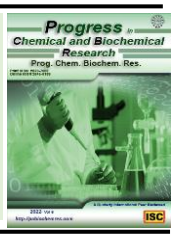
Progress in Chemical and Biochemical Research

Journal homepage: www.pcbiochemres.com



Original Research Article

Qualitative Phytoconstituents and In Vitro Antimicrobial Study of Some Solvent Leaf Extracts of *Acalypha wilkesiana* Mull. Arg. (Euphorbiaceae)

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ARTICLE INFO

Article history

Submitted: 2023-03-13
Revised: 2023-05-12
Accepted: 2023-05-28
Published: 2023-06-09
Manuscript ID: PCBR-2303-1258
DOI: 10.22034/pcbr.2023.389660.1258

KEYWORDS

Plant
Phytochemical
Microbes
Pathogens
Extract

ABSTRACT

This research aimed to investigate *in vitro* antimicrobial property of *Acalypha wilkesiana* against some selected pathogenic microbes that are resistant to drugs. The crude and defatted methanol extracts were screened for the presence of secondary metabolites as well as analysed for *in vitro* antimicrobial activity using agar-well diffusion method. The phytochemicals in the leaf include flavonoids, terpenoids, carbohydrates, tannins, saponins, cardeinolides, and cardiac glycosides. The methanol leaf extract showed activity against some microbes in a concentration-dependent manner, with highest inhibition zone against *Salmonella typhi* (24.67±0.33mm) at 500 mg/ml with insignificant difference as compared with to the inhibition zone of the standard drug, Ciprofloxacin (20 mg/ml) [25.00±0.57] and the lowest zone of inhibition against *Streptococcus pyogenes*, *Bacillus subtilis*, *Salmonella typhi*, and *Klebsiella pneumoniae* had the least zones of inhibitions of 7.00±0.00, 7.00±0.00, 7.00±0.00, and 8.00±0.00, respectively, at 100 mg/ml. *S. aureus, E. coli*, *and C. albicans* had no zones of inhibition and no zone of inhibition shown at any concentrations on *Aspegillus niger.* The antimicrobial susceptibility test shows that *Acalypha wilkesiana* had the highest activity against *C. albicans* (19.00±0.57), while no activity against *Escherichia coli* and *Klebsiella pneumonia.* Although the residual portion had the best antimicrobial effect, thus, this study has provided guide that the compound(s) responsible for the antimicrobial effect could be polar in nature.

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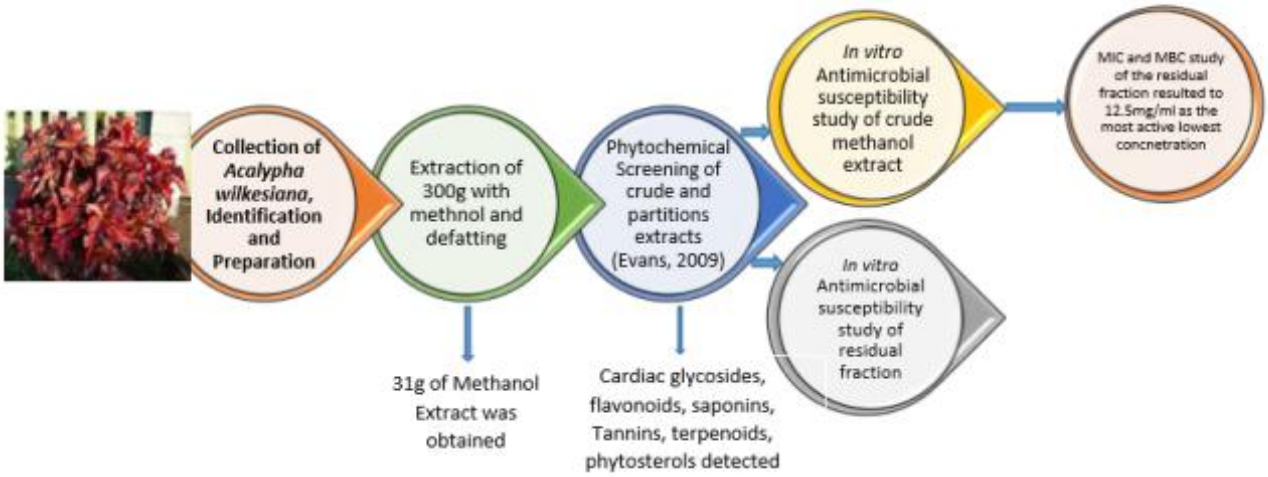
GRAPHICAL ABSTRACT



1- Introduction

Medicines derived from plants are the oldest health care products [1]. Recently, due to the increase in industrialization, most plants with therapeutic value are being formulated into useful pharmaceutical dosage forms such as creams, ointments, tablets, lotions, and syrups, pharmaceutical industries have come to consider traditional medicines as sources of bioactive agents that can be used in the preparation of medicines, many of pharmacologically interesting medicinal plant species in use around the world are employed in more than one community, and often in more than one country for multiple uses [2].

