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Antibacterial Activity and Phytochemical Screening of *Acalypha wilkesiana* (Copperleaf) Leaf Extract on Some Clinical Isolates

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ABSTRACT

Ethanolic extract of fresh leaves of *Acalypha wilkesiana* was screened for its phytochemical and in-vitro antibacterial properties on four clinical isolates *Staphylococcus aureus, Escherichia coli* , *Shigella* sp , and *Klebsiella pneumonia*. The disc diffusion technique was used to assay for the antimicrobial properties. The results showed that the extract of concentrations between 50mg/ml to 200mg/ml inhibit the growth of all test organisms. The Minimum Inhibitory Concentration (MIC) determined by tube dilution method were 150mg/ml for *Staphylococcus aureus* and *Klebsiella pneumonia*, 100mg/ml for *Escherichia coli* and *Shigella* sp respectively, while the Minimum Bactericidal Concentration (MBC) determined using the media dilution technique showed that at 200mg/ml *Escherichia coli* and *Shigella* sp were killed. The phytochemical screening of the leaf extract revealed the presence of Saponins, Tannins, Alkaloids, Phlobatins, Phenolics and Cardiac Glycosides. Some of which may have contributed to the observed anti-bacterial activity.

Keyword: *Acalypha wilkesiana,* Antibacterial, Phytochemical, Susceptibility

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INTRODUCTION

Plants have been a source of medicine in the past centuries and today scientists and the general public recognize their value as a source of new or complimentary medicinal products [1]. This plant-based, traditional medicine system continues to play an essential role in health care, with about 80% of the world's inhabitants relying mainly on traditional medicines for their primary health care [2]. Long before mankind discovered the existence of microbes, the idea that certain plants had healing potential and that they contained what we would currently characterize as antimicrobial principles, was well accepted. Since antiquity, man has used plants to treat common infectious diseases and some of these traditional medicines are still included as part of the habitual treatment of various maladies [3].

During the last two decades, there has been a considerable increase in the study and use of medicinal plants all over the world especially in advanced countries. Medicinal plants have been used in Africa before the introduction of antibiotics and other modern drugs [4].

The success story of chemotherapy lies in the continuous search for new drugs to counter the challenge posed by resistant strains of microorganisms. The investigation of certain indigenous plants for their antimicrobial properties may yield useful results. Many studies indicate that some plants have substances such as peptides, unsaturated long chain aldehydes, alkaloids, essential oils, phenolics, as well as different ethanol, chloroform, methanol and butanol soluble compounds. These plants have emerged as plants with compounds possessing significant therapeutic potential against human pathogens, including bacteria, fungi or virus [5].

Nigeria has a great variety of natural vegetation, which is used in trado-medicine to cure various ailments [6]. Among the plants used for medicinal purpose in Africa, particularly in Nigeria is *A. wilkesiana, is* an evergreen, tropical shrub that has been cultivated in Nigeria for decades.

Acalypha wilkesiana belongs to the Euphorbiaceae Family comprises about 570 species. Some of the common names of *A. wilkesiana* include: Fijian Fire Plant, Fire Dragon Plant, Beefsteak Plant, Hudling , Redleaf, Josephs Coat, Hoja de Cobre Huu-Krataai, Aworoso, and Match- Me-If-You-Can. It is believed to be originally native to South Pacific Islands. It is a tropical shrub and grows best in the southern and central parts of Florida. Most Copperleaf cultivars grow 5-7 feet tall. It is grown for its colored foliage, many cultivars with different leaf shapes, sizes and colors have been developed and grow best in fertile, well drained soil [7].

A. wilkesiana has been known to be used in most part of Nigeria to treat antifugal and antibacterial ailment especially in the western part of Nigeria. The vast use of *A. wilkesiana* in the treatment of various bacteria ailment in western Nigeria gives a great course for study. Also, there is a need to identify novel substances that are active towards microorganisms of concern.

The aim of this study is to compare antibacterial activity of ethanolic extract of *A. wilkesiana* with already identified antibiotics and also to determine the phytochemical components of the plant.

MATERIALS AND METHODS

Collection of Plants Materials

Fresh plants leaves were collected from the premises of Salem University Lokoja. The plant was identified and authenticated in the Botany Unit of the Department of Biological Science, Kogi State University Ayingba, Nigeria.

Test Organism

Four bacteria species were collected from Zankli Medical Centre Mabushi Abuja Nigeria. The bacteria used were *Escherichia coli, Shigella* sp*, Staphylococcus aureus and Klebsiella pneumonia.*

Extraction and Preparation of Dried Ethanolic Extracts

Plant extract was prepared according to [8]. 15g of powdered dried leaf sample was weighed into a thimble and placed in the soxhlet extractor. The extraction was carried out at 70 – 100^{0}C. The filtrate was concentrated using vacuum evaporator , the concentrated extract was stored at room temperature for further use. The yield of residue was noted, a portion of it was used to test for the following constituents: alkaloids saponins phlobatins steroids triterpenoids glycosides and phenolics.

