked in 200mls of sterile distilled water (hot and cold) for 24 hours with agitation at intervals. The mixture was filtered using Whatman's no 1 filter paper. The filtrate was concentrated by drying at  $37^{\circ}\text{C}$  (Hussain *et al.*, 2011). The extract was divided into two portions: the first part was for antimicrobial assay and the second for phyto-chemical analysis.

### **2.4. Collection of clinical isolates**

The test organisms (fifty isolates each of *E. coli* and *S. aureus*), used were clinical isolates from patients with urinary tract infections attending Federal Medical Centre Owerri and Imo Specialist Hospital Umuguma, Owerri West LGA, Imo State. The isolates had previously been collected and stored in their laboratory. Out of the fifty isolates, fourteen and thirty six isolates of *E. coli*, and sixteen and thirty four isolates of *S. aureus* were from male and female patients respectively, and both within the age ranges of 0 – 90 years. The identities of the organisms were confirmed using standard cultural, morphological and biochemical methods. They were then maintained on nutrient agar slants at  $4^{\circ}\text{C}$  as described by Cheesbrough (2004) until needed.

### **2.5. Antimicrobial susceptibility test using antibiotic disc**

Kirby-Bauer modified disc diffusion method was used for susceptibility testing in accordance with the Clinical and Laboratory Standard Institute (Cheesbrough, 2004). This was done by placing antibiotic discs on a plate of Mueller Hinton agar uniformly inoculated with standardized pure cultures each of the hundred test organisms, and allowed for about 10

minutes for the surface of the agar to dry. Oxoid antibiotic discs; amoxi-clav (30µg), ciprofloxacin(5µg), nalidixic acid(30µg), cefadroxil (30µg), ceftriaxone (30µg), nitrofurantoin (300µg), vancomycin (30µg) and amikacin (30µg) were aseptically placed and evenly distributed on the inoculated plates and lightly pressed with sterile forceps to ensure its contact with the agar. The plates were then aerobically incubated overnight at 37°C and observed for susceptibility (zone of inhibition) or otherwise. The zones of inhibition were measured after incubation and interpreted according to the recommendation of the National Committee for Clinical Laboratory standards (NCCLS). The multi-drug resistant isolates were determined from the results of the antibiotic susceptibility testing. Isolates that were resistant to two or more drugs were considered to be multidrug resistant (Jyoti *et al.*, 2014). They were preserved on nutrient agar slants and used for antimicrobial testing of plant extracts.

## 2.6. Antimicrobial activity assay of the plant extracts

The antibacterial susceptibility testing of the different plant extracts was done by the well in agar diffusion method as described by Usman *et al.* (2007) and Dahiya (2012). A total of 15 extracts of *Loranthus micranthus* (from different host trees) were tested against the multi-drug resistant isolates. The stock concentration (500mg/mL) of each plant extract was prepared by dissolving 1gram of plant extract in 2mLs of 1% dimethyl sulfoxide. This was further diluted with sterile distilled water to obtain concentrations of 250, 125, 62.5 and 31.25 mg/mL. Sterile Mueller Hinton agar plates were inoculated with 0.1ml of the standardized organisms (24hr old suspension) respectively. Subsequently wells of 5mm diameter were bored into the inoculated plates using a sterile cork borer. The holes were filled with different concentrations of the extract (0.5mL) and allowed to diffuse at room temperature for 2 hours. The plates were then incubated in the upright position at 37°C for 24 hours. Standard antibiotics; amoxi-clav and amikacin were used as positive controls and water as a negative control.

## 2.7. Determination of the minimum inhibitory concentration

The MIC of the aqueous and ethanolic extracts of *L. micranthus* harvested from the five different host trees was determined using the broth dilution technique as described by Gedam *et al.* (2007) and Auwal *et al.* (2013). The MIC was determined by adding 2mLs of a specific concentration (500, 250, 125 or 31.25mg/ml) of the plant extract and 0.1mL of standardized test organism into a test tube containing 2mls of sterile nutrient broth. Isolates were diluted according to 0.5 McFarland standards which was equal to  $0.5 \times 10^8$  cfu/ml. The culture was then incubated at 37°C for 24hours and the least concentration of the extracts with no visible turbidity was taken as the MIC.

## 2.8. Determination of the phytochemical constituents

The ethanolic and aqueous leaf extracts of *Loranthus micranthus* harvested from the five different host trees were subjected to various standard phytochemical analysis respectively to identify the chemical constituents such as tannins, alkaloids, flavonoids, saponins, cardiac glycosides, steroids, anthraquinones, polyphenols, and terpenoids, as described by Usman *et al.* (2007) and Auwal *et al.* (2013).

