BOOK OF PROCEEDINGS
(Available at: http://acsnigeria.org/publications/proceedings)

Antimicrobial Screening of the Ethanol Extract and Alkaloid Fraction from the Root of *Acalypha hispida*

Bilkisu A. Adedoyin[1], Fagge A. Khadija[1], and Garba Dauda[2]
[1]Applied Chemistry Research Laboratory, Department of Chemistry, University of Abuja, Nigeria
[2]Department of Pharmaceutical Chemistry, Faculty of Pharmaceutical Science, University of Abuja. Nigeria

Corresponding Author's email: bilkisu.adedoyin@uniabuja.edu.ng

ABSTRACT

Acalypha hispida is a fast-growing tropical shrub belonging to the family *Euphorbiaceae*. The plant is used in traditional medicine to treat various ailments including leprosy, skin rashes, ulcers, wounds, diarrhoea, and gonorrhoea. This study was aimed at screening of *Acalypha hispida* plant for its antibacterial activities. The cytotoxicity was carried out using a brine shrimp lethality test (BSLT) assay. Antimicrobial activity of the ethanol root extract and an alkaloid fraction of *Acalypha hispida* against clinical isolated species of Staphylococcus *species, gonorrhoea, Bacillus anthracis, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia, Aspergillus actinomycete,* and *Candida albicans* were investigated using micro dilution agar method MIC and MBC methods. The result obtained from the BSLT gave LC_{50} of 31.41 µg/L for alkaloid fraction and LC_{50} of 62.28 µg/L for ethanol extracts indicating higher toxicity of alkaloid fraction to brine shrimp larva. The alkaloid fraction showed high antimicrobial activity against all the microorganisms tested indicating a broad spectrum of activity. The result from the ***in-vitro*** inhibitory activity revealed that the alkaloid fraction was more active against the Gram-negative bacteria (*E. coli* and *K. pneumonia*) with inhibition zones of 17 -33 mm. The high antimicrobial activity observed in the alkaloid may be due to the presence of some phytochemicals with high antimicrobial activity.

KEYWORDS: Medicinal plant, Microorganisms, Alkaloids, Cytotoxicity, Phytochemicals

1. INTRODUCTION

Medicinal plants contain substances that are of great importance to the health of individuals and communities.[1] They play a significant role in providing primary health care services to rural people. They also serve as therapeutic agents and important raw materials for the preparation of traditional therapies and some modern drugs.[1] Plants are rich sources of secondary metabolites, such as alkaloids and saponins which have been found in *in-vitro* studies of *A. hispida* to possess antimicrobial, anti-inflammatory, and anti-diabetic properties[2]. These metabolites are low molecular weight substances that are essential for producing constituents that are inhibitors of other organisms which compete for food supply or regulators of the cellular differentiation process[3]. Alkaloids are naturally occurring chemical compounds containing basic nitrogen atoms. The name alkaloid is derived from the word alkaline and is used to describe any nitrogen-containing compound. [4]Alkaloids are heterocyclic nitrogen compounds that are reported to be useful in treatment of numerous diseases including Human Immunodeficiency Virus (HIV) Infection, trypanosomiasis,_malaria among others[5]. They are also reported to have antimicrobial and anti-diarrhoea activity due to their effect on transit time in the small intestine and their ability to interact with microbial deoxyribonucleic acid (DNA) [6,7]. The increase in and over use of antibiotics in the treatment of microbial infections is preserving bacterial resistance against available antibiotics.[8] Thus, newer more efficacious antibiotics are needed to treat microbial infections. Approximately 20% of the world's plants have been subjected to pharmacological or biological tests as natural products of plant origin are important sources of constituents that could be developed into drugs, dyes, fragrances, and pesticides.[9] *Acalypha hispida*, (Chenille plant) is a flowering shrub belonging to the family Euphorbiaceae, subfamily *Acalyphinae*, and genus *Acalypha* is the fourth largest genus of the Euphorbiaceae family. The plant is also known as the "Philippines Medusa", "red hot cat's tail" and "fox tail'' in English. The root and flower decoction are used for kidney ailments and as a diuretic. Leaf poultice is used as a cure for leprosy, the decoction of leaves and the flowers are taken internally as laxative and for treatment of gonorrhoea. Bark is used as an expectorant for asthma[10]. Phytochemicals

component in the plant show the presence of alkaloids, anthraquinones, saponins, phenolics, steroids, coumarins, and glycosides which exhibit antifungal, anti-ulcer, and antitumor properties have been previously investigated. The plant contains ellantogitannis such as acylpyridines M_1, M_2 and D_1, anthocyanins namely, cyanidin 1-O-(2''-galloyl-β-galactopyranoside), cyanidin 3-O-(2''-galloy-β-galactopyranoside) and cyanidin3-O-β-galactopyranoside. [11, 21]. In the present study, we report the antimicrobial activities of the ethanol and the alkaloid extract of the root of *Acalypha hispida* grown in northern part of Nigeria.

