

PHYTOCHEMICAL COMPOSITION OF ACALYPHA WILKENSIANA AND ITS ANTIMICROBIAL ACTIVITY

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Abstract

This study was designed to assess the phytochemical composition as well as the antimicrobial efficacy of *Acalypha wilkensisiana* leaf extracts.

Antimicrobial activity of the leave extracts were determined by measurement of the zone of inhibition of the extract on the test organisms, measurement of minimum bactericidal concentration (MBC) and minimum inhibitory concentration (MIC). The antimicrobial activity was performed by agar well diffusion method.

The phytochemical analysis of the methanol leaf extract showed the extract contains anthraquinones, flavonoids, phenols and alkaloid (being the chief constituent). The leaf extract showed high microbial activity on the bacteria at higher concentration and comparatively lower antifungal activity on the studied fungi. The highest antimicrobial effect was observed on the highest concentration (200 mg/ml) of the extract on *P. aeruginosa* which was more than the activity of the standard drug streptomycin. Activity of the extracts on the organism was bactericidal except on *E. coli* (where MBC: MIC ratio > 4).

A. wilkensisiana contains bioactive compounds and possess antimicrobial activity on both gram positive and negative bacteria tested as well as the fungi. It has greater potential to be harnessed as an antimicrobial agent against *P. aeruginosa*.

Keywords: *Acalypha wilkensisiana*; *A. wilkensisiana*; antimicrobial activity; antimicrobial potential.

Introduction

The practice of seeking medicinal remedies via plant parts such as leaves, barks, roots has been in practice in Africa since ancient times.¹ The struggle between man and illness as well as drugs resistance has led to the “look back” to nature in the form of medical plant.^{2,3} Plant based antimicrobials represent a vast untapped source.⁴ *Acalypha wilkensisiana* belongs to the spurge family (Euphorbiaceae) and belongs to the genus *Acalypha*. It is commonly called Copper leaf, Joseph’s coat and Fire dragon.⁵ The genus *Acalypha* comprised about 570 species which are mainly weeds while the rest are ornamental plants.⁶ *A. wilkensisiana* is a popular outdoor plant native to Fiji and surrounding Islands in the pacific, but has spread to other parts of the world especially tropics of Africa, Asia and America.⁷ It is a fast growing evergreen shrub that tints the landscape with bronze red to mute red while the leaves have heart-shape with combinations of colours such as green, purple, yellow, white, pink, orange.⁸ The leaves are compound, alternate, coordinate, or heart shape and petiolated.⁶

Report has it that local populace in Nigeria uses *A. Wilkensisiana* leaves for herbal remedy for treatment of undefined skin infection in children.⁹ Consequently, this study sets off to establish the phytochemical constituent as well as antimicrobial activities of *A. Wilkensisiana*.

Methods

Isolation of bacterial and fungal species

Bacterial and fungal culture of the test organisms; *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Aspergillus niger* and *Candida albicans* were obtained from the microbiology laboratory, University of Calabar Teaching Hospital, Calabar, Nigeria. The test organisms were confirmed via microscopic, morphological, culture and biochemical analysis as described by Cheesbrough et al., taxonomical guide and standard operational procedures.^{10,11,12}

Collection and identification of plant material

Fresh leaves of *A. Wilkensisiana* were harvested from a domestic garden at satellite town, Calabar, Nigeria. Same was identified at the department of Botany, University of Calabar, Nigeria. There was no requirement of permission for plant collection. The plant was formally identified at the Botany Department, University of Calabar and a voucher specimen deposited.

Preparation of Leaves Extract

The harvested leaves were air-dried and grounded into powder. Following grinding, 200 g of the powder were suspended in 1200 ml of ethanol (98.67% BDH) and agitated properly for 10 minutes using an electric blender. The suspension was stored in a refrigerator at 4°C for 24 hours. Next, the mixture was filtered using chess cloth and the resultant filtrate concentrated in vacuo (rotary evaporator) to 10% of the original volume at 40 °C. The resulting concentrate was kept at 37 °C in water bath for complete dryness. The extract was constituted for the various concentration required for the antimicrobial assessment.