### 3. Results and Discussion

The results obtained in this study showed that the prevalence of urinary tract infection (UTI) isolates was higher in female patients between the ages of 20-35 years (47.2%) and the age range 36-90 (47.1%) for *E. coli* and *S. aureus* respectively, than male patients within the same age groups (Table 1). This is in line with the findings of Oladeinde *et al.*, (2011) who recorded 73% in females and 30% in males, and that of Sibi *et al.*, (2011) who recorded 57.8% and 25% in the females and males in his study population respectively. This indicates a significant relationship between UTI in females from different studies. The high prevalence of UTIs in females has been attributed to so many factors including: the shortness of the female urethra which allows for quicker access of the organism to the bladder, closeness of the female urethral opening to the anus and vagina which predisposes a woman to infections of the urinary tract, high sexual activity which is a predisposing factor to UTI, poor personal care, use of contraceptives and the absence of the bacteriostatic properties of prostatic secretions that is present in males (Griebing, 2007; Ani and Mgbechi, 2008; Gould *et al.*, 2010).

The study also demonstrated the inhibitory effects of some known antibiotics on *Escherichia coli* and *S. aureus*. The antibiotic susceptibility profile of *E. coli* revealed that the organism was highly sensitive to amikacin (76%) and nitrofurantoin (58%) (Table 2). This is in line with the findings of Goetsh *et al.*, (2000) and Sibi *et al.*, (2011) which confirmed the inhibitory effects of these drugs on *E. coli* isolates. Nitrofurantoin plays a significant role in controlling the growth of the isolates hence it is among the choice drugs in the treatment of UTIs caused by *E. coli*. The antibiotic susceptibility profile of *S. aureus* revealed that the organism was most sensitive to amoxiclav (86%) and most resistant to ciprofloxacin (60%). This is in contrast to the findings of Kim and Joseph (2012) and Oladeinde *et al.* (2011) which stated that fluoroquinolones which are broad spectrum antibiotics are highly effective in the treatment of UTIs. The resistant rates exhibited by *E. coli* to ciprofloxacin (52%) in the present study, is also in contrast with the findings of Kim and Joseph (2012) and Sibi *et al.* (2011). The reason for these variations could be as a result of the abuse of ciprofloxacin in the study areas. The results of the present study however are in conformity with the report of Goetsh *et al.* (2000) and Farhan *et al.* (2012) in which resistance to ciprofloxacin was also highlighted. Ali *et al.* (2013) reported that UTI bacterial pathogens have demonstrated resistance to antibiotics over the years which could also be a reason for the resistance of *S. aureus* and *E. coli* to ciprofloxacin observed in this study

The *E. coli* isolates demonstrated a high resistant rate of 70% to nalidixic acid which is one of the choice drugs for treatment of UTIs. This is in contrast to the findings of Sibi *et al.* (2011) that recorded high sensitivity rates for the same organism. Increased resistance of *E. coli* to nalidixic acid could be attributed to the increasing pathogenicity and virulence of the organism (Silvio, 2015). *E. coli* expressed moderate sensitivity rates (52%) to Ceftriaxone and amoxiclav, broad spectrum antibacterial agents known to be highly effective against UTIs. It was observed that amikacin which is a narrow spectrum drug had the best effect on *E. coli* isolates while amoxiclav had the best effect on *S. aureus*.

Despite the availability of antibiotics for its treatment, UTIs remain the most common bacterial infections in human populations (Khan *et al.*, 2012), and with resistance to conventional antibiotics being on the increase, it becomes absolutely necessary to screen plant extracts with known medicinal properties, for activity against the multidrug resistant

bacterial pathogens isolated in this study. Antibacterial compounds from natural sources could serve as alternatives to overcoming the problem of drug resistance by these bacteria.

The ethanolic extracts of *Loranthus micranthus* parasitic on the five different host trees inhibited the growth of the isolates at varying concentrations. Comparatively, the ethanolic extracts of *Loranthus micranthus* parasitic on *Garcinia kola*, *Vitis vinifera* and *Pentaclethra macrophylla* demonstrated significant inhibitory effects on both *E.coli* (50%, 38.8% and 33.3% respectively) and *S. aureus* (62.5%, 56.2 and 50% respectively) compared to that of *Kola acuminata* and *Dialium guinnense*. All extracts of mistletoe parasitic on *Pentaclethra macrophylla* demonstrated a moderate inhibitory effect on the isolates while those from *Dialium guineense* were least effective, with no activity against most of the isolates (Tables 3 and 4).

