Antibiogram and Evaluation of Antibacterial Activity of Stem Bark Extract of *Artocarpus heterophyllus* Lam on Clinical Isolates

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Abstract: The growing resistance of bacteria to antibiotics calls for means of circumventing such negative trend through other means. This study evaluated the antibacterial activity of *Artocarpus heterophyllus Lam* against multi drug resistant bacteria. One hundred and ten pure cultures of bacteria collected from State Hospital, Ijebu-Ode, Ogun State, were re-identified by sub-culturing on selective media and by biochemical tests. Antibiotic susceptibility was carried out using Kirby Bauer disk diffusion method. Phytochemical analysis was carried out using qualitative method. The antibacterial activity of the extract was determined by agar well diffusion. *Escherichia coli* (65) and *Klebsiella pneumoniae* (45) were re-identified. All isolates showed resistance to three or more antibiotics. Phytochemical analysis depicts the presence of alkaloid, saponin, flavonoid, steroids, phenol, anthraquinone and cardiac glycosides. The result of the antibacterial activity of the extract revealed that some of the tested isolates were concentration dependent. All isolates of *E. coli* did not respond to the inhibitory effect of the extracts at lower concentrations (12.5 – 50 mg/ml), while some isolates of *K. pneumoniae* responded to the inhibitory effect of the extract at 25 – 100 mg/ml. The antibacterial activity ranged from 3 -12 mm, with *E. coli* having highest zones of inhibition. The result obtained in this study validated the traditional use of *A. heterophyllus* in treating bacterial infections. Stem bark of the tested plant showcased antibacterial activity *in vitro*, hence, can be used in the treatment of infection due to the tested bacteria.

Keywords: Antibiotic resistance, *Artocarpus heterophyllus*, Multi drug-resistance, Antibacterial activity, Phytochemical.

INTRODUCTION

Microbial infection is a major health problem all over the world with high mortality and morbidity along with increasing antibiotic resistance (CDC, 2015). Bacteria can cause several infections ranging from urinary tract infections, wound infections and diarrhea infections. Urinary tract infection (UTI) can be defined as condition in which bacteria are multiplying and attacking the urinary tract regardless of the position along the tract (Sule and Abdullahi, 2016). Urinary tract infections are one of the most common bacterial infections affecting people in hospitals as well as in the community. Data from the combined National Ambulatory Health Care Surveys in the US for 2009–2010 showed that UTI accounted for approximately 9.8 million visits to health care settings such as primary care, outpatient and emergency departments (CDC, 2015).

Wound infection is the presence of pus in a lesion as well as the general or local features of sepsis such as pyrexia, pain and induration. Infection is believed to occur when virulence factors expressed by one or more microorganisms in a wound out-compete the host natural immune system (Pondei *et al*., 2013). Wound infection is one of the most common hospital acquired infection that results in sepsis, limb loss, long hospital stays, higher costs and is responsible for significant human mortality and morbidity worldwide (Mahat *et al*., 2017).

Diarrheal disease is the second leading cause of death among children under five globally; accounting for 9 percent of all deaths in 2012, with about 1,600 children dying each day or more than 580,000 children yearly (WHO, 2013). These deaths mainly occur in south Asia and sub-Saharan Africa (UNICEF, 2012).