The medicinal value of plants lies in chemical substances that have therapeutic importance which serves as the precursors for the synthesis and production of medicines and drugs [3]. These chemical substances are called Phytochemical ('Phyto' means plant in Greek). They are naturally occurring and biologically active chemical compounds produced by plants during the secondary metabolism [4] which serves as protective agents doe the plants against damage, disease, and contribute to the plant's physiological properties which include colour, aroma, and flavour [5,6]. Recently, it is clearly known that they have roles in the protection of human health, when their dietary intake is significant. Scientific information has shown that about than 4,000 phytochemicals have been recorded and catalogued [7].

Acalypha wilkesiana (Figure 1) belongs to the family of plants known as Euphorbiaccae and genus Acalypha. It is called Fire Dragon, Joseph's coat, and Copper leaf in English [8]. The leaves of *A. wilkesiana* are used locally for the management of and treatment of disease which includes hypertension, gastrointestinal disorders, fungal-skin infections, and diabetes mellitus [9].

Resistance of the effect of drugs by microorganisms is one of the world's leading health concern. Infections from bacterial resistance are becoming increasingly overwhelming, this is due to the fact that some pathogens have even become resistant antibiotics, class of drug also known as antimicrobials. They are known for the effectiveness in treating microbial related-diseases [10]. The loss of effective antibiotics has

been undermining human efforts to fight infectious diseases [10].

Some micro-organisms that were previously controlled by antibiotics have undergone mutations and developed resistance to these antibiotics. Thus, to overcome this resistance by micro-organism, which has become complicated since the emergence of HIV/AIDS, there is a need for continuous search for new drugs. Since many people rely on herbal medicine, researchers should investigate some of plants used as herbal medicine to prove and validate their medicinal potentials [10].

Fig1. *Acalypha wilkesiana* plant and its natural habitat

2- Materials and Methods

2-1- Sample collection and identification

Fresh leaves sample of *Acalypha wilkesiana* were collected from Unimaid Garden, University of Maiduguri, in Borno State, Nigeria. The plant's identity was verified a Taxonomist. A voucher specimen of the leaf was deposited at the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, and University of Maiduguri for future reference. The leaves sample were neatly cleaned by removing unwanted materials and debris using distilled water and shade dried for 7 days before were pulverized using wooding mortar and pestle. The powdered material was then parked in an appropriate container for further used.

2-2- Sample extraction and partitioning

Three hundred grammes (300 g) of *Acalypha wilkesiana* leaf was fine powdered and weighed, 2 L of 85% methanol was added to the sample in a 5 L conical flask and sonicated for 6hr for a period of 3 days. The mixture obtained was filtered with a Muslin cloth, the Whatman filter paper No. 1. The filtrate was allowed to dry at reduced pressure, weighed, encoded as AWM, and stored.

The extract obtained from the leaves was exhaustively defatted in a separating funnel using absolute n-hexane (1 L). The fractions obtained (n-hexane and residual portions) were concentrated to dryness under reduced pressure, encoded (AWH and AWR), and stored.

2-3-Preliminary phytochemical screening

The crude methanol, n-hexane, and residual fractions of leaf of *Acalypha wilkesiana* were screened qualitatively for the presence of phytochemicals which include alkaloids, anthraquinones, flavonoids, saponins, tannins, terpenoids, cardiac glycosides, and steroids, using methods as described by Evans [11] and Yakubu *et al*. [12].

2-4-In vitro Antimicrobial Studies

Test for organisms

The antimicrobial effects of the fractions were carried out on clinically isolated microorganism obtained from the Department of Veterinary Microbiology, Faculty of Veterinary Medicine, and University of Maiduguri. This include: *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli*, *Klebsiella*, *pneumoniae*, *Salmonella typhi*, *Bacillus subtilis*, *Candida albicans,* and *Aspergillus niger*.