Antibacterial Assay of Selected Plant

The agar diffusion technique was used. Twenty milliliters of sterile Muller Hinton Agar in Petri dishes were seeded with standardized innocula using sterile swab stick. Wells of 6.0 mm in diameter were cut on the seeded plate and each of them filled with the prepared leaf extract of different concentration. The extract was left to diffuse into the medium for 1hour and the plates incubated for 24 hours at 37^{0}C. The ethanol was used as a control [9].

Minimal Inhibitory Concentration (MIC) of Extract

The minimal inhibitory concentration (MIC) of extract was determined using the tube dilution method [10]. Different concentration of the extract ranging between 10mg/mi and 200mg/ml were prepared and standardized inoculums of the test organisms added. Control cultures without extract were set up, both control and experimental tubes incubated at 37^{0}C for 24hrs. The MIC was reported as the lowest concentration extract that showed no visible growth.

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Minimum Bactericidal Concentration

All the MIC tubes with no visible growth were plated out on Muller Hinton Agar and incubated at 37^0C 24 hours. The MCB was taken to be the lowest concentration that did not produced colonies on Muller Hinton Agar [11].

Sensitivity Test

The antibiotics susceptibility of the isolate was determined by the disc diffusion method on Muellar Hinton Agar. The antibiotic multi-disc containing for Gram positive (G-ve); septrin, Chloranphenicol, sparfloxacin, ciprofloxacin, amoxicillin, augmentin, gentamycin, pefloxacin, tarivid, streptomycin and for Gram +ve; pefloxacin, gentamycin, ampiclox, zinnacef, amoxicillin, rocephin, ciprofloxacin, streptomycin, septrin, erythromycin, was used. The inoculum was standardized by adjusting it's density to equal of a barium sulphate ($BaS0_4$) at 0.5 McFarland turbidity standards, and incubated at 37^oC for 18hrs. The diameter of the zone of clearance was measured to the nearest millimeter (mm) [10].

Phytochemical screening of ethanolic extract of *Acalypha wilkesiana*

The methods described by [12] were used to test for the presence of tannin saponins phenolics and alkaloids while [13] was adapted for the presence of steroid, tritepens , phlobatins and glycoside

RESULTS

The data obtained in Table 1 shows antibacterial activity of the leaf extracts of *A. wilkesiana.*. The extract shows an effective level of susceptibility to the organisms; *Staphylococcus aureus* and *Klebsiella pneumonia*. at a concentration of 150 - 200 mg/ml. *Escherichia coli* and *Shigella* sp were susceptible at a concentration of 100-200 mg/ml. The MIC and MBC ranged between 100 to 200mg/ml (Table 2). Tables 3a and 3b show the result of the sensitivity of the test organisms to commercial antibiotics. The test result shows that the organisms of study are resistant to most of the orthodox/regularly clinically used antibiotics. However *Shigella* sp showed a relatively high susceptibility to these antibiotics than other organisms.

The result of the phytochemical analysis obtained from the aqueous leave extract of *A.wilkesiana* (Table 4) indicated that tannins and phenolics were highly present in the extract. Phlobatanins, cardiac glycosides, saponins and alkaloids were present but in mildly.

Table 1: Antibacterial activity of the leaf extract of *Acalypha wilkesiana*

Organism/ Concentration of extract (mg/ml)	Zones of inhibition of extract (mm)					
	10	50	100	150	200	Control Ethanol
Shigella sp	0.0	8.0	10.0	11.0	15.0	0.0
Klebsiella pneumonia	0.0	7.0	10.0	13.0	17.0	0.0
Staphylococcus aureus	0.0	0.0	10.0	13.0	17.0	0.0
Escherichia coli	0.0	0.0	0.0	12.0	13.0	0.0

Values are means of replicate

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Table 2: Bactericidal and Bacteristatic effect of ethanolic extract of *Acalypha wilkesiana*

Organisms\ concentration of extract(mg/ml)	Concentration of extract (mg/ml)						Effect of extract
	0	10	50	100	150	200	
Escherichia coli	-	-	-	+	+	+	BC
Klebsiella pneumonia	-	-	-	-	+	+	BS
Staphylococcus aureus	-	-	-	-	+	+	BS
Shigella sp	-	-	-	+	+	+	BC