2. MATERIALS AND METHODS

2.1. Plant collection

Fresh roots of *A. Hispida* were obtained from the biological Garden of Ahmadu Bello University, Zaria, Kaduna state, Nigeria. August 2023. The identity of the plant was authenticated in the herbarium section of the biological Sciences Department Ahmadu Bello University Zaria by comparing it with the herbarium Voucher specimen and voucher number ABU02643 was obtained for future reference.

2.2. Sample Preparation

The root samples were carefully air-dried under a shed in the laboratory at room temperature. Pulverized into uniform powder using a wood mill machine and the powder was kept in a jar at room temperature.

2.3. Extraction of Plant Material.

The powdered plant materials were extracted using absolute ethanol. About 100 g of the pulverized root sample was soaked in 500 ml of absolute ethanol for 72 hours using maceration method of extraction and the mixture was filtered using two folds of whatt man No. 1 filter paper. The extract was concentrated at 40°C using a rotary evaporator.

2.4. Phytochemical Analysis

The ethanol extract was subjected to preliminary phytochemical tests using standard methods as described. [12, 13]

2.5. Alkaloid Extraction

Alkaloids were extracted following the method reported by [7] the dried powder of the plant root (50 g) was mixed with 20% ^{v}/v ethanol with distilled water and dried at room temperature before the extraction of total alkaloids. The granulated powder was extracted with benzene for 6h. The extract was shaken with three successive portions of 25 mL of 5% sulphuric acid and decolorized by heating with activated charcoal. The hot solution was then filtered using Whatman No.1 filter paper. The filtrate was percolated and the solvent was evaporated under vacuum. The residue was then dissolved in water/hydrochloric acid mixture at pH 2.5, and allowed to seep. The collected solution was adjusted to pH 8 with ammonia and washed (6 x 150ml) of dichloromethane. Then, the dichloromethane was evaporated and the resulting residue was concentrated to dryness under reduced pressure to yield the solid alkaloid extract which was confirmed using Dragendorff reagent to give purple colour.[15]

2.6. Brine shrimp lethality test

Brine shrimp toxicity test: A portion of 70 g of Brine shrimp (*Artemia salina*) eggs was added to 250 ml ^{of} seawater in a beaker and kept for 48 hours for the eggs to hatch into shrimp larvae. A portion of 0.2g of each extract dissolved in 2ml of its various solvents of extraction. 50, 5, and 1µ/L of each solution were drawn into vials and allowed to evaporate within 24 hr. Two drops of DMSO were added and made up to 2ml with distilled water corresponding to concentrations of 1000, 100, and 10 µg/ ml respectively. Each dosage was prepared in triplicates including the control. Ten (10) shrimp larvae were added to each vial. The number of the surviving shrimp at each dosage and the control was recorded after 24hrs

and the $LC_{50,}$ was computed using Finney probit analysis Software. In this assay, a drop of dimethyl sulphoxide (DMSO) was added to test and control vials to enhance the solubility of the test materials. All tests and analysis were carried out in triplicates and the results obtained were averaged.

2.7. Antimicrobial Assay

The diluted concentrations range of 0.5-5.0 $x10^2$µg/cm^3 of extracts was prepared and were tested for their antimicrobial properties using the agar-well technique.[15] The assay for antimicrobial activity was carried out using standard clinical isolate of *Staphylococcus aureus, Lactobacillus species, Staphylococcus faecalis, Bacillus subtills, Bacillus anthracis, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi, Klebsiella pneumonia,* and *Staphylococcus diderm* and fungal isolates used included A*spergillus funigatus, Aspergillus actinomycete,* and *Candida albicans*. The antimicrobial susceptibility test was conducted using the method earlier described by [15]. The tests were carried out using a stock concentration of 100 mg/ml prepared by dissolving 1 g of the crude extract into 10 ml of sterile distilled water. The dilution ratio for Gram-positive bacteria and Gram-negative bacteria was 1:1000 and 1:5000 respectively using peptone water (15). About 0.5 ml of the dilute cultures were aseptically inoculated on the surface of sterile Petri dishes containing sterile solid nutrient agar. Discs impregnated with the crude extract at the concentration of 5 mg/disc were aseptically mounted on agar and thereafter incubated at 37°c for 24 hours, the inhibition zone was observed and then recorded in millimetres using a transparent meter rule. The standard drugs used are penicillin and ciprofloxacin respectively.