2-5- Screening for Antimicrobial activity

The crude and residual fraction of the leaf were subjected to preliminary *in vitro* antimicrobial test using the agar well diffusion technique as described by Usman *et al*. [13]. Wells were bored on the media using 6 mm cork-borer and were filled with 0.2 mL aliquots of various concentrations of the extracts (500 mg/ml, 400 mg/ml, 300, and 200 mg/ml, for the crude and 100 mg/ml, 50 mg/ml, 25 mg/ml, and 12.5

mg/ml. The plates containing the agar were kept in an incubator at 37 °C for 24 hrs. The diameters of the zones of inhibition zone after incubation for methanol extract and fractions were measured in millimetres using a transparent ruler calibrated in meter, and the tests were carried out in triplicates.

2-6- Minimum inhibitory concentration (MIC) study

This study was carried using the nutrient broth dilution technique as described by Vollekova *et al.* [14]. The study was conducted for microorganisms sensitive to the methanol and residual fraction. The extract was initially diluted to the highest concentration of 100 mg/ml in 95% methanol in distilled water (v/v) and were inoculated with 1 ml suspension of the organisms and thereafter incubated at 35 °C.

2-7- Minimum Bactericidal Concentration (MBC) study

The method was used as described by Vollekova *et al*. [15] and as adopted by Yakubu *et al*. [15]. A loopful of each test tube containing methanol extract and residual fraction were inoculated by streaking on a solidified nutrient agar plate and then incubated at 35 °C for 24 hours and observed for bacterial growth. The lowest concentration of the sub-culture that shows no bacterial growth was considered the minimum bactericidal concentration.

2-8- Statistical Analysis

The data obtained from the antimicrobial analysis was analysed using Graphpad Prism version 8.4.3 for windows, followed by Turkey-Kramer's multiple comparison test. Values of $p<0.05$ were considered and were expressed in mean and standard error of mean (SEM).

3- Results and Discussion

3-1- Extraction profile of *Acalypha wilkesiana*

The weight of crude methanol extract obtained by maceration method yielded 32.0 g, with a percentage yield of 10.66 %. The 23.0 g which was defatted with n-hexane yielded 23.10 g with a 72.18% yield while the residual partitioned fraction was 8.9 g with a 27.82% yield. The extraction profile of the extraction is presented in Table 1.

3-2- Qualitative phytochemical analysis of *Acalypha wilkesiana*

The phytochemical test of the crude, n-hexane, and residual extracts revealed the presence of flavonoids, terpenoids, carbohydrates, tannins, saponins, and cardenolides, while alkaloids and anthraquinones were notably absent from the crude and partitioned fractions. It was observed that most of the phytochemicals were not detected in the n-hexane fraction as compared to the crude and residual portions. The results of phytochemical screening are listed in Table 2.

3-3- Antimicrobial activity of *Acalypha wilkesiana* methanol leaf extract

The methanol leaf extract showed activity of the extract against some microbes in a concentration-dependent manner. The extract showed highest inhibition zone against *Salmonella typhi* (24.67±0.33 mm) at 500 mg/ml with insignificant difference as compared with to the inhibition zone of the standard drug, Ciprofloxacin (20 mg/ml) [25.00±0.57] and lowest zone of inhibition against *Streptococcus pyogenes, Bacillus subtilis, Salmonella typhi,* and *Klebsiella pneumoniae* had least zones of inhibitions of 7.00±0.00, 7.00±0.00, 7.00±0.00, and 8.00±0.00, respectively at 100 mg/ml. *S. aureus, E. coli, and C. albicans* had no zones of inhibition and no zone of inhibition shown at any concentrations on *A. niger*. The results of this study are provided in Table 3.

3-4- Antimicrobial activity of *Acalypha wilkesiana* of residual fraction

The antimicrobial susceptibility test shows that *Acalypha wilkesiana* had highest activity against *C. albicans* (19.00±0.57) which is a gram positive bacterium, and also had activity on *candida albicans, Streptococcus pyogenes, Aspergillus, and*

Staphylococcus aureus which are gram negative bacteria, but there was no effect against *Escherichia coli* and *Klebsiella pneumonia.* The effect of the residual fraction against some of the microbes was also concentration-dependent. The results for the antimicrobial activity are indicated in Table 4.

3-5- Minimum Inhibitory Concentration (MIC) study of *Acalypha wilkesiana* leaf residual fraction of methanol extract

The minimum inhibitory test (MIC) of the residual fraction of *A. wilkesiana* showed *a* bacteriostatic effect on *Bacillus subtilis, Staphylococcus aureus, Streptococcus pyogenes* and bactericidal effect on *Echerichia coli, Candida albicans and Klebsiella pneumonia,* the results is shown in Table 5.