Values are means of replicate

Key

+ = Strong growth inhibition of the organism

- =No growth inhibition of the organism

BC = Bactericidal: when low concentration inhibited bacteria growth

BS = Bacteristatic when high concentration kills bacteria growth

Table 3a: Antibiotic sensitivity of gram negative isolates

Organism	Zones of inhibition in Antibiotics disc (mm)									
	SXT 30 µg	CH 30 µg	SP 10 µg	CPX 10 µg	AM 30 µg	AU 30 µg	CN 10 µg	PEF 30 µg	OFX 10 µg	S 30µg
Escherichia coli	R	8.0	R	R	R	R	R	R	R	R
Klebsiella pneumonia	R	12.0	11.0	R	R	13.0	R	R	R	R
Shigella sp	17.0	18.0	16.0	16.0	16.0	10.0	15.0	18.0	18.0	17.0

Values are means of replicate

Key:

SXT---Septrin, CH----Chloranphenical, SP---Sparfloxacin, CPX---Ciprofloxacin

AM----Amoxacillin, AU----Augmentiin, CN----Gentamycin, PEF---Pefloxicin

OFX----Tarivin, S---Stretomycin, R--- Resistant

Table 3b: Antibiotic sensitivity of gram positive isolates

Organism	Zones of inhibition in Antibiotics disc (mm)									
	PEF 10 µg	CN 10 µg	APX 30 µg	Z 20 µg	AM 30 µg	RO 25 µg	CPX 10 µg	S 30 µg	SXT 30 µg	E 10µg
Staphylococcus aureus	12.0	12.0	R	R	R	R	R	5.0	R	R

Values are means of replicate

Key:

R---Resistant, Ro ----Rocephin, E----Erythromycin, AM---Amoxacillin , Z---Zinnacef

CPX---Ciprofloxacin, CN---Gentamycin, PEF----Pefloxin, S---Streptomycin,

SXT----Septrin, APX----Ampiclox

Table 4: Results of Phytochemical screening of ethanolic extract of *Acalypha wilkesiana*

S/N	Chemical constituents	Present or Absent
1	Saponins	+
2	Tannins	++
3	Phenolics	++
4	Alkaloids	+
5	Phlobatins	+
6	Cardiac glycosides	+

Key:

+ = Present

++ = Strongly Present

DISCUSSION

Many studies have established the usefulness of medicinal plants as a great source for the isolation of active principles for drug formulation [14]. In most cases, the traditional preparation of crude drugs from medicinal plants for the treatment of diseases involves cold or hot water or ethanolic extractions of parts of the plants such as roots, stem, barks and leaves [15]. Have indicated that the efficiency of the extraction procedure depends upon the acceptability of the constituents to the solvents. Local or traditional extractions of plant materials for medicinal use involve the local gin (ogogoro). Local gin which is from fermented palm wine distillation is known to contain high concentration of alcohol, when these solvents are used as herb extractants, it may be possible that bioactive substances that are less soluble in water would then be dissolved by the solvents.

Several investigation on medicinal plants have indicated that organic solvents such as alcohols are extensively used for crude extraction before being re-extracted to obtain purified active compounds using some other organic solvents [16,17].

Several species of the genus *Acalypha* have been studied and it has been demonstrated that they have antioxidant, wound healing, post-coital antifertility, neutralization of venom, antibacterial, antifungal and antitrypanosomal activities [18].

The results of this study support the antibacterial and activities of *A. wilkesiana* as a broad spectrum antibacterial agent since it inhibited the growth of Gram-positive (*S. aureus*, and gram negative bacteria (*E. coli, K. pneumonia and Shigella* sp). The fact that the Ethanolic extract of *A. wilkesiana* and its fractions showed activity against most of the test organisms is a major breakthrough in appreciating the medicinal potential of the plant especially in the management of clinic community acquired and nosocomial associated infections.

Comparison of the extract with the commercial antibiotics shows that the extract shows high level of inhibition towards the tested organisms as the commercial antibiotics. Some of the organism that was resistant to most of these antibiotics was susceptible to the extract. This shows that the ethanolic extract of *A. wilkesiana* can be used as a broad spectrum antibiotic.

Results of the phytochemical screening show the presence of some bioactive components. Some of them include tannins and phenolics. Some of these components however, were present in minute quantities; saponins alkaloids, phlobatins and cardiac glycolysis. Several plants which are rich in phenolics compounds and alkaloids have been shown to possess anti-microbial activities against a no of microorganisms

CONCLUSION

It seems reasonable to conclude that *A. wilkesiana* possess anti-bacterial active principles capable of inhibiting the growth of a number of common pathogenic microorganisms as shown by the four tested bacteria. If these active chemical principles are

trapped they will be a great asset to drug development for the purpose of health care delivery, especially in Nigeria.

This finding is significant because most bacteria have been reported to be completely resistant to the action of most antibacterial drugs available in Nigeria. Further study needs to be carried out on the isolation of the bioactive components of the tested plants and their effect vivo.

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