International Journal of Science Academic Research
Vol. 04, Issue 09, pp.6324-6329, September, 2023
Available online at http://www.scienceijsar.com

ISSN: 2582-6425

Research Article

PHYTOCHEMICAL CONSTITUENTS AND ANTIMICROBIAL ACTIVITY OF EXTRACTS OF *ACALYPHA WILKESIANA*

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Received 24th July 2023; **Accepted** 28th August 2023; **Published online** 30th September 2023

Abstract

The present study was conducted to evaluate the phytochemicals, antimicrobial activity of n-hexane and ethanol extracts of *Acalypha wilkesiana.* The micro-organisms used for the antimicrobial assay were eight clinical pathogens, four bacteria: *Klebsiella pneumoniae, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa,* and four fungi: *Aspergillus niger, Penicillium notatum, Piedra hortae and Melassezia furfur* using agar well diffusion method. The minimum inhibition concentration was determined for the bacteria and fungi. The result of the phytochemical screening showed that both n-hexane and ethanol extracts contains alkaloids, terpenoids and eugenol. Tannins, glycosides and saponins are present in ethanol extract only. Phenolic compound and steroids are present in n-hexane extract only. The antimicrobial screening revealed that both extracts used in this study have good antibiotics properties against various bacteria and fungi pathogens tested. The result of this study showed that the plant *A. wilkesiana* contained phytochemical constituents which suggest the application of the plant as supplementary sources of antimicrobial agent for man.

Keywords: n-hexane, *Acalypha wilkesiana,* Phytochemicals, Antimicrobial, MIC, MBC.

INTRODUCTION

Plant provides a variety of resources that contribute to the fundamental needs of both human being and animals such as food, clothing and shelter. Among plants of economic importance are medicinal plants. Plants have been utilized as therapeutic agents since time immemorial in both organized and unorganized forms [1]. Natural products have been an inherent part of the ancient traditional medicinal system, e.g. Chinese and Egyptian [2]. According to World Health Organization [3], medicinal plant is defined as any plant which in one or more of its organs (example leaves, roots, rhizomes, stems, barks, flowers, fruits, grains or seeds), contains substances that can be used for curative purposes, or which are one of the substance used for chemo-pharmaceutical semi-synthesis. Such a plant will have its parts used in the control or treatment of a disease condition and therefore contains chemical components that are often referred to as phytochemicals ('phyto-' from the Greek word *–phyto* meaning 'plant') or phyto-constituents and are responsible for protecting the plant against microbial infections or infestations by pests [4, 5, 6, 7]. *Acalypha wilkesiana* belongs to the family *Euphorbiaceae* (spurge family). It is a plant of great ornamental value due to its showily coloured foliage and is widely cultivated in the tropical and subtropical countries. It is an evergreen Shrub. It grows 3 metres (9.8 ft) high and 2 metres (6ft 7in) across. The leaves are coppery green with red splashes of colour, its other names including: A. *amentaceae* and A. *tricolor*. It is popularly called copper leaf, Joseph's coat, fire dragon and match-me-if-you-can [8]. The Hausas of the Northern Nigeria call it "Jiwene" and "Jinwinini", while the Yoruba of the Southern Nigeria call it "aworoso". It is native to Fiji and nearby islands in the South Pacific, but has spread to most parts of the world, especially the tropics of Africa, America and Asia. Many cultivars are available with different leaf forms and colours: *A. wilkesiana* 'Godseffiana' has narrow, drooping, green leaves with creamy-white margins, 'Marginata' has coppery-green leaves with pink or crimson margins, 'Macrophylla' has larger leaves, variegated with bronze, cream, yellow and red, while 'Musaica' has green leaves that are mottled with orange and red [9, 10]. Phytochemistry on the other hand, is the study of natural products. Phytochemicals have been isolated and characterized from fruits such as almond and apples, vegetables such as broccoli and onion, spices such as turmeric, ginger, beverages such as green tea and red wine, as well as many other sources [7, 11]. For example, the use of bearberry (*Arctostaphylos uva-ursi)* and cranberry juice (*Vaccinium macrocarpon)* to treat urinary tract infections is reported in different manuals of phytotherapy, while species such as lemon balm (*Melissa officinalis),* garlic (*Allium sativum)* and tee tree (*Melaleuca alternifolia*) are described as broad-spectrum antimicrobial agents [12].