On the other hand, the hot and cold water extracts of mistletoe demonstrated less antimicrobial activity to the isolates than the ethanolic extracts. The hot water extracts from *Vitis vinifera* expressed the highest activity to *E. coli* at 50% while that of *Garcinia kola* was highest at 56.2 % for *S. aureus* (Tables 5 and 6), while the cold water extracts had 33.3% and 43.75% activity for *Vitis vinifera* for both *E. coli* and *S. aureus* (Tables 7 and 8).

A comparative investigation on antimicrobial activities of *Loranthus micranthus* leaves parasitic on six different host trees by Osadebe *et al.* (2004) recorded antimicrobial activity of plant extracts harvested from *K. acuminata* and *P. macrophylla*. In another comparative study by Yusuf *et al.* (2013) the antibacterial effect of *L. micranthus* parasitic on *Kola acuminata* on *E. coli* and *S. aureus* was also confirmed. Benjamin *et al.* (2016) reported antibacterial activity of *L. micranthus* parasitic on *P. macrophylla* on *E. coli* isolates.

It is important to note that the effects of extracts on isolates are also dependent on the extraction methods and the concentration used. A comparison of the antibacterial activities of both aqueous and ethanolic extracts showed that the ethanolic extracts demonstrated a higher antibacterial activity than the aqueous extracts, similar to previous findings by Fagbohun *et al.*, (2013). Ethanol is an organic solvent and will dissolve organic compounds better and as such will liberate the active ingredients required for antimicrobial activity.

The varying effect of the extracts from the different host trees might be as a result of the medicinal properties of the host tree for example *Garcinia kola* is known for its wide range of medicinal uses and has been proven to possess antibacterial and anti-inflammatory activities (Galam *et al.*, 2013). *L. micranthus* as a parasitic plant are dependent on their host tree for nutrition, hence the antimicrobial effects of its extracts. The observed variations of the activities of these extracts may be due to the different host plant from which the samples were collected and different geographical locations as suggested by Osadebe *et al.* (2004).

The zones of inhibition of the control antibiotics on the multi-drug resistant isolates were found to be higher than those of the extracts. However, it has been suggested that plant extracts exhibiting diameter of zones of inhibition > 10mm are to be considered active (Usman *et al.*, 2007). The bioactive components and concentration of the antibiotics may be a contributing factor as antibiotics are products of large scale industrial fermentation. The products of such processes are usually pure due to the good manufacturing processes and quality control which guarantees standard (Yusuf *et al.*, 2013), compared to the extracts, even though they contain medicinal properties as a result of the presence of bioactive

components, are in crude and unrefined forms. The molecular sizes of the antibiotics aid in their solubility. This could also enhance their penetration through the cell wall into the cytoplasm of the test organisms (Mailard, 2002).

Phytochemical analysis of the mistletoe extracts demonstrated the presence of phytochemicals including flavonoids, saponins, tannins, cardiac glycosides, steroids. Comparatively, it was observed from the study that the phyto-constituents were higher in the ethanolic extracts compared to the aqueous extracts (Table 10). According to Whitney *et al.* (2002), these phytochemicals are important for their pharmacological properties. From the study, the ethanolic extracts of mistletoe parasitic on *G. kola*, *P. macrophylla* and *K. acuminata* contained cardiac glycosides and tannins in abundance. Cardiac glycosides are organic compounds used in treatment of heart failure and certain irregular heartbeats. Tannins, steroids, saponins and terpenoids were present in moderate quantities in mistletoe harvested from *G. kola*, *P. macrophylla* and *Kola acuminata* but absent in *Vitis vinifera* and *Dialium guineense*.

Phyto-chemicals are secondary metabolites present in the extract which may confer *L. micranthus* with its therapeutic activities (Whitney *et al.*, 2002). For instance, saponin is an antioxidant phytochemical whereas tannins have biological activities that may enhance the prevention and management of many ailments (Njoku and Akumefula, 2007). In previous works, the ethanolic and aqueous leaf extracts of *L. micranthus* parasitic on *K. acuminata* and *P. macrophylla* have been reported to contain tannins, terpenoids, flavonoids, and alkaloids which have been implicated in various pharmacological activities of the plant including antibacterial and antidiabetic properties (Osadebe and Ukwueze, 2004; Osadebe and Omeje, 2009; Obatomi *et al.*, 1994)

#### 4. Conclusion

The experimental results obtained from this study indicated that the Eastern Nigerian mistletoe leaf extracts sourced from five different host trees demonstrated antibacterial activity against both gram positive and gram negative multidrug resistant bacteria implicated in UTI and could be said to be broad spectrum in action. The present study proved the efficacy of the active components present in *L. micranthus* parasitic on *Garcinia kola* and *Vitis vinifera* for the treatment of UTI and could be further explored for commercial use. The findings from the present work further support the traditional belief that mistletoe from different host trees vary greatly in their usage for the treatment of ailments and also that the therapeutic potential of mistletoe is dependent on the type of host plant.

