Journal of Pharmaceutical, Chemical and Biological Sciences
ISSN: 2348-7658
CODEN: JPCBBG
Impact Factor (GIF): 0.701
Impact Factor (SJIF): 3.905
June- August 2017; 5(2):103-107
Published on: 16 July 2017

Research Article

Phytochemical Screening and Antibacterial Activity of *Acalypha wilkesiana* and *Maerua angolensis*

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Received: 31 March 2017 **Revised:** 30 May 2017 **Accepted:** 06 June 2017

ABSTRACT

The crude ethanol extract from *Acalypha wilkesiana* and *Maerua angolensis* were fractionated with n-hexane, chloroform, distilled water, ethylacetate and methanol to afford six soluble fractions. The fractions were tested for antibacterial activity using agar diffusion method and the result revealed some promising activities against the bacterial strains. Phytochemical analysis showed the presence of alkaloids, saponins, tannins, and flavanoids.

Keyword: *Acalypha wilkesiana*; *Maerua angolensis;* phytochemicals; antibacterial activity

INTRODUCTION

Antibiotics continued to provide the basis for the treatment of bacterial infections. Unfortunately however, there are several reports of antibiotic resistance of human pathogens to the available antibiotics [1]. This is largely due to the high genetic variability of bacteria which enables them to quickly circumvent the action of the antibiotics by developing resistance. Hence, the need for new and efficient antibiotics is of paramount importance [2]. Plants have proved to be the source of many pharmaceutical products that are currently used as therapy for various diseases [3-5]. The inspiration which led to the discovery of those plant-derived drugs has always been based on their traditional use as remedy by different cultures around the globe [6]. *Acalypha wilkesiana* is an evergreen shrub which grows in tropical and sub-tropical regions, its ointment is widely used in Nigeria to treat fungal skin diseases [7]. *Maerua angolensis* is a tall tree that grows in tropical Africa and arid regions, its parts are widely used traditionally to treat skin rashes, sores, womb cleansing, and sexually transmitted diseases [8]. The efficacy of these plants in the cure of such infections has attracted our attention to further establish more scientific basis for their application.

MATERIALS AND METHODS

Plant Material

Acalypha wilkesiana and *Maerua angolensis* were collected from Katsina metropolis and its outskirt, respectively. The plants were identified and authenticated at the Department of Biology, Umaru Musa Yar'adua University, Katsina, Nigeria.

Extraction

The ground samples of *Acalypha wilkesiana* and *Maerua angolensis* (200g each) were extracted by

percolation with 800ml of re-distilled ethanol for a period of two weeks. Each extract was concentrated and evaporated to dryness on a rotary evaporator at 40°C to afford ethanol extract [9].

Fractionation of Crude Extracts

The crude extract was prepared as mentioned above and label as F_1. The residue, F_1 was macerated four times with 20mL of n-Hexane and the soluble fraction evaporated to afford n-Hexane fraction, F_2. The insoluble residue was further macerated three times with 20mL each of distilled water and chloroform. The chloroform soluble fraction; F_3, the water soluble fraction; F_4, were separately evaporated to dryness. The chloroform and water insoluble residue was macerated four times with 20mL of ethylacetate. The ethylacetate soluble fraction, F_5, was also evaporated to dryness. While the insoluble residue was further macerated four times with 20mL of methanol and the soluble fraction, F_6, evaporated to dryness.

Phytochemical Screening

Test for Alkaloids

Each extract (0.5g) was stirred with 5mL of 1 per cent aqueous hydrochloric acid on a steam bath; 1mL of the filtrate was treated with a few drops of Mayer's reagent and a second 1mL portion was treated similarly with Dragendorff's reagent. Turbidity or precipitation with either of these reagents was taken as evidence for the presence of alkaloids in the extract being evaluated [10].

Test for Saponins

Each extract (0.5g) was shaken with water in a test tube. Frothing which persists on warming was taken as evidence for the presence of saponins [11].

Test for Tannins

Each extract (0.5g) was stirred with 10mL of distilled water. This was filtered and ferric chloride reagent was added to the filtrate, a blue-black precipitate was taken as evidence for the presence of tannin [12].

Test for Flavonoids

A portion of each extract was heated with 10mL of ethylacetate over a steam bath for 3min. The mixture was filtered and 4mL of the filtrate was shaken with 1mL of dilute ammonia solution. A yellow coloration was taken as evidence for the presence of flavonoids [13].

Test for Anthraquinones

0.5g of crude extract was shaken with 10mL of benzene and was filtered. 0.5mL of 10% ammonia solution was added to the filtrate and the mixture was shaken well and the presence of the violet colour in the layer phase indicates the presence of the anthraquinones [10].