The plants are administered in different forms such as poultices, concoctions of different plant mixtures, infusions as teas or tinctures or as component mixtures in porridges and some administered in different ways including oral, nasal (smoking, snuffing or steaming), tropical (lotions, oils or creams), bathing or rectal (enemas). Different plant parts and components (roots, leaves, stem barks, flowers or their combinations) have been employed in the treatment of infectious pathologies in the respiratory system, urinary tract, gastrointestinal and biliary systems, as well as on the skin [13, 14]. Numerous methods have been utilized in drug discovery, including isolation of compounds from plants and other natural sources, synthetic chemistry, combinatorial chemistry and molecular modeling [15, 16] despite the recent interest of pharmaceutical companies and funding organizations in molecular modeling, combinatorial chemistry and other synthetic chemistry techniques, natural products (in particular,

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medicinal plants), remain an important source of new drugs, new drug leads and new chemical entities (NCEs) [17, 18]. Between 2001 and 2002, approximately one quarter of the best-selling drugs worldwide were natural products or were derived from natural products [18]. Approximately 28 % of NCEs that appeared between 1981 and 2002 were natural products or natural product-derived [19].

MATERIALS AND METHODS

Study Area

This study was carried out in the Faculty of Physical Sciences, Department of Chemistry, University of Benin, Benin city, located in Ovia-North East Local Government area of Edo State. Predominant occupation among the people is farming, despite the availability of reliable medical service, the local populaces still rely on the use of herbs as medicines for both curative and prophylaxis purpose.

Collection and identification of plant sample

Fresh samples of *Acalypha wilkesiana* leaves were obtained from local gardens within Benin City and authenticated by a Taxonomist in the Department of Plant Biology and Biotechnology, University of Benin, Benin City.

Preparation and Treatment of the Plant Sample

The leaves were collected, properly washed, air-dried and ground into fine powder and stored in air-tight plastic container prior to extraction/use.

Extraction Procedures (Preparation of Extracts)

The extracts of *A. wilkesiana* were obtained using different solvents (ethanol and n-hexane). 120g of dried and milled plant material was extracted with 500ml of n-hexane using a Soxhlet extractor equipped with a reflux condenser. For the ethanolic extract, 110g of milled plant material was used. The two extracts were concentrated separately by evaporating to dryness on a water bath (50 ℃) to give the crude extract, with their various masses determined.

Qualitative Determination of Phytochemicals

The samples (ethanol extract and n-Hexane extract) of *Acalypha wilkesiana* leaves were analyzed for the presence of alkaloids, saponins, tannins, cardiac glycosides, eugenols, steroids, phenols, flavonoids, and terpenoids according to standard methods by Trease & Evans, [20] and Lawal *et al*, [21].

Test for Steroids (Salkowski's Test)

About 0.2 g of the extracts was dissolved in 2 ml of acetic anhydride. Dilute sulphuric acid was carefully added. A change in colour from violet to green was observed, an indication of the presence of steroids.

Test for Cardiac-Active Glycoside (Keller-Killani Test)

About 0.2 g of the extracts was dissolved in 2 ml of glacial acetic acid containing one drop of ferric chloride solution followed by the addition of 1 ml of concentrated sulphuric acid. A brown ring at the interface confirmed the presence of cardiac glycoside.

Antimicrobial assays

Microorganisms

The microorganisms (bacteria and fungi) employed in this study were procured from the University of Benin Teaching Hospital, Benin City which includes clinical isolates of *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Malassezia furfur, Piedra hortae, Aspergillus flavus* and *Penicillium notatum.*

Preparation of Culture Media

Nutrient broth and Nutrient agar, all product of Himedia Laboratories Mumbai (India) were used for the bacteria study while Potatoes Dextrose Agar and Potatoes Dextrose Broth were used for the fungi in this study. The composition of the medium was Beef extract 3.0g, peptone 5.0g, sodium chloride 8.0g, agar 15.0g.