Phytochemical analysis of the extract

Qualitative Analysis

The qualitative analysis of the phytochemical component of *A. Wilkensisiana* were performed using Dragendorff method for alkaloid; flavonoids, by sodium hydroxide method; anthraquinones by modified Borntrager test; Saponin by emulsifying and frothing test; quinonechlorimide method for phenols and ferric chloride and lead acetate methods for tannins. The full details of the methods is contained in our previous article.¹

Quantitative analysis

- i. Determination of anthraquinone content: Approximately 5 ml of the plant extract was soaked on 50 ml of distilled water for 16 hours. The suspension was then heated at 70°C for one hour using the water bath. After the suspension was cooled, 5 ml of 50% methanol was then added and filtered. The clear solution was then

- measured by UV Spectrophotometer at a wavelength of 450 nm and then compared with a standard solution containing 1 mg/100 ml purpurin with the absorption maximum 450nm.¹³.
- ii. Determination of total phenolic content: The modified Folin-ciocalteau method was used. Approximated 2.5ml of 1% Folin-ciocalteau reagent and 2% sodium carbonate solution were added to 1 ml of the leaf extract. The resultant mixture was incubated for 30 minutes at room temperature. The resultant mixture was assayed for Phenolic content spectrophotometrically at 765 nm using Gallic acid as standard¹⁴.
 - iii. Determination by total flavonoids: The alluminium chloride method was used in the assay of the total flavonoid content involving little modification. Approximately 1 ml of the leaf extract was added to the following reagents; 3 ml methanol, 0.2 ml of 10% AlCl₃, 0.2 ml of 1 m potassium acetate and 5.6 ml of distilled water and kept at room temperature for 30 minutes. This was followed by colometric reading at 420nm using quercetin as standard.¹⁴
 - iv. Determination of Total alkaloids: The total alkaloids contents was estimated using the UV-spectrometer method¹⁵. Approximately 5 ml of the plant extract was dissolved in 2N HCl and then filtered. One milliliter (1 ml) of the resulting filtrates was then transferred to separating funnel and washed with 10 ml chloroform. The pH of the buffer solution was adjusted to neutral using 0.1N NaOH. Consequently, 1 ml of the resultant solution was transferred to separating funnel followed by addition of 5ml bromocresol solution and 5 ml of phosphate buffer. The mixture was shaken and the resultant complex was fractioned by vigorous shaking with chloroform. The resulting fractions were collected in a 10 ml volumetric flask followed by dilution to volume using chloroform. The absorbance of the complex in chloroform was measured spectrophotometrically at 470 nm.¹⁶.
 - v. Determination of total saponins: Ten milliliter (10ml) of the sample was transferred into a conical flask and 50 cm³ of aqueous ethanol added. The mixture was then heated over a water bath for 4 hours with continuous stirring at about 55°C. The resultant mixture was filtered and the residue re-extracted with another 100 ml 20% ethanol.¹⁷

Determination of antimicrobial activity

The antimicrobial activity of the ethanol leaf extract was performed using agar well diffusion method. This was determined by measuring the zones of inhibition against the test organism. The test organism was inoculated on sterile Mueller-Hinton agar plate (for the bacteria) and Sabouraud dextrose agar (for the fungi) using swab stick. Wells were then made on the inoculated plates using a sterile cork borer. Exactly 50 µL of the respective concentration of the extracts were transferred into the wells using sterile micropipette. The controls (positive and negative) were treated the same way in separate wells. The diameter of the zones of inhibition were measured (in mm) after incubation of the plates for 24 hours at 37°C for the bacteria and 3 days for the fungi. The zone of inhibition is marked by clear areas without growth around the well.^{10,18} Each assay were repeated twice.

Determination of minimum inhibition concentration (MIC)

The minimum inhibitory concentration of the ethanol leaf extracts of *A. wilkensisiana* was performed using the selected bacterial and fungi separately. Concentrations ranging from 50 mg/ml to 200 mg/ml of the extracts were prepared and 500 µL of each dilution were

incubated with 5ml of Mueller Hinton broth and Sabouraud dextrose agar containing 0.1 ml of each of the bacterial and fungal suspension respectively at 37°C for 24 hours (for the bacteria) and 3 days at 30 °C (for the fungi). Following incubation, the tubes were examined for bacterial and fungal growth by observing turbidity of the broth. The minimum inhibitory concentration was determined as the minimum concentration that showed no visible growth.¹⁹

Determination of minimum bactericidal concentration (MBC)

It is determined by re-culturing (sub-culturing) broth dilutions with no visible growth from MIC (i.e. those at or above MIC). Exactly 10 µL of each culture medium for bacteria and fungi with no visible growth were removed from each well and inoculated in duplicates in nutrient agar plates, respectively. Following incubation for 24 hours at 37°C for bacterial and 3 days at 30°C for fungi. Minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) are defined as the lowest extract concentration at which 99.9% of the microorganism are killed. Literally, it is the lowest broth dilution of the antimicrobial that prevents growth of the organism on the agar plate. Failure of the organism to grow on the agar plate means that only non-viable organism were present^{20,21}.