Table 1: Occurrence (%) of the isolates among male and female patients

Age (years)	Gender/Organism			
	Male		Female	
	<i>Escherichia coli</i> (n=14)	<i>Staphylococcus aureus</i> (n=16)	<i>Escherichia coli</i> (n=36)	<i>Staphylococcus aureus</i> (n=34)
0-7	1(7.1)	2(12.5)	2(5.6)	2(5.9)
8-19	2(14.3)	2(12.5)	5(13.9)	4(11.8)

20-35	5(35.7)	5(31.3)	17(47.2)	13(38.2)
36-90	6(42.9)	7(43.8)	13(36.1)	15(47.1)

Table 2: Antibiotic susceptibility profile (%) of UTI implicated *E. coli* and *S. aureus* isolates

Antibiotics	Sensitive		Resistant	
	<i>E. coli</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>S. aureus</i>
	(n=50)	(n=50)	(n=50)	(n=50)
Amoxi – Clav	26(52)	43(86)	24(48)	7(14)
Ciprofloxacin	24 (40)	20 (40)	26(52)	30(60)
Ceftriaxone	26(52)	24(48)	23(46)	26(52)
Amikacin	38(76)	ND	12(24)	ND
Nalidixic acid	15(30)	ND	35(70)	ND
Nitrofurantoin	29(58)	26(52)	21(42)	24(48)
Vancomycin	ND	34(68)	ND	16(32)
Cefadroxil	ND	30(60)	ND	20(40)

Key: ND; Not determined

Table 3: Antimicrobial activity profile of ethanolic extracts of *Loranthus micranthus* on *E. coli*

Host tree	Concentration (mg/ml)/No(%) of sensitive isolates (n=18)				
	500	250	125	62.5	31.25
<i>Garcinia kola</i>	9(50)	9(50)	7(38.8)	6(33.3)	5(27.7)
<i>Kola acuminata</i>	6(33.3)	6(33.3)	5(27.7)	5(27.7)	5(27.7)
<i>Vitis vinifera</i>	7(38.8)	7(38.8)	7(38.8)	7(38.8)	6(33.3)
<i>P. macrophylla</i>	6(33.3)	6(33.3)	5(27.7)	5(27.7)	5(27.7)
<i>D. guineense</i>	5(27.7)	5(27.7)	4(22.2)	3(16.7)	3(16.7)

Table 4: Antimicrobial activity profile of *Loranthus micranthus* ethanolic plant extracts on *S. aureus*.

Host trees	Concentration (mg/ml)/No(%) of sensitive isolates (n=16)				
	500	250	125	62.5	31.25
<i>Garcinia kola</i>	10(62.5)	10(62.5)	10(62.5)	9(56.25)	7(43.7)
<i>Kola acuminata</i>	6(37.5)	6(37.5)	4(25)	3(18.75)	3(18.5)
<i>Vitis vinifera</i>	9(56.2)	9(56.2)	8(50)	8(50)	7(43.7)
<i>P. macrophylla</i>	8(50)	8(50)	7(43.7)	7(43.7)	6(37.5)
<i>D. guineense</i>	4(25)	4(25)	3(18.75)	2(12.5)	1(6.25)

Table 5: Antimicrobial activity profile of *Loranthus micranthus* hot water plant extracts on *E. coli*.

Host trees	Concentration (mg/ml)/No(%) of sensitive isolates (n=18)				
	500	250	125	62.5	31.25
<i>Garcinia kola</i>	6(33.3)	6(33.3)	5(27.7)	4(22.2)	4(22.2)
<i>Kola acuminata</i>	6(33.3)	5(27.7)	5(27.7)	5(27.7)	4(22.2)
<i>Vitis vinifera</i>	9(50)	8(44.4)	8(44.4)	8(44.4)	7(38.8)
<i>P. macrophylla</i>	5(27.7)	5(27.7)	5(27.7)	4(22.2)	4(22.2)
<i>D. guineense</i>	4(22.2)	4(22.2)	3(16.7)	3(16.7)	2(11.1)

Table 6: Antimicrobial activity profile of *Loranthus micranthus* hot water plant extracts on *S. aureus*.