Agar Well Diffusion Assay

The antimicrobial activity of the extracts was determined by using the agar well diffusion technique. Nutrient agar plates were each seeded with 0.1ml of an overnight culture of each bacterial (). The 24hrs broth culture of each bacterium were used to seed sterile molten nutrient agar at , allowed to set and well made by sterile standard cork borer (6.0mm in diameter) and 200g (0.2ml) of 15mg/ml solution of extract added into each well. The bacterial plates incubated at 37 ℃ for 24hours, after which diameter of the zones of inhibition were measured for the two extracts (n-hexane and ethanol) [22].

Potato Dextrose Agar

The medium was prepared by suspending 18.4 g of the agar powder in 500 ml of sterilized deionized water in a conical flask and stir to dissolve properly. The conical flask was covered with cotton wool and aluminium foil paper and autoclaved at 121 ^{o}C for 15 minutes. The medium was allowed to cool before pouring into plates aseptically in the required amount. The plates were covered and allowed to solidify.

Mueller Hinton Agar

About 39 g of Mueller Hilton agar were dissolved in 1000 ml of distilled water in a conical flask corked with cotton wool and foil paper and allowed to dissolve in 1000 ml of distilled water in a conical flask. The medium was the placed in an autoclave to sterilize it for 15 minutes at 121^{o}C at a pressure of 15psi. After sterilization, the flask was allowed to cool before it was poured into Petri dishes aseptically [22].

Determination of Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentration is the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation. Minimum inhibitory concentrations are important in diagnostic laboratories to confirm the resistance of microorganism to an antimicrobial agent and also to monitor the activity of a new antimicrobial

agent. The MIC values of each plant extracts were determined using two-fold micro-dilution to prepare concentrations of 100mg/mol, 50mg/mol, 25mg/mol, 12.5mg/mol of each extract and a drop of the bacterial suspension that had been previously diluted to about cfu/ml were aseptically incorporated into molten Nutrient agar and allowed to set. The plates were incubated at 37 $^{\circ}C$ for 24 hours. The lowest concentration preventing visible growth for each of the test organisms was recorded as the MIC. The experiments were carried in triplicate for each extract concentration and ciprofloxacin as positive control while distilled water was used as the negative control.

Determination of Minimum Bactericidal Concentration (MBC)

Minimum bactericidal concentration is the lowest concentration of antibacterial agent required to kill a particular bacterium. It can be determined from broth dilution. The MBC is identified by determining the lowest concentration of antibacterial agents that reduces the viability of the bacterial inoculums is greater than or equal to 99.9%. Antimicrobial agents are usually regarded as bactericidal, if the MBC is no more than four times the MIC. Nutrient agar plates were divided into different sections and labeled with the different concentration on the base of the plates; these were used to plate out the content of lowest MIC plate in the respective sections of the plates. The plates were incubated aerobically at 37 $^{\circ}C$ for 18-24hours after which the MBC were recorded. The lowest concentration of MIC tubes with no visible bacterial growth on solid medium was regarded as MBC.

RESULTS AND DISCUSSION

Table 1. % Yield of Extracts of *Acalypha wilkesiana*

Powdered Plant Sample (g)	Solvent	Mass of Crude Extract (g)	% Yield
120	Hexane extract	7.59	6.33
110	Ethanol	11.52	10.47

$$\% \text{ yield} = \frac{Mass of crude\ extract}{Mass of powdered plant sample} \times 100$$