Ethics consideration

This study was approved by Health Research Ethical Committee (HREC) of the University of Calabar Teaching Hospital.

Statistical Analysis

Data were analysed using SPSS version 20 (Armonk, NY. IBM Corp.). Percentage, description statistics were used to report the data.

Results

Table 1 shows the qualitative phytochemical composition of *A. wilkensisiana*. The phytochemical analysis showed that the leave extract contains alkaloid, flavonoid,

anthraquinone, saponin and phenol. Tannin and saponin were absent.

Table 2 shows the quantitative phytochemical composition of *A. wilkensisiana*. The result showed that alkaloids are contained in large amount (24.00 ± 0.16) compared to other phytochemical constituents. However, the result were comparative below for Anthraquinones (0.04 ± 0.03 mg/100g), flavonoids (0.40 ± 0.06 mg/100g) and phenols (0.48 ± 0.04 mg/100g).

The methanol extracts of *A. wilkensisiana* showed significant antimicrobial effect (inhibitory effect) to the growth of gram positive and gram negative bacteria as well as the fungi. The mean zone of inhibition for the gram positive bacteria (*S. aureus*) ranged from 0 to 15.5 mm in a dose dependent manner. On the other hand, the mean zones of inhibition of the gram negative bacteria (*Pseudomonas aeruginosa* and *Escherichia coli*) ranged from 5.5 mm to 22.00 mm that was also in a dose dependent manner. Overall, *A. wilkensisiana* extracts showed high antimicrobial activity at higher concentration (200 mg/ml) even above the inhibition activity of the standard drug/positive control (streptomycin; 20.00 mm). However, it performed comparatively poor in antifungal activity (Table 3).

The MIC of the gram positive bacteria; *S. aureus* was 100 mg/ml while the MBC was 250 mg/ml. On the other hand, the MIC of the gram negative bacteria (*P. aeruginosa* and *E. coli*) were both 50 mg/ml whereas their MBC were 200 mg/ml and 250 mg/ml, respectively. The MIC of the fungi (*C. albicans* and *A. niger*) were 100mg/ml and 200 mg/ml, respectively while their MFC were 300 mg/ml and 400 mg/ml, respectively (table 3). Using the MBC: MIC (for bacterial) and MFC: MIC (for the fungi) ratios, we observed that the inhibitory effect of *A. wilkensisiana* on *S. aureus*, *P. aeruginosa*, *C. albicans* and *A. niger* were bactericidal whereas the effect on *E. coli* is bacteriostatic (Table 3).

Discussion

Phytochemicals are non-nutritive components present in plant that exert protective/disease preventing effect or treatment of chronic diseases²². The phytochemical analysis of *A. wilkensisiana* showed the presence of phenols, volatile organic matter, flavonoid, alkaloid and anthraquinone with alkaloids occurring in comparatively higher quantity. Unlike in our study, Evangelene et al.,²³ in an earlier study reported presence of saponin, and triterpenes in addition to the finding of this study but reported the absence of alkaloids.

In the current study, the *A. wilkensisiana* extract showed promising antimicrobial activity against *S. aureus*. The *A. wilkensisiana* extracts showed maximum 15.50 mm zone of inhibition in the 200 mg/ml concentration which is highly promising considering the zone of inhibition of the standard drug (25.00 mm). The MIC for *A. wilkensisiana* extract on *S. aureus* was 100 mg/ml while the MBC was 200 mg/ml giving an MBC:MIC ratio of 2.5 which is an indication of a bactericidal activity. Bactericidal activity has been defined as a ratio of MBC:MIC > 4.²⁴

A. wilkensisiana leaf extract showed maximum inhibition zone (22.00 mm) in the 200 mg/ml followed by the 20.00 mm in the 100 mg/ml on the *P. aeruginosa* which was 2mm larger than that of the standard drug Streptomycin itself. The MBC was 200 mg/ml while the MIC was 50 mg/ml with an MBC:MIC ratio of 4:1, indicating that *A. wilkensisiana* extract exerted a very potent bactericidal effect at both higher and lower concentration of the extract than in any other tested bacteria.