Sources of Microorganisms

Two bacterial strains were used in this study; one gram negative and one gram positive, namely *staphylococcus aureus* and *Escherischia* coli, respectively. All the tested strains were obtained from Federal Medical Centre, Katsina and brought to the Department of Microbiology, Umaru Musa Yar'adua University, Katsina. These bacterial cultures were maintained in nutrient agar slant for further investigation.

Antibacterial Susceptibility Test

Three concentrations for each fraction of the plants extract were prepared as 500µg/mL, 250µg/mL and 125µg/mL. Thus, the stock solution of 500µg/mL of the plant extract was firstly prepared by dissolving 1g of each fraction in 2ml Dimethylsulphoxide (DMSO). Subsequently, 250µg/mL and 125µg/mL were prepared by taking 0.6mL and 0.2mL of the stock solution and then dissolved in 0.4mL and 0.8mL of DMSO, respectively. These concentrations were used for the antibacterial susceptibility test against the selected organisms.

Sensitivity of different bacterial strains to various extracts was measured in terms of zone of inhibition using agar diffusion assay described by Bauer et al [14]. The plates containing nutrient agar were spread with 0.2 mL of the inoculum. Wells were cut out from agar plates using a sterilized stainless steel borer and filled with 0.1 ml of the extract. The plates inoculated with different bacteria were incubated at 37°C up to 48 h and diameter of any resultant zone of inhibition was measured.

RESULTS AND DISCUSSION

Phytochemical screening was employed as a guide in describing the large number of secondary metabolites found in the various extracts of plant. The results (Table 1) revealed the presence of alkaloids, saponins, tannins and flavanoids in all

the fractions. While alkaloids were found to be present in all the fractions of both plants, anthraquinones were found only in one fraction of *Maerua angolensis*. Saponins, tannins and flavanoids were found present in some fractions and absent in some for both plants.

Table 1: Result of Phytochemical Screening

Plant (part)	Fraction	Constituents				
		Alk	Sap	Tan	Ant	Fla
Acalypha wilkesiana (whole plant)	F_1	+	-	+	-	+
	F_2	+	-	-	-	-
	F_3	+	-	-	-	-
	F_4	+	+	+	-	-
	F_5	+	+	+	-	+
	F_6	+	-	+	-	+
Maerua angolensis (leaves)	F_1	+	+	+	-	+
	F_2	+	+	-	-	+
	F_3	+	+	-	+	-
	F_4	+	+	+	-	+
	F_5	+	+	+	-	+
	F_6	+	-	-	-	+

Alk = Alkaloids, Sap = Saponins, Tan = Tannins, Ant = Anthraquinones, Fla = Flavonoids
F_1= ethanol F_2= n-Hexane, F_3= chloroform, F_4= water, F_5= ethylacetate, F_6= methanol
+ = Present, - = Absent

Antibacterial susceptibility test (Table 2 and 3) showed the zones of inhibition measured in millimetre (mm) on the bacteria susceptible to the plant extracts. When these values were translated into a graphical representation, it could be seen that the anti Staphylococcus aureus activity was much observed at 200 µg/mL and 500 µg/mL concentrations for both plants (Figure 1). Specifically an excellent inhibition was found in the F_1 (ethanol) fraction of Acalypha wilkesiana (Figure 1). Similarly the anti Escharischia coli activity depicted on Figure 2 reveals that the activity of both plants appeared to be more potent at higher concentrations (200µg/mL and 500µg/mL). The ethanol fraction (F_5) gave the highest zone of inhibition which is quite agreeable with the anti Staphyloccus aureus activity.

Table 2: Anti *Staphylococcus aureus* activity

Plant Part	Fraction	500	250	125
Acalypha Wilkesiana (whole plant)	F_1	18	12	NA
	F_2	14	12	NA
	F_3	16	15	11
	F_4	16	11	NA
	F_5	12	12	NA
	F_6	16	16	10
Maerua angolensis (leaves)	F_1	12	NA	NA
	F_2	14	14	12
	F_3	16	11	NA
	F_4	NA	NA	NA
	F_5	10	NA	NA
	F_6	14	10	NA
Ciprofloxacin (Standard Drug)		24	22	22

Diameter Zones of inhibition (mm); Concentration (µg/mL); F_1 = ethanol;F_2 = n-Hexane; F_3 = chloroform; F_4 = water; F_5 = ethylacetate; F_6 = methanol; NA= not active