The phytochemical screening result (Table 2) shows or indicates that both n-hexane and ethanol extract contain alkaloids, terpenoids and eugenol. Tannins, glycosides and saponins are present in ethanol extract only. Phenolic compound and steroids are present in n-hexane extract only. Secondary metabolites of plant are mainly responsible for different pharmaco-logical properties and their therapeutic benefits. Apart from their potential antibacterial activity, compounds present in this study such as alkaloids are known as anti-malarial agents, analgesics and can act as stimulants. Glycoside moieties such as saponins, cardiac glycosides and flavonoids can inhibit tumor growth, act as an anti-parasitic agent, and can be used as an anti-depressant. The presence of these phytochemicals is an indication that the plant is a rich source of medicinal constituents [23].

n-Hexane extracts

In table 3, the effect of *A. wilkesiana* n-hexane extract at 50mg/ml on *Escherichia coli* was 14mm compared to *S. aureus* which was 11mm.

Table 2. Phytochemical screening of hexane and ethanol extract of *Acalypha wilkesiana*

S/N	Phytochemical constituents	Name of Test	Hexane Extract	Ethanol Extract
1.	Alkaloids	Picric acid Test	+	+
2.	Flavonoids	Lead Test	-	-
3.	Phenolics	Ferric chloride	+	-
4.	Tannins	Ferric chloride	-	+
5.	Eugenol	KOH/HCl	+	+
6.	Steriods	Acetic acid/	+	-
7.	Terpenes	Salkowski Test	+	+
8.	Saponins	Foam Test	-	+
9.	Glycosides	General Test	-	+

KEYS: — = Absent,+ = Present

The 100mg/ml concentration, had the best observed minimum inhibitory effect on tested bacterial isolates such as *E. coli* (16mm), *P. aeruginosa* (13mm), *K. pneumoniae* (11mm) and *S. aureus* (14mm). While 12.5mg/ml, had the less observed antimicrobial effect on *E. coli* (10mm), *P. aeruginosa* (8.1mm), *K. pneumoniae* (7mm) and *S. aureus* (8.9mm) respectively. The n-hexane extract of *A. Wilkesiana* showed antibacterial activity against all the bacterial isolates studied which is in agreement with Kebede *et al*., [24].

Table 3. Minimum Inhibitory Concentration (MIC) of n-hexane extract of *A. wilkesiana* on bacterial isolates

Microorganisms (Bacteria isolates)	Minimum inhibitory concentration (MIC) (mg/ml)			
	100	50	25	12.5
	Zone of inhibition (mm)			
E. coli	16	14	13	10
Klebsiella pneumoniae	11	10	8	7
Staphylococcus aureus	14	11	10	8.9
Pseudomonas aeruginosa	13	10	9	8.1

In table 4, the effect of A. *wilkesiana* n-hexane extract at 50mg/ml on *P. hortae* was 10mm compared to *M. furfur* which is 8.2mm. The 100mg/ml concentration, had the best observed minimum inhibitory effect on tested fungi isolates such as *A. flavus* (10mm), *P. hortae* (12.1mm), *M. furfur* (12mm) and *P. notatum* (9mm). While 12.5mg/ml, had the less observed antimicrobial effect on *A. flavus* (7mm), *P. hortae* (7.3mm), *M. furfur* (6.5mm) and *P. notatum* (7.1mm) respectively. This is similar to Kebede *et al*., [24].

Table 4. Minimum Inhibitory Concentration (MIC) of n-hexane extract of *A. wilkesiana* on fungi isolates

Microorganisms (Fungi isolates)	Minimum inhibitory concentration (MIC) (mg/ml)			
	100	50	25	12.5
	Zone of inhibition (mm)			
Melassezia furfur	12	8.2	6.2	6.5
Piedra hortae	12.1	10	8	7.3
Aspergillus flavus	10	9	8	7
Penicillum notatum	9	8	7.9	7.1

In table 5, The effect of *A. wilkesiana* on n-hexane extract at 50mg/ml on *Klebsiella pneumoniae* was 13mm compared to *P. aeruginosa* which was 11mm. The concentration 100mg/ml, had the best observed minimum bactericidal effect on tested bacterial isolates such as *E. coli* (10mm), *P. aeruginosa* (11.5mm), *K. pneumoniae* (14mm) and *S. aureus* (9mm). While 12.5mg/ml concentration had no observable effect on tested bacterial isolates respectively.