Host trees	Concentration (mg/ml)/No(%) of sensitive isolates (n=16)				
	500	250	125	62.5	31.25
<i>Garcinia kola</i>	9(56.2)	9(56.2)	9(56.2)	7(43.7)	7(43.7)
<i>Kola acuminata</i>	6(37.5)	6(37.5)	6(37.5)	5(31.25)	4(25)
<i>Vitis vinifera</i>	8(50)	8(50)	8(50)	8(50)	7(43.7)
<i>P. macrophylla</i>	6(37.5)	6(37.5)	5(31.25)	5(31.25)	2(12.5)
<i>D. guineense</i>	4(25)	4(25)	3(18.75)	1(6.25)	1(6.25)

Table 7: Antimicrobial activity profile of cold water extracts *Loranthus micranthus* on *E. coli*.

Host trees	Concentration (mg/ml)/No(%) of sensitive isolates (n=18)				
	500	250	125	62.5	31.25
<i>Garcinia kola</i>	5(27.7)	5(27.7)	5(27.7)	3(16.7)	3(16.7)
<i>Kola acuminata</i>	4(22.2)	4(22.2)	4(22.2)	3(16.7)	3(16.7)
<i>Vitis vinifera</i>	6(33.3)	6(33.3)	6(33.3)	5(27.7)	2(11.1)
<i>P. macrophylla</i>	4(22.2)	4(22.2)	3(16.7)	2(11.1)	0(0)
<i>D. guineense</i>	3(16.7)	3(16.7)	2(11.1)	2(11.1)	0(0)

Table 8: Antimicrobial activity profile of *Loranthus micranthus* cold water plant extracts on *S. aureus*.

Host trees	Concentration (mg/ml)/No(%) of sensitive isolates (n=16)				
	500	250	125	62.5	31.25
<i>Garcinia kola</i>	4(25.5)	4(25.5)	4(25.5)	3(18.7)	1(6.25)
<i>Kola acuminata</i>	4(25)	4(25)	4(25)	3(18.7)	2(12.5)
<i>Vitis vinifera</i>	7(43.75)	7(43.75)	6(37.5)	3(18.75)	2(12.5)
<i>P. macrophylla</i>	4(25)	4(25)	4(25)	3(18.75)	2(12.5)
<i>D. guineense</i>	3(18.75)	3(18.75)	2(12.5)	2(12.5)	1(6.25)

Table 9: Minimum inhibitory concentration of *L. micranthus* plant extracts from different host trees on *E.coli* and *S. aureus*

Host tree	MIC (mg/ml)/ Solvent			Isolate
	EE	HE	CE	
<i>G. kola</i>	31.25	62.5	125	<i>E. coli</i>
	31.25	62.5	125	<i>S. aureus</i>
<i>K. acuminata</i>	125	125	125	<i>E. coli</i>
	62.5	125	125	<i>S. aureus</i>
<i>V. vinifera</i>	62.5	125	125	<i>E. coli</i>
	31.25	125	125	<i>S. aureus</i>
<i>P. macrophylla</i>	62.25	125	250	<i>E. coli</i>
	125	125	250	<i>S. aureus</i>
<i>D. guineense</i>	125	250	250	<i>E. coli</i>
	125	250	250	<i>S. aureus</i>

KEY: EE; Ethanolic extracts; HE; Hot water extracts; CE; Cold water extracts

Table 10: Phytochemical analysis of *L. micranthus* ethanolic plant extract from different host trees

Parameters	<i>Garcinia kola</i>	<i>Vitis vinifera</i>	<i>Pentaclethra macrophylla</i>	<i>Dialium guineense</i>	<i>Kola acuminata</i>	Solvents
Flavonoids	++	++	++	-	++	EE
	+	++	+++	-	+	AQE
Alkaloids	+	-	+	+	++	EE
	+	-	+	+	++	AQE
Saponins	+	++	+	++	+	EE
	+	+	+	-	+	AQE
Cardiac glycosides	+++	+++	++	++	+++	EE
	+++	++	+++	+	+++	AQE
Steroids	++	-	++	+	++	EE
	+	-	++	+	++	AQE
Anthraquinones	+	++	++	-	++	EE
	+	+	-	-	-	AQE
Polyphenols	++	-	++	++	+	EE
	+	-	+	+	+	AQE
Terpenoids	++	+	++	++	++	EE
	+	-	+	+	++	AQE
Tannins	+++	+++	+++	+++	+++	EE
	+++	+++	+++	+++	+++	AQE

Key: - =absent, + = present, ++ = moderate, +++ = high. EE; Ethanolic extracts; AQE; Aqueous extracts

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