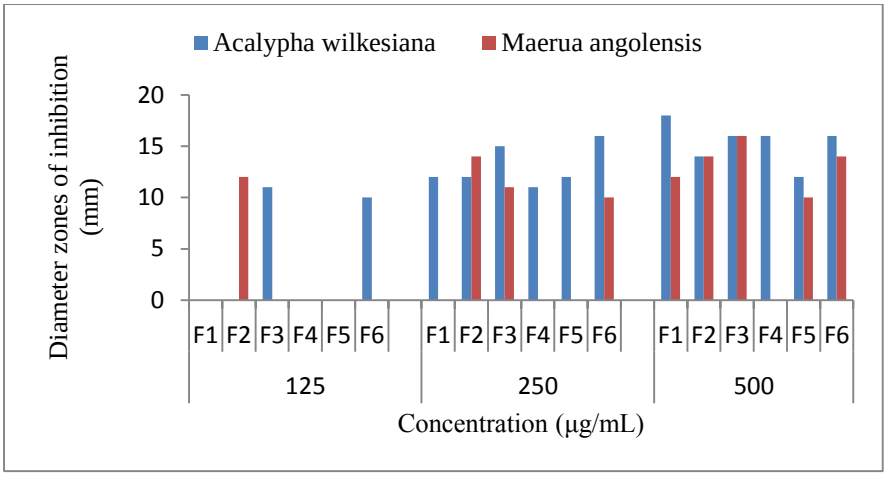

Fig.1. Effect of concentration on the inhibition of *Staphylococcus aureus*

Table 3: Anti *Escharischia coli* activity

Plant Part	Fraction	500	250	125
Acalypha Wilkesiana (whole plant)	F_1	16	12	NA
	F_2	12	09	NA
	F_3	13	NA	NA
	F_4	12	10	NA
	F_5	22	10	NA
	F_6	14	10	NA
Maerua angolensis (leaves)	F_1	13	12	NA
	F_2	12	10	NA
	F_3	14	13	09
	F_4	NA	NA	NA
	F_5	14	10	NA
	F_6	16	12	NA
Ciprofloxacin		30	28	28

Diameter Zones of inhibition (mm); Concentration (µg/mL); F_1 = ethanol; F_2 = n-Hexane; F_3 = chloroform; F_4 = water
F_5 = ethylacetate F_6 = methanol NA= not active

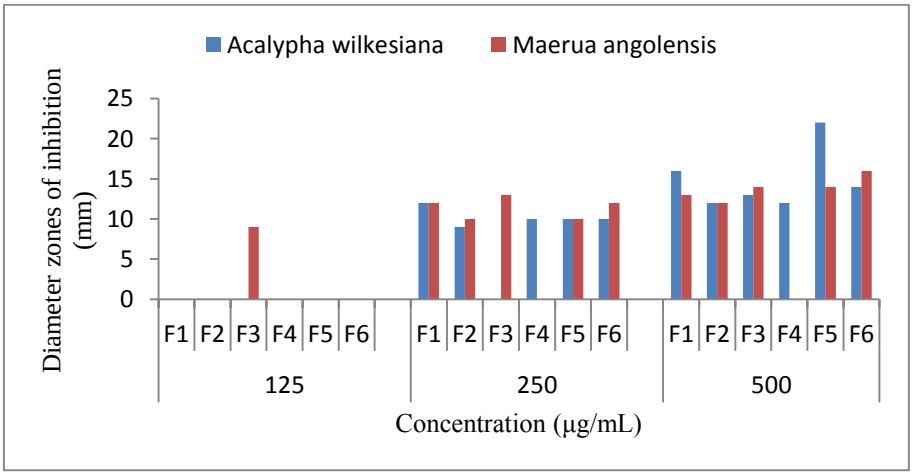

Fig. 2. Effect of Concentration on the inhibition of *Escharischia coli*

CONCLUSIONS

The promising result displayed by the *Acalypha wilkesiana* and *Maerua angolensis* extracts in the antibacterial bioassay provides a scientific basis for the traditional application of these plants in the treatment of different body infections. This further indicates that the fractions are potential source of antibacterial agent(s) that could be effective in the treatment of various bacterial infections.

ACKNOWLEDGEMENTS

The authors are grateful to Fatima Abdulkadir for assisting in the collection of *Acalypha wilkesiana*, Mustapha Haruna for the identification of the plants and Mannir Kabir for the bioassay.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interests.

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Cite this article as:
Amina Sada Yusuf, Ibrahim Sada, Yusuf Hassan, Ibrahim Lawal Kane. Phytochemical Screening and Antibacterial Activity of *Acalypha wilkesiana* and *Maerua angolensis*. J Pharm Chem Biol Sci 2017; 5(2):103-107