Artocarpus heterophyllus Lam, (*Moraceae* family) is one of the most important trees in tropical home gardens and perhaps the most widespread and useful tree in the significant genus *Artocarpus*. The tree is reportedly a native to the rainforests of Malaysia, the Western Ghats of India and also found in Central and Eastern Africa, south-eastern Asia, the Caribbean, Florida, Brazil, Australia, Puerto Rico and many Pacific Islands (Madhavi *et al*., 2013). The generic name comes from the Greek words 'artos' (bread) and 'karpos' (fruit). The fruits are eaten and are commonly called breadfruit. In Nigeria, its cultivation has not been encouraged, although they are said to be found in the south-coastal parts of the country where they grow wild or semi-conserved, and in this area, the fruits are boiled and eaten by children (Ajayi and Adewale, 2013). However, this practice has stopped, and the fruit now substantially go to waste (Bello and Olawore, 2012). It has great commercial, nutritional and medicinal value and is cultivated mainly for its fruit, which is utilized in traditional medicine for treating anemia, asthma, wound healing, ulcers, dermatitis, diarrhea and cough (Jagtap and Bapat, 2010). Different Parts of *A. heterophyllus* have been reported to be used as food or as medicine and have been found in other parts of Africa, Asia and India (Madhavi *et al*., 2013). The antimicrobial activities of *A. heterophyllus* has been reported by various researchers (Thapa *et al*., 2016; Jitendra *et a*l., 2014; Hafid *et al.*, 2017; Binumol and Sajitha, 2013), however, it is not yet popularized and still an underutilized plant in Nigeria, hence has not attained natural or worldwide importance. There is paucity of data and research on the antibacterial potency, phytochemical constituents and GC-MS of the stem bark extract of *A. heterophyllus*.

Hence, this study evaluated the phytochemical constituents of the plant and the antibacterial activity with the view of authenticating the traditional uses of the plants for its antimicrobial uses. The study also evaluated the antibiogram of the isolates used in the study.

MATERIAL AND METHODS

Collection, Identification and Extraction of Plant Material

Stem Bark of *Artocarpus heterophyllus* was collected from Enugu state. The plant was authenticated by a botanist in the Department of Plant Science, Olabisi Onabanjo University, Ago –Iwoye, Ogun State. The stem bark of *A. hererophyllus* was rinsed in sterile water and spread on a sterile bench and left to dry in a cool dry place for three weeks. The dried sample was ground by mortar and pestle and powdered into a fine sample by an electric grinder. The powdered plant was kept in a sterile plastic bottle and stored in the refrigerator at 4^{o}C for further analysis. One hundred (100 g) of *A. heterophyllus* was soaked in 1000 ml ethanol for 3 days, after which the extract was filtered using a sterilized cotton-wool plugged into a buchner funnel. The filtrate was again filtered thrice using Whatmann No 1 filter paper and concentrated by water bath at 60^{o}C. The extract was then stored in an airtight container and kept in the refrigerator at 4°C until needed. Percentage yield was then calculated as

$$\text{Percentage Yield} = \frac{\text{Weight of Extract}}{\text{Weight of Crude Drug}} \times 100$$

Test organisms

A total of 110 pure clinical cultures of *Escherichia coli* and *Klebsiella pneumoniae* that were isolated from urine, wound and stool samples were obtained from Microbiology laboratory of Ogun State General Hospital, Ijebu-Ode, Ogun State, Nigeria. They were identified based on their morphological appearances on Eosin methylene blue agar, Gram stain reaction and biochemical reactions.

Preliminary Phytochemical analysis

The stem bark extract of *A. heterophyllus* was subjected to qualitative analysis using the standard method of Harborne as described by Adeeyo *et al*. (2018).

Antibiotic Susceptibility Screening

All the 110 clinical isolates were screened for antibiotic susceptibility. Cultures of the isolates were grown on nutrient agar plates overnight at 37°C. Approximately five colonies were emulsified in 2 ml of sterile saline and the turbidity was adjusted to a 0.5 McFarland standard. Sterile swabs were dipped into the suspension and squeezed against the side of the suspension tube to remove excess inoculums, and streaked across pre-dried Mueller Hinton agar plates, three times for each plate, with the plate rotated approximately 90° between each streaking. After 10 to 15 minutes, to allow absorption of excess moisture into the agar, antibiotics were placed on the agar plate using a sterile forceps. The sensitivity discs were placed on the agar plates with reasonable distant apart to prevent overlapping of zone of inhibition. The plates were incubated at 37ºC for 24 hours (Hudzicki, 2009). The diameter of the inhibition zone was determined according to the CLSI guidelines (2015) for bacteria by measuring the clear zones with a metre rule and recorded in millimeter. Seven antibiotics (Oxoid, ThermoScientific, United Kingdom), were used, (100 µg Ampiclox, 3 µg Ceftrazone 10 µg Gentamicin, 5 µg Ciprofloxacin, 5 µg Trimetoprim, 1.25/23.75 µg Trimethoprim-sulphamethazaxole and 30 µg Chloramphenicol).