Bacteriostatic (MBC : MIC of 5.0) activity of *A. wilkensisiana* leaf extract was observed in the *E. coli* isolate with 200 mg/ml concentration which is 6 mm shy away from the inhibition zone of the standard drug streptomycin (20.00 mm). However, the lower concentration performed poorly (7.50 mm for 100 mg/ml and 6.20 mm for the 50 mg/ml).

Though with lower zone of inhibition, the *A. wilkensisiana* leaf extracts showed promising antifungal potential against the two fungal species (*C. albicans* and *A. niger*) performing better than the standard antibacterial drug streptomycin. Both showed fungicidal activity (*C. albicans*: MBC : MIC = 3.0; *A. niger*: MBC: MIC = 2.0) at high concentration (200 mg/ml) but performed poorly at low concentration.

The findings of this study is in tandem with previous studies^{25,26} that reported antimicrobial activities of *A. wilkensisiana* especially on *P. aeruginosa*, *S. aureus*, *E. coli*, *Candida* spp and *Aspergillus* spp. This activity could be attributed to bioactive phytochemical contents.

The excellent antimicrobial activity of the *A. wilkensisiana* leaf extract on *P. aeruginosa* opens up a new leaf of opportunity on alternative to already available synthetic antibiotics considering the bad history of sensitivity of *P. aeruginosa* to antibiotics. *P. aeruginosa* has been known to resist many of the currently available antibiotics. The World Health Organization in not too long time listed carbapenem-resistant *P. aeruginosa* as one of the three bacterial species in which there is a critical need for the development of new antibiotics to treat infection.^{28,29}

In conclusion, the result of this study, ethanol extracts of *A. wilkensisiana* plant contains bioactive phytochemical and possess antimicrobial activity at higher concentration against some selected gram positive and gram negative bacteria and fungi. This observation has substantiated the logic behind the use in folk medicine and open up a new leaf for harnessing the plant as an antimicrobial agent.

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Table 1: Qualitative phytochemical screening of ethanolic leaf extract of *A. wilkensisiana*

Phytochemicals	Ethanol extract
Phenols	+
Volatile organic matter	++
Flavonoid	+
Alkaloid	+++
Anthraquinone	+
Saponin	-
Tannin	-

+++ : present in appreciable quantity; ++ : present; + : present in low quantity; - : absent

Table 2: Quantitative phytochemical screening of methanolic leaf extract of *A. wilkensisiana*

Phytochemicals	Concentration (mg/100g)
Phenols	0.48 ± 0.04
Volatile organic matter	3.68 ± 0.02
Flavonoid	0.40 ± 0.06
Alkaloid	24.00 ± 0.16
Anthraquinone	0.04 ± 0.03

Values are expressed as mean standard ± deviation
Result performed in triplicates (n=3)

Table 3: Antimicrobial activity of methanolic leaf extract of *A. wilkensisiana*

Organism	MIC (mg/ml)	MBC (mg/ml)	MBC:MIC ratio	Antimicrobial mode	Zone of inhibition of extract (mm)			Zone of inhibition of control	
					200mg/ml mean \pm SD	100mg/ml mean \pm SD	50mg/ml mean \pm SD	Streptomycin (positive control)	Solvent (negative control)
<i>S. aureus</i>	100	250	2.5	Bactericidal	15.50 \pm 0.71	11.50 \pm 0.71	R	25.00	R
<i>P. aeruginosa</i>	50	200	4.0	Bactericidal	22.0 \pm 0.00	20.00 \pm 0.00	18.50 \pm 0.71	20.00	R
<i>E. coli</i>	50	250	5.0	Bacteriostatic	14.0 \pm 0.00	7.50 \pm 0.71	6.00 \pm 0.00	20.00	R
<i>C. albicans</i>	100	300*	3.0#	Fungicidal	13.50 \pm 0.71	5.50 \pm 0.71	R	0.00	R
<i>A. niger</i>	200	400*	2.0#	Fungicidal	8.50 \pm 0.71	R	R	0.00	R

MBC: minimum bactericidal concentration; MIC: minimum inhibitory concentration; * :represents (minimum fungicidal concentration (c)); # : represents MFC: MIC