2.7.1. Test of Minimum Inhibitory Concentration (MIC)/Minimum Bactericidal Concentration (MBC)

The MIC and MBC of the extract were determined by the micro dilution agar method. About 2-fold serial dilution of the extracts prepared in sterile distilled water to achieve a decreasing concentration ranging from 160 to 1.25 mg/ml in 9 sterile tubes labelled 1 to 9. A sterile cork borer of 8 mm diameter was used to bore well in the resolidified Mueller Hinton agar (MHA) plates and 100 µL of each dilution was added aseptically to the wells in triplicate that had microbe isolate seeded with the standardized inoculums (1.5 X 10^8 CFU/ml). 100 µ methanol was introduced into the well-in-place extract as the control. All the test plates were incubated at 37 ^{0}C and were observed for growth after 24 hours. The lowest concentration of an extract showing a clear zone of inhibition was considered as the MIC. In the determination of MBC, a 100µl aliquot from the tube showing MIC was placed on an MHA plate and spread over the plate. After incubation at 37 ^{0}C for 24 hours, the plates were examined for the growth of a bacterium to determine the concentration of the extract at which 99.9% killing of bacterial isolates was achieved.

3. RESULTS AND DISCUSSION

3.1. Results

The results from the studies are presented in Tables 1 to 4.

The result obtained from the brine shrimp lethality test BSLT (Table 1) gave LC_{50} of 61.4 µg/L for alkaloid fraction and LC_{50} > 100µg/L for ethanol extracts respectively.

Table 1: Brine–Shrimp lethality test result of *Acalypha hispida stem bark,*

Plant Extracts	LC_{50} µg/ml
Ethanol Extract (EE)	62.28
Alkaloid Fraction (AK)	31.14

The results of the phytochemical screening of the ethanol and alkaloid extracts showed the presence of different secondary metabolites constituents (Table 2) present in the plant.

Table 2: Phytochemical Constituents of the Ethanol Extract of *Acalypha hispida* Root

Phytocompounds	Ethanolic extract
Alkaloids	+
Anthraquinones	+
Carbohydrates	-
Saponins	+
Phenolics	+
Tannins	+
Phlobatannins	+
Terpenoids	+
Steroids	+
Flavonoids	+
Glycosides	+
Coumarin	+

Key: + = present, - = absent

The result of the antimicrobial activity presented in Table 3, revealed that the alkaloid fraction was active more against the Gram-negative bacteria (*S. aureus, E. coli* and *K. pneumonia*) with inhibition zones of 17-33 mm, while the ethanol extract was active against all other test microorganisms, indicating a broad spectrum of activity (Table 3).

Table 3: Zone Inhibition of ethanol and alkaloid extract of *Acalypha hispida* Root against microorganism

Microorganism	Conc. x10²µg/cm³	EE	AK	PEN	CP	*C
Staphylococcus aureus	0.5	30	28	N	25	N
Lactobacillus spp	1.0	14	15	N	23	N
Staphylococcus faecalis	1.5	33	23	N	18	N
Gonorrhoea	2.0	23	19	N	21	N
Bacillus anthracis	2.5	19	N	20	31	N
Escherichia coli	3.0	12	33	24	N	N
pseudomonas aeruginosa	3.5	14	12	26	33	N
salmonella typhi	4.0	24	21	23	N	N
Klebsiella pneumonia	4.5	25	33	25	31	N
Staphylococcus epiderm	5.0	21	17	19	21	20

N= No Inhibition *C= Negative control EE= Ethanol extract, AK=Alkaloid extract, PEN= Penicillin, CP= Ciprofloxacin.

The result of the antifungal activity presented in Table 4, revealed that the ethanolic extract was active against the tested fungi stain with inhibition zones of 15-33 mm, while the alkaloid fraction gave no inhibition against all tested fungi strain (Table 4).