3-6- Minimum Bactericidal Concentration (MBC) study of *Acalypha wilkesiana* leaf residual fraction of methanol Extract

The minimum bactericidal concentration (MBC) shows that *Acalypha wilkesiana* has turbidity on *Candida albicans, Bacillus subtilis* and shows no turbidity on *Pseudomonas aeruginosa* and *Staphylococcus aureus.* The result is shown in Table 6.

Table1. The extraction profile of methanolic leaf extract of *A. wilkesiana*

Extract	Mass (mg)	Yield	% Yield	Colour	Texture
Methanol	300	32.00	10.66	Greenish-brown	Gummy
n-Hexane	-	23.1	72.18	Dark green	Gummy
Residual	-	8.9	27.82	Reddish brown	Amorphous

Table 2. Phytochemical constituents of methanol, N-Hexane, and residual partitioned fractions of *A. wilkesiana* leaf extract

Phytochemical	AWM	AWH	AWR
Alkaloids	-	-	-
Flavonoids	+	-	+
Terpenoids	+	+	+
Carbohydrates	+	-	+
Tannins	+	-	+
Saponins	+	-	+
Cardenolides	+		+
Glycosides	-	-	-
Cardiac glycosides	+	+	+

**Keys: (+) = presence, (-) = absence; AWM= A. wilkesiana methanol crude extract, AWH=A. wilkesiana n-hexane fraction fraction; and AWR=A. wilkesiana residual fraction*

Table 3. Antimicrobial susceptibility activity of methanol leaf extract of *A. wilkesiana*

		Concentrations (mg/ml) /diameter of inhibition zone (mm) as Mean±SEM					
S/N	**Test Organism**	**500**	**400**	**300**	**200**	**100**	**CIP (20)**
1	*S. aureus*	19.67±0.33^{a}	15.00±0.00^{b}	10.33±0.33^{c}	8.00±0.00^{d}	0.00±0.00^{e}	31.00±0.57^{f}
2	*S. pyogenes*	22.00±0.00^{a}	18.00±0.00^{b}	13.33±0.33^{c}	9.33±0.33^{d}	7.00±0.00^{e}	35.00±0.57^{f}
3	*B. subtilis*	21.33±0.33^{a}	17.00±0.00^{b}	12.33±0.33^{c}	9.66±0.33^{d}	7.00±0.00^{e}	32.00±0.57^{f}
4	*E. coli*	19.00±0.00^{a}	15.00±0.00^{b}	11.67±1.20^{c}	7.00±0.00^{d}	0.00±0.00^{e}	12.00±0.57^{cf}
5	*S. typhi*	24.67±0.33^{a}	20.00±0.00^{b}	15.33±0.33^{c}	10.33±0.33^{d}	7.00±0.00^{e}	25.00±0.57^{af}
6	*K. pneumoniae*	24.33±0.33^{a}	19.00±0.00^{b}	15.00±0.00^{c}	11.00±0.00^{d}	8.00±0.00^{e}	45.00±0.57^{f}
7	*C. albicans*	21.67±0.33^{a}	17.00±0.00^{b}	12.00±0.00^{c}	8.00±0.00^{d}	0.00±0.00^{e}	NT
8	*A. niger*	0.00±0.00^{a}	0.00±0.00^{ab}	0.00±0.00^{ac}	0.00±0.00^{ad}	0.00±0.00^{ae}	NT

***Note:** Values on the same row with different alphabetical subscripts are statistically significant P<0.05; n=3; and NT: Not Tested*

Table 4. Antimicrobial susceptibility pattern of residual fraction extract of *A. wilkesiana*

		Concentrations (mg/ml) /diameter of inhibition zone (mm) as Mean±SEM				
S/N	**Test Organism**	**100**	**50**	**25**	**12.5**	**CIP (20)**
1	*S. aureus*	10.00±0.57^{a}	8.00±0.57^{aef}	7.00±0.57^{beg}	6.00±0.57^{cfj}	31.00±0.57^{d}
2.	*S. pyogenes*	11.00±0.57^{a}	10.00±0.57^{ad}	9.33±0.33^{ae}	7.00±0.57^{bde}	35.00±0.57^{c}
3.	*B. subtilis*	11.00±0.57^{a}	9.00±0.57^{aef}	8.00±0.57^{beg}	7.00±0.57^{cfg}	32.00±0.57^{d}
4.	*E. coli*	16.67±0.33^{a}	13.33±0.33^{befh}	12.00±0.57^{degi}	13.67±0.33^{dfj}	12.00±0.57^{dfij}
5	*S. typhi*	7.66±0.33^{a}	0.00±0.00^{b}	0.00±0.00^{cb}	0.00±0.00^{db}	25.00±0.57^{e}
6	*K. pneumonia*	10.00±0.57^{a}	0.00±0.00^{bf}	0.00±0.00^{cf}	0.00±0.00^{df}	45.00±0.57^{e}
7	*C. albicans*	19.00±0.57^{a}	15.67±0.57^{bfg}	14.00±0.57^{efh}	14.00±0.57^{dgh}	NT
8	*A. niger*	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	NT