Table 5. Minimum Bactericidal Concentration (MBC) of n-hexane extract of *A. wilkesiana* on bacterial isolates

Microorganisms (Bacteria isolates)	Minimum Bactericidal Concentration (MBC) (mg/ml)			
	100	50	25	12.5
	Zone of inhibition (mm)			
E. coli	10	9	7	-
Klebsiella pneumoniae	14	13	12	-
Staphylococcus aureus	9	8	7	-
Pseudomonas aeruginosa	11.5	11	-	-

In table 6, the effect of *A. Wilkesiana* ethanol extract at 50mg/ml only had effect on *M. furfur* (7.7mm) compared to other fungi isolates which had no inhibition value. The concentration 100mg/ml, had the best observed minimum fungicidal effect on tested fungi isolates such as *A. flavus* (7mm), *P. hortae* (8mm), *M. furfur* (10mm) and *P. notatum* (6mm). While 12.5mg/ml concentration had no observable effect on tested fungi isolates respectively.

Table 6. Minimum Fungicidal Concentration (MFC) of n-hexane extract of *A. Wilkesiana* on fungi isolates

Microorganisms (Fungi isolation)	Minimum Fungicidal Concentration (MFC) (mg/ml)			
	100	50	25	12.5
	Zone of inhibition (mm)			
Melassezia furfur	10	7.7	-	-
Piedra hortae	8	_	-	-
Aspergillus flavus	7	_	-	-
Penicillium notatum	6	_	-	-

Table 7. Summary of the MIC and MBC n-hexane extract of *A. wilkesiana* on bacterial isolates

Microorganisms (Bacteria isolates)	MIC (mg/ml)	MBC (mg/ml)
E. coli	10	7
Klebsiella pneumoniae	7	12
Staphylococcus aureus	8.9	7
Pseudomonas aeruginosa	8.1	11

Table 8. Summary of the MIC and MFC n-hexane extract of *A. wilkesiana* on Fungal isolates

Microorganisms (Fungi isolates)	MIC (mg/ml)	MFC (mg/ml)
Melassezia furfur	6.5	7.7
Piedra hortae	7.3	8
Aspergillus flavus	7	7
Penicillum notatum	7.1	6

Ethanol extracts

The minimum inhibitory concentration (MIC) of ethanol crude extract of *A. wilkesiana* (Table 9) indicated a significant activity with zones of inhibition 12mm (*E. coli),*10mm (*S. aureus*), 11mm (*P. aeruginosa*), 16mm (*K. pneumoniae*) at a high dose of 100mg/ml. however, as the concentration of the crude extract decreased, the zones of the inhibition were also reduced.

Table 9. Minimum Inhibitory Concentration (MIC) of ethanol extract of *A. wilkesiana* on bacterial isolates

Microorganisms (Bacteria isolates)	Minimum inhibitory concentration (MIC) mg/ml)			
	100	50	25	12.5
	Zone of inhibition (mm)			
E. coli	12	10	9	8.5
Klebsiella pneumoniae	16	14	13	11
Staphylococcus aureus	10	9	8.3	8
Pseudomonas aeruginosa	11	10	9	8. 6

This indicated that the extract showed a dose dependent activity. This result was in agreement with the work of Lawal *et al*, [21]; Gulay *et al*., [25]; Jacob & Sumathy, [26]; Vivek *et al*., [27]. That the extract inhibited the growth of the isolates is an indication that they contain substance(s) that are active against bacterial species [6, 28].