Table 4. Zone Inhibition (mm) against fungal isolates using Ethanol and Alkaloid *Acalypha hispida* Root extracts

	Zone of inhibition (mm)		
Microorganism	**EE**	**AK**	**x10²µg/cm³**
Aspergillus funigatus	32	N	6
Aspergillus actinomycete	32	N	6
Candida albicans	33	N	6

N= No Inhibition EE= Ethanol extract, AK=Alkaloid extracts

3.2. Discussion

The result obtained from the brine shrimp lethality test BSLT gave LC_{50} of 61.4 µg/L for alkaloid fraction and LC_{50}> 100µg/L for ethanol extracts indicating high toxicity of alkaloid fraction to brine shrimp larva. The results of the phytochemical screening of the ethanol and alkaloid extracts showed the presence of different active constituents like flavonoids, terpenoids, tannins, phlobatannins, alkaloids, saponins, phenolics, anthraquinones, steroids, glycosides and coumarins (Table 2). These Phyto-compounds have been reported to be responsible for the antimicrobial properties displayed by many medicinal plants[17]. The result from the *in- vitro* inhibitory activity revealed that the alkaloid fraction was active more against the Gram-negative bacteria (*E. coli* and *K. pneumonia*) with inhibition zones of 17-33 mm, while the ethanol extract was active against all other test microorganisms, indicating a broad spectrum of activity (Table 3). The results of the effect of the extracts on the fungal isolates are shown in table 4. The ethanol extract inhibited all the tested fungal isolates with inhibition of (32-33mm), while the alkaloid had no effect on the tested microbes (see Table 4). Thus, their presence in this plant may be responsible for the remarkable antimicrobial effect observed. The results of the *in- vitro* inhibitory activity of the alkaloid fraction show that the ethanol extract are quite appreciable when compared to the mean inhibition zones produced by the control antibiotics and the fact that the standard antibiotics are in the purified and concentrated form whereas the extracts are crude and may harbour both pharmacologically and non-pharmacologically active compounds (see Table 2-4). In the test of Minimum Inhibitory Concentration (MIC)/Minimum Bactericidal Concentration (MBC), the plates were examined for the growth of a bacterium to determine the concentration of the extract at which 99.9% killing of bacterial isolates was achieved.

The high activity exhibited by ethanol extract against tested strains of microbes shows that the root extract could be used as an alternative medicine in treating *staphylococcal* infections pending when the active ingredients of this plant would be isolated, chemically identified, and purified for further evaluation. Alkaloids generally have been noted for their ant malarial and antibacterial activities although it seems their mechanism of action on microbes remains unclear.[7, 17] The high activity observed more on the Gram-negative bacteria by the alkaloid extract is noteworthy and could have been a result of a possible interaction between these alkaloids and some constituents of the Gram-negative cell wall thereby causing cytotoxic damage to this group of bacteria. This suggestion is on the basis that structurally, Gram-positive and Gram-negative bacteria differ only in their cell wall composition.[18] Therefore, the alkaloid extract could be explored as a narrow-spectrum herbal drug especially in cases of gastrointestinal infections since the tested bacteria strains are clinical isolates. The inhibitory mechanism of the phytochemicals extracted with ethanol on the radial growth of the filamentous fungi could be that of blocking the synthesis is of the cell wall constituents or possible interference with the replication of genetic material to prevent cell division since it was recorded that the Molds did not grow at all.[19]

4. CONCLUSION

The maximum antimicrobial activities exhibited by the ethanol root extract of *A. hispida* against bacteria (Gram-positive and negative) and fungi respectively suggest that the extract may be used for the treatment of typhoid, malaria, boil, respiratory tract, and other diseases caused by the test organisms. The alkaloid fraction significantly inhibited the growth of Gram-negative bacteria, and thus could serve as a good first-line drug for infections caused by Gram-negative bacteria.

REFERENCES

(1) Sofowora, A. Medicinal Plants and Traditional Medicines in Africa. Spectrum Books Ltd., Ibadan, 2013, Pages: 289.
(2) Tiwari, P., Kumar, B., Kaur, M., Kaur, G., and Kaur, H.. Phytochemical screening and extraction: A review. *InternationalePharmaceutica Sciencia*, 2011, 1(1), 98-106
(3) Matthias E, Daniel J.K. Plant secondary metabolites as Defenses, Regulators, and primary metabolite: the Blurred Functional Trichotomy. Plant physiology, 2019, vol 184: 39-52