***Note:** Values on the same row with different alphabetical subscripts are statistically significant P<0.05; n=3; and NT=Not Tested.*

Table 5. The Minimum Inhibitory Concentration (MIC) activity of *A. wilkesiana* methanol leaf partitioned residual extract

	Concentration (mg/ml)			
Organisms	100.00	50.00	25.00	12.50
E. coli	-	-	-	-
C. albicans	-	-	-	+
P. aeruginosa	-	-	-	+
S. aureus	-	-	+	+
B. subtilis	-	-	-	+
K. pneumonia	-	-	-	-
S. pyogenes	-	-	-	-

**Key: + = Bactericidal and - = Bacteriostatic*

Table 6. Minimum Bactericidal Concentration (MBC) activity of *A. wilkesiana* methanol leaf partitioned residual extract

	Concentration (mg/ml)			
Organisms	100.00	50.00	25.00	12.5.0
C. albicans	-	-	-	+
P. aeruginosa	-	-	-	-
S. aureus	-	-	-	-
B. subtilis	-	-	-	+
S. pyogenes	-	-	-	-

4- Discussion

The preliminary screening of secondary metabolites of the leaf extract showed the presence of classes of phytochemicals with reported therapeutic importance. This includes the alkaloids, carbohydrates, cardenolides flavonoids, terpenoids, and saponins. The result correlates slightly with previous study of Awe and Eme [15], Aladejimokun *et al.* [16], Madziga *et al.* [17], and Omage *et al*. [18]. These plant chemicals have been scientifically reported to possess analgesic, antioxidant, anti-obesity, antibacterial, antifungal, antidiabetic, ant-hypertensive, anticholesterol antimalarial, antiarrhythmic, hypnotic and anticonvulsant, antiemetic, anticancerous, antiparasitic, diuretic, anti-HIV activity, and hepatoprotective activity [19].

Both methanol extract and residual fraction of *Acalypha wilkesiana* showed antimicrobial property by inhibiting the growth of most of the microbes on nutrient agar plates at different concentrations. The residual fraction was active (MIC and MBC) at lowest concentrations of 12.5 mg/ml against *C. albicans*. The resistance of the *A. niger* against the tested concentrations those not indicate a total inactivity of the extract, but rather a resistance of working concentrations. It was noticed that the antimicrobial activity of residual partitioned fraction was significantly increased when compared with concentration of the crude methanol extract. This shows that the defatting could have served as a purification process. Hence, the tendency of having higher activity as the extract is further purified.

5- Conclusion

This study also shows that *Acalypha wilkesiana* contains the used bioactive principles could be a source of chemotherapy for treatment of microbial related illnesses in both humans and animals, since both the crude and partitioned fractions exhibited significant antimicrobial property that can potentially inhibit the growth of enteric pathogens. The purification of methanol extract by defatting indicates the possibility of presence of highly effect antimicrobial agent(s) for the treatment of *C. albicans*-related diseases.

Acknowledgments

The authors appreciate the technical assistance of Messrs. Fine Akawu and Isah Gulani of the Departments of Pure and Applied Chemistry and Veterinary Microbiology.

Conflict of interest

The authors declared no conflict of interests.

Ethical consideration

Ethical issues such as data fabrication, falsification, plagiarism, and misconduct are observed by the authors.

Funding/Support

This study was self-sponsored by the authors.

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HOW TO CITE THIS ARTICLE

James Yakubu et al. Qualitative Phytoconstituents and In Vitro Antimicrobial Study of Some Solvent Leaf Extracts of Acalypha wilkesiana Mull. Arg. (Euphrivceae). Prog. Chem. Biochem. Res, 6(2) (2023) 161-169.

DOI: 10.22034/pcbr.2023.389660.1258

URL: https://www.pcbiochemres.com/article_172308.html