Table 10. Minimum Inhibitory Concentration (MIC) of ethanol extract of *A. wilkesiana* on fungi isolates

Microorganisms (Fungi isolates)	Minimum inhibitory concentration (MIC) (mg/ml)			
	100	50	25	12.5
	Zone of inhibition (mm)			
Melassezia furfur	11	9.2	8.1	7.5
Piedra hortae	9.5	9.1	8.4	7. 6
Aspergillus flavus	8.2	7	6.5	6
Penicillum notatum	7.8	7	6.1	5.7

The minimum bactericidal concentration (MBC) of ethanol crude extract of *A. wilkesiana* (table 11) indicated that the extract showed a dose dependent activity. At 100mg, ethanol crude extract of *A. wilkesiana was* significantly active against the bacteria isolates used in this study. As the concentration decreases, the activity of the crude extract decreases. The extract inhibited the growth of the isolates is an indication that they contain substance(s) that are active against bacterial species [6, 28].

Table 11. Minimum Bactericidal Concentration (MBC) of ethanol extract of *A. wilkesiana* on bacterial isolates

Microorganisms (Bacterial isolates)	Minimum Bacteriocidal Concentration (MBC) (mg/ml)			
	100mg	50mg	25mg	12.5mg
	Zone of inhibition (mm)			
E. coli	8.1	7.4	5.1	_
Klebsiella pneumoniae	11	9	8	
Staphylococcus aureus	7.5	6.8	_	_
Pseudomonas aeruginosa	9	7	_	_

Table 12. Minimum Fungicidal Concentration (MFC) of ethanol extract of *A. Wilkesiana* on fungi isolates

Microorganisms (Fungi isolation)	Minimum Fungicidal Concentration (MFC) (mg/ml)			
	100mg	50mg	25mg	12.5mg
	Zone of inhibition (mm)			
Melassezia furfur	9.1	7. 6	_	_
Piedra hortae	7.4	7	_	_
Aspergillus flavus	6.8	_	_	_
Penicillium notatum	6.2	_	_	_

Table 13. Summary of the MIC and MBC ethanol extract of *A. wilkesiana* on bacterial isolates

Microorganisms (Bacteria isolates)	MIC (mg/ml)	MBC (mg/ml)
E. coli	8.5	5.1
Klebsiella pneumoniae	11	8
Staphylococcus aureus	8	6.8
Pseudomonas aeruginosa	8. 6	7

Table 14. Summary of the MIC and MFC ethanol extract of *A. wilkesiana* on Fungal isolates

Microorganisms (Fungi isolates)	MIC (mg/ml)	MFC (mg/ml)
Melassezia furfur	7.5	7. 6
Piedra hortae	7. 6	7
Aspergillus flavus	6	6.8
Penicillium notatum	5.7	6.2

KEYS: MIC: Minimum inhibitory concentration; MBC: Minimum Bactericidal Concentration; mm: millimeter

(-) = no activity; <10mm = non-significant activity or resistant; >10mm = high activity or sensitive for the bacteria while <5mm = non-significant activity or resistant; >5mm = high activity or sensitive for the fungi (Clinical and Laboratory Standards Institute, CLSI, [29, 30, 31, 32].

Conclusion

The result of the analysis carried out, supports the use of *Acalypha wilkesiana* in folkloric medicine as an antibiotic and in the treatment of other light ailments. The pharmacological effect of the phytochemical constituents such as alkaloids, glycoside and flavonoids as well as the antimicrobial activity of the plant can explain the rationale for the use of this plant in the treatment of infections in traditional medicine. It is expected that using natural products as therapeutic agents will probably not elicit resistance in microorganisms. Therefore, the outcome of this research work suggest that the plant could probably be a veritable and cheaper substitute for conventional drugs since the plant is easily obtainable and the extract can easily be made through a simple process.

Conflict of Interest

The authors declare no conflicts of interest. The authors alone are responsible for the content and the writing of the paper.

Funding

This research did not receive any grant from funding agencies in the public, commercial, or not-for-profit sectors.

Authors' Contributions: The entire study procedure was conducted with the involvement of all writers.

Acknowledgements

The authors would like to thank all the Laboratory and staff of the Department of Chemistry, University of Benin, Benin City, Edo State, Nigeria and St Kenny Research Consult, Ekpoma, Edo State for their excellent assistance and for providing medical writing support/editorial support in accordance with Good Publication Practice (GPP3) guidelines.

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