(4) Ishtiyak P., Hussain S.A. Traditional use of medicinal plants among tribal communities of Bangus Valley, Kashmir Himalaya, India. *Stud. Ethno-Med.* 2017; 11:318–331
(5) Rahman, M. A., & Mossa, J. S. Alkaloids: Future prospective to combat leishmaniasis. Natural Product Research. 2018, 32(19), 2228-2237. doi:10.1080/14786419.2017.1387793.
(6) Mishra, A. P., Saklani, S., Salehi, B., Parcha, V., Sharifi-Rad, M., Milella, L., Iriti, M., Suleria, H. A. R., & Kumar, N. Alkaloids and their pharmacological potentials: An overview. *European Journal of Medicinal Chemistry*, 2021, 223, 113632. doi:10.1016/j.ejmech.
(7) Garba, S. and S. O. Okeniyi (2011). Antimicrobial activities of total alkaloids extracted from some Nigerian medicinal plants. *Journal of Microbiology and Antimicrobials*. 4(3); pp. 60-63.
(8) Lee, C., & Ventola, M. S.. The antibiotics resistance crisis. *Pharmacy & Therapeutics*, 2015 40, 277-283.
(9) Gad HA, El-Ahmady SH, Abou-Shoer MI, Al-Azizi MM. Application of chemometrics in authentication of herbal medicines: a review. Phytochem Anal. 2013;24(1):1–24. https://doi.org/10.1002/pca.2378
(10) Md. AfjalusSiraj, Jamil A. Shilpi, Md. Golam Hossain, Shaikh Jamal Uddin, Md. Khirul Islam, IsmetAra. Anti-inflammatory and Antioxidant Activity of *Acalypha hispida* Leaf and Analysis of its Major Bioactive Polyphenols by HPLC. Advanced Pharmaceutical Bulletin. 2016:6(2), 275-283.
(11) Prajna P. P *, Reshmi R., Shana K. M, Vishnupriya V. V, Fahmeeda P. P. and E. Tamil J. Traditional uses, phytochemistry and pharmacology of Acalypha hispida burm: A systematic review. World Journal of Biology Pharmacy and Health Sciences, 2022, 12(02), 087–093.
(12) Tiwari, P., Kumar, B., Kaur, M., Kaur, G., and Kaur, H. Phytochemical screening and extraction: A review. *Internationale Pharmaceutica Sciencia*, 2011, 1(1), 98-106
(13) Bapna, S., Choudhary, P.K., Satvekar, T., Ramaiya, M. and Chowdhary, A. Antl-plasmodial potential of crude alkaloid extract of three plants used in traditional medicine in India. *JPBS* 2014, 9(5): 48-51.
(14) Basheer, L., Zroudi, M., Awadallah, H., & Al-Khatib, S. "Antimicrobial activity of essential oils from medicinal plants against multidrug-resistant bacteria using the agar well diffusion method." *Journal of Genetic Engineering and Biotechnology*. 2023, 21(1), 45-52. doi:10.1186/s43141-023-00317-1.
(15) Trudic, A., Ranin, L., &Opavski, N. Antimicrobial Susceptibility Testing: A Comprehensive Review of Currently Used Methods. *Antibiotics*, 2022. 11(4), 427. https://doi.org/10.3390/antibiotics11040427
(16) Mukherjee, P.K.; Harwansh, R.K.; Bahadur, S.; Banerjee, S.; Kar, A. Evidence-based validation of Indian traditional medicine: Way forward. In *From Ayurveda to Chinese Medicine*; World Scientific Publishing Co. Pte Ltd.: Singapore, 2017; pp. 137–167. [Google Scholar]
(17) Lv, L., Gao, B., & Li, M. "Research Progress on Antibacterial Activities and Mechanisms of Natural Alkaloids: A Review". *Antibiotics*, 2021, 10(3), 318. DOI: 10.3390/antibiotics10030318
(18) Silhavy, T. J., Kahne, D., & Walker, S. The bacterial cell envelope. *Cold Spring Harbor Perspectives in Biology*, 2010. 2(5), a000414. https://doi.org/10.1101/cshperspect.a000414 19.
(19) Gizaw, A., Kena, B., Babele, D. A., Borena, B. M., Boneya, G., Siraj, M., Shiferaw, S., Terfa, W., Tadesse, M., Bayu, M. D., Tadese, N. D., Sarba, E. J., &Bhaludra, C. S. S. Phytochemical screening and in vitro lousicidal and acaricidal activities of *Justicia schimperiana* (Hochst. ex Nees) T. Anderson leaf in West Showa Zone, Ethiopia. *Advances in Pharmacology and Pharmacy*, 2022, 11(2), 289-308 https://doi.org/10.54085/ap.2022.11.2.88
(20) Liu, C., Wang, Y., & Liu, X. Application of alkaloids in the treatment of Gram-negative bacterial infections: A review. *Antibiotics*, 2021.10(3), Article318. https://doi.org/10.3390/antibiotics10030318.
(21) MdAfjalus S, Jamil, A.S, Golam, H, Shaikh, J.U, MdKhiru, I, IsmetAra, J, Hemayet, H, Anti-inflammatory and Antioxidant Activity of Acalypha hispida Leaf and analysis of its major Bioactive polyphenols by HPLC: *Adv Pharm Bull.* 2016,30:6(2);275-283doi:10.15171/apb.2016.039.