Antibacterial activity of stem bark of *Artocarpus heterophyllus*

Suspension of the paste crude extract was made by dissolving 5 g of the crude extract into 10 ml of 70 % ethanol to give a concentration of 500 mg/ml. Further serial dilutions were made by to get 100 mg/ml, 50 mg/ml 25 mg/ml and 12.5 mg/ml respectively. The antibacterial activity of *A. heterophyllus* was determined using agar well diffusion. The 110 stocked bacterial isolates were re-cultured into a Mueller Hinton agar and incubated at 37^{o}C for 24 hours. Suspension of 0.1 ml of the test inoculums that matched 10^5 McFarland standard were inoculated onto Mueller Hinton Agar and spread evenly with the aid of sterilized cotton swab. Holes of 6 mm were bored into the inoculated media with the aid of a sterilized cork borer. This was carried out by dipping the cork borer into ethanol and flaming it in-order to sterilize it. Fifty microgram (50 µg) of the plant extracts of different concentration (100 mg/ml, 50 mg/ml, 25 mg/ml and 12.5 mg/ml) were dispensed into each holes and Ceftriaxone powder of concentration of 100 mg/ml was used as the positive control. The plates were incubated for 24 hours at 37^{o}C. The zones of inhibition were measured with the aid of a metre rule and recorded in millimeter.

RESULTS

Numbers of re- identified organisms

A total of 110 *E. coli* and *K. pneumoniae* isolates from urine, stool and wound samples were identified through their morphological appearances on Eosin methylene blue agar, Gram reaction and biochemical test, with *E. coli* having the highest occurrence (59.1 %) and *K. pneumoniae* having 40.9 % (Table 1).

Table1: Numbers of re- identified organisms

Organisms	No of isolates (n)	Percentage occurrence (%)
E. coli	65	59.1
K. pneumonia	45	40.9
Total	110	100

Antibiotic susceptibility test

Percentage resistance of all the isolates (110) to the tested antibiotics was determined to ascertain the antibiotics that have the highest and lowest sensitivity against the tested isolates. (Figure1). The resistance pattern of *E. coli* and *K. pneumoniae* isolates were also determined (Table 2).

These profiles were determined from the interpretation of the diameter of the zones of inhibition of these antibiotics on the organisms according to the guidelines of CLSI, (2015).

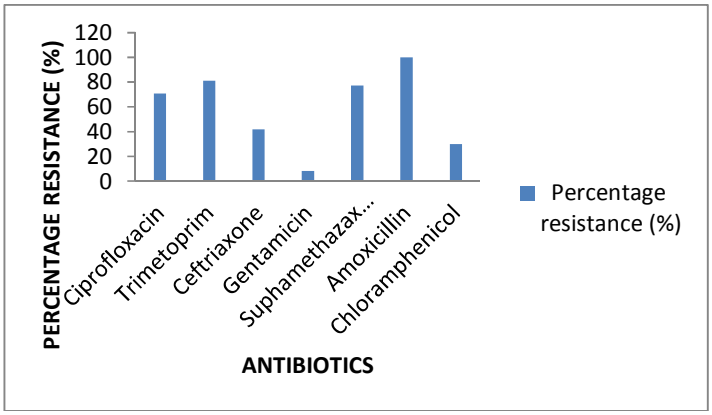

Figure 1: Percentage resistance pattern of all isolates to the tested antibiotics

Table 2: Antibiotic resistance pattern of the *E. coli* and *K. pneumoniae*

Organism	Percentage resistance of the isolates (n) %						
	CIP	W	CRO	CN	SXT	AX	C
E. coli	65 (100)	65 (100)	11 (16.9)	0 (0)	65 (100)	65 (100)	33 (50.8)
K.pneumoniae	8 (17.8)	24 (53.3)	35 (77.8)	9 (20)	20(44.4)	45 (100)	0 (0)

KEYS: CIP- Ciprofloxacin, CN- Gentamicin, SXT-Trimetoprim-sulphamethazaxole, AX- Amoxillin, CN- Ceftriaxone, C- Chloramphenicol, W-Trimetoprim.

Percentage yield and qualitative analysis of stem bark of *Artocarpus heterophyllus*

The percentage yield of the extract was 6.1g. The qualitative phytochemical analysis of the extract revealed the presence of some bioactive compounds. Those with greenish blue colour were reported as glycosides, blue-green precipitate were reported as steroids and a reddish brown precipitate formation indicated the presence of alkaloids. The formation of green, blue or blue-black colour indicated the presence of tannins with the formation of red- violet colour as terpenoids. A deep yellow colour mixture which became colourless upon the addition of few drops of diluted H_2SO_4 were reported as flavonoids and a yellowish green precipitate observed in the mixture were reported as anthraquinones. Development of bluish green colour was taken as positive for phenol, whereas those with froth formation were regarded as saponin (Table 3).

Table 3: Phytochemical constituent of stem bark of *A. heterophyllus*

Phytochemical	*A. heterophyllus*
Alkaloid	+
Saponin	+
Tannin	–
Antraquinone	+
Flavonoids	+
Terpenoids	–
Steroids	+
Cardiac glycoside	+
Phenol	+

KEYS: (+) = Present, (-) = Absent.

Antibacterial activity of stem bark of *A. heterophyllus*

Of all the 110 *E. coli* and *Klebsiella pneumoniae* that were subjected to the stem bark extract of *A. heterophyllus,* it was observed that there were varied zones of inhibition as the isolates were concentration dependent. All the isolates of *E. coli* did not respond to the inhibitory effect of the extracts at lower concentrations (12.5- 50 mg/ml) when compared to the control drug at 100 mg/ml (17 mm- 26 mm). However, at 100 mg/ml, there was evidence of inhibitory effect with the highest inhibition of 12 mm and lowest of 3 mm (Table 4). On the contrary, some of the isolates of *Klebsiella pneumoniae pneumoniae* responded to the inhibitory effect of the extracts at 25 mg/ml (5 mm – 7 mm) and at 50 mg/ml (6 mm – 8 mm). At 100 mg/ml, highest inhibitory effect observed was 9 mm and lowest at 6 mm (Table 4).

Table 4: Distribution of the Isolates based on their response to the extract at different concentrations

Organisms	Total number of isolates tested	Concentration of the extract (mg/ml) / number of isolates that responded			
		12.5 mg/ml	25 mg/ml	50 mg/ml	100 mg/ml
E. coli	65	00	00	00	35
K. pneumoniae	45	00	07	07	24
Total	110	00	07	07	59

DISCUSSION

In this study, amongst the isolates from urine, stool and wound, *E. coli* was found to be the most prevalent, at 59.1 %, followed by *K. pneumoniae (*40.9 %). *E. coli* being the most predominant, was is in accordance with the study of Joan *et al*., 2013 who recorded 70 % of *E. coli* in UTI cases. Among the 110 bacterial isolates, 70.9 %, 80.9 %, 41.8 %, 8.18 %, 77.3 %, 100 %, 30 % were found to be resistant to Ciprofloxacin, Trimetoprim, Ceftriaxone, Gentamicin, Trimetoprim-Sulphamethazaxole, Amoxyllin and Chloramphenicol respectively. Among the antibiotics tested Amoxylin was found to be least effective, this could be due to the fact that it is the most widely used because of its easy access, while Gentamicin was the most effective, this also could be due to the fact that Gentamicin is parenteral in nature, hence it is not easily abused by users. The antibiotic susceptibility pattern of *K. pneumoniae* is similar to that of (Azar and Ebadi, 2017 and Osagie *et al*., 2017). *A*ll isolates of *E. coli* showed 100 % resistance to Amoxillin, Trimethoprim, Trimetoprim-sulphamethazole, is in agreement with the report of Reuben and Owuna, (2013). From this study it can be inferred that Amoxillin, Trimetoprim and Trimetoprim-sulphamethazole could not be used in the treatment of *E. coli*. One hundred (100 %) resistance to Ciprofloxacin was also reported which is similar to the findings of Omololu-Aso *et al*. (2017a) who reported 92.8 % to Ciprofloxacin, and Zare *et al*. (2018) reported 35.5 % resistance.

The percentage yield of *A. heterophyllus* is 6.13 g. The phytochemical analysis of *A. heterophyllus* revealed the presence of flavonoids, saponins, alkaloids, steroids, cardiac glycoside, anthraquinone, and phenol. These findings concurred with those obtained in a previous study carried out in Nigeria, by Ajiboye e*t al*. (2018). However, the finding was contrary to those obtained in a study carried out in Sri Lanka by Sivagnanasundaram and Karunanayake, (2015*),* who reported that the extract from stem bark of *A. heterophyllus* does not contain alkaloids, but terpernoid. This difference could be associated with the geographical and environmental factors of the area from which the plant was collected.

The result also agreed with the findings of other studies that reported the presence of Saponins, Tannins, Amino acids, Proteins, Cardiac glycosides, Alkaloids, Carbohydrates and Flavonoids (Muthukumar *et al*., 2018; Khobe *et al.*, 2017).

Extract of *A. heterophyllus* showed antibacterial inhibitory effect against some of the isolates tested with varied zones of inhibition as the isolates were concentration dependent. All the isolates of *E. coli* did not respond to the inhibitory effect of the extracts at lower concentrations (12.5- 50 mg/ml) when compared to the control drug at 100 mg/ml (17 mm- 26 mm). However, at 100 mg/ml, there was evidence of inhibitory effect with the highest inhibition of 12 mm and lowest of 3 mm. The inhibitory effect of the plant extracts against *E. coli* is similar to the report of Sivagnanasundaram and Karunanayake, (2015), who found out that the ethanoic stem bark extracts (30 mg/ml) of *A. heterophyllus* and *A. altilis* possessed significant antibacterial activity against *Escherichia coli* with 9.50 ± mm zone of inhibitions. On the contrary, some of the isolates of *Klebsiella pneumoniae* responded to the inhibitory effect of the extracts at 25 mg/ml (5 mm – 7 mm) and at 50 mg/ml (6 mm – 8 mm). At 100 mg/ml, highest inhibitory effect observed was 9 mm and lowest at 6 mm. The antibacterial activity of the plant in this study could be attributed to the presence of phytochemicals which have been reported to poses antimicrobial properties (Falowo *et al*., 2017).

CONCLUSION

The extracts had a dose - dependent inhibitory properties against all bacterial isolate from this study. The findings in this work have justified the traditional use of this plant in ethno medicinal treatment of oral infection, dysentery, fever, diarrhea, wound, tooth decay, and various stomach related problems which are caused by some of these organisms used in this study. The antibacterial activity of the extract was further supported by the presence of phytochemicals in the extract which are known to possess antimicrobial properties. Further research on isolation and characterization of the specific chemical compounds that is responsible for the antibacterial activity of the extract is of great importance. The result of the study will also need to be confirmed using in vivo models and also the toxicities of the active compounds should be assessed in order to establish their practical applications. Determination of the possible mechanism of antibacterial action of the extracts is important and most importantly to popularize the use and utilization of *A. heterophyllus* in Nigeria by planting and creating